IntechOpen

Leishmaniases as Re-emerging Diseases

Edited by Farhat Afrin and Hassan Hemeg

LEISHMANIASES AS RE-EMERGING DISEASES

Edited by **Farhat Afrin** and **Hassan Hemeg**

Leishmaniases as Re-emerging Diseases
http://dx.doi.org/10.5772/intechopen.71150
Edited by Farhat Afrin and Hassan Hemeg

Contributors

Gul Shahnaz, Hafiz Shoaib Sarwar, Masoom Yasinzai, Ana Lucia Abreu-Silva, Solange Araújo Melo, Tatiane Aranha Penha-Silva, Renata Mondego De Oliveira, Juliana Ide Aoki, Sandra Marcia Muxel, Lucile Maria Floeter-Winter, Juliane Cristina Ribeiro Fernandes, Abdoulaye Kassoum Kone, Ogobara Keguerem Doumbo, Mahamadou Ali Thera, Babacar Faye, Bartira Rossi-Bergmann, Ariane Sousa-Batista, Sujit Bhattacharya, Sudeshna Ganguly, Sandipan Ganguly, Sabahat Azim, Ajanta Ghosal, Shanta Dutta, Eltahir Awad Khalil, Sushma Singh, Magdalena M. Aguirre-Garcia, Laila Gutierrez Kobeh, Alma Reyna Escalona-Montaño, Arturo Alfredo Wilkins-Rodriguez, Anabela Cordeiro-Da-Silva, Pedro Cecilio, Luiz Oliveira, Adelina Jiménez, Gabriel Alfonso Gutiérrez Rebolledo, Rosalba León-Díaz, Farhat Afrin, Hassan Hemeg

First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Leishmaniases as Re-emerging Diseases
Edited by Farhat Afrin and Hassan Hemeg

p. cm.

Print ISBN 978-1-78984-101-5

Online ISBN 978-1-78984-102-2

eBook (PDF) ISBN 978-1-83881-521-9

Meet the editors

Dr. Farhat Afrin received her Ph.D. from the Indian Institute of Chemical Biology, Kolkata, India. Earlier, she served at the Department of Biotechnology, Hamdard University, New Delhi, India, for 16 years. She also worked at the National Institutes of Health, Bethesda, Maryland, USA, and at the Centre for Immunology and Infection, University of York, UK. She is a recipient of several honors, including American Association of Immunologists Young Faculty Travel Grant, Commonwealth Academic Staff Fellowship, and Department of Biotechnology Overseas Associateship. Her research interest is parasite immunology with an emphasis on vaccination and immunotherapeutics of infectious diseases. She has published over 56 papers in journals of international repute and is an academic editor, editorial board member, and reviewer of several journals and editor of a number of books.

Dr. Hassan A. Hemeg completed his Masters in Pathological Science from Sheffield University, UK, and his Ph.D. from King Abdulaziz University, Jeddah, Saudi Arabia. He earned several honors such as Fellow of the Institute of Biomedical Science, UK, and Certified Canadian Accreditation Specialist for Health Care Facilities. He acquired training in microbiology from Sheffield and Bristol universities, UK, and the US Department of Labor, Occupational Safety and Health Administration. His research interest is in the field of antimicrobial resistance. He has published several papers in journals of international repute and is editor of a number of books. Presently, he is an associate professor in the Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Taibah University, Madina, Saudi Arabia, where he also served as vice dean.

Contents

Preface

Leishmaniasis comprises a broad spectrum of neglected vector-borne diseases ranging in severity from self-healing but disfiguring and stigmatizing cutaneous lesions to mucocutaneous and fatal visceral manifestations, depending on the species and host characteristics. The visceralizing species is the most devastating and listed as one of the major neglected tropical diseases. The syndrome primarily afflicts the impoverished population of low-income countries, mainly in the tropics and subtropics. Despite intensive research, live vaccines are the only effective treatment till date against cutaneous leishmaniasis, while none exists for the visceral form. Moreover, there is an upward trend in development of resistance to most of the currently available chemotherapeutic arsenal. Combinations of drugs have also been explored. Absence of vaccines, progressive emergence of HIV/*Leishmania* coinfection, and relapse after treatment delineate the gravity of leishmaniasis affliction.

Invasion of host macrophages by *Leishmania* triggers a multitude of signaling circuits to eliminate the pathogen. However, the parasite has evolved stratagems to neutralize macrophage defensive arsenals, the very heart of the immune system's defensive machinery, creating a safe niche for its survival. Identification of new drug targets can contribute towards designing inhibitors and strengthening the pipeline for disease elimination. Natural products from medicinal plants have also shown leishmanicidal effects that in some cases are potentiated by immunomodulation. A plethora of nanoparticles has been reported to induce protection with modulation of the immune response. Another major challenge in the mitigation of this endemic disease is to achieve safe, efficacious, and low-cost prophylactic and/or therapeutic vaccines with long-lasting protection.

This book encompasses the epidemiology of leishmaniasis, immune evasion strategies employed by the *Leishmania* parasite, and current vaccination and immunotherapeutic approaches, including prophylactic as well as therapeutic vaccines. The prospects of new drug targets and inhibitors and natural product-based antileishmanial drugs and nanomedicines have also been exemplified.

I would like to take this opportunity to express my deepest gratitude to my revered colleague Dr. Hassan Hemeg for his valuable guidance, perseverant support, and constructive criticism that gave impetus to the success of this book.

Farhat Afrin and Hassan Abdullah Hemeg
Department of Clinical Laboratory Sciences
Faculty of Applied Medical Sciences
Taibah University
Madina, Kingdom of Saudi Arabia

Section 1

Introductory Chapter

Chapter 1

Introductory Chapter: Leishmaniasis: An Emerging Clinical Syndrome

Farhat Afrin and Hassan A. Hemeg

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79662

1. Introduction

Leishmaniasis comprises a broad-spectrum of neglected vector-borne diseases ranging in severity from the self-healing but disfiguring and stigmatizing cutaneous lesions to mucocutaneous and fatal visceral manifestations, depending on the species and host characteristics. This syndrome primarily afflicts the impoverished population of low-income countries falling in the tropics and subtropics. Globally, 0.7–1.2 million new cases of cutaneous leishmaniasis (CL) occur every year while for visceral leishmaniasis (VL), 200,000–400,000 new cases and 20,000–40,000 deaths are reported each year, with 95% of fatal cases occurring in only six countries, namely, India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil [1]. The disease is transmitted by the bite of female *Phlebotomus* sandflies that transmit the promastigotes, which are then transformed into amastigotes within the mammalian macrophages. The goal of World Health Organization is to eliminate this public health problem in the South-east Asia Region by 2020 [2]. Despite intensive research, live vaccines are the only effective vaccines till date against CL while none exists for the visceral form that is the most severe of the various clinical forms of leishmaniasis. Moreover, there is an upward trend in development of resistance to most of the currently available drugs [3]. The chemotherapeutic arsenal is associated with need for hospitalization and prolonged periods of treatment, coupled with high toxicity, which limits the application and patient compliance. Combinations of drugs have also been explored. Absence of vaccines, progressive emergence of HIV-*Leishmania* co-infection and relapse after treatment delineate the gravity of leishmaniasis affliction [4]. A recent report indicated relapse of post kala-azar dermal leishmaniasis (PKDL) 1 year after successful treatment of VL with miltefosine and paromomycin [5]. Antimony therapy is also not advised in elderly patients with CL due to severe adverse side effects [6]. The potential of

the visceralizing species, *Leishmania donovani* to cause localized cutaneous lesions is also not fully understood [7].

This chapter gives a brief glimpse of the recent advances in immunopathogenesis and immune evasion strategies employed by the *Leishmania* parasite, vaccination and immunotherapeutic approaches, natural product-based drugs, nanomedicines, therapeutic targets and diagnosis of leishmaniasis. We have included citations of the latest research articles presenting the most recent results.

2. Immunopathogenesis and immune evasion strategies

Invasion of host macrophages by *Leishmania* triggers a multitude of signaling circuits to eliminate the pathogen. However, the parasite tries to subvert these defense mechanisms to create a safe haven for their survival. *Leishmania* secretes effector molecules to modulate the host immune transcriptome resulting in alterations in the host epigenome to alter cytokine and chemokine levels, their cross talks and downstream signaling hubs. This adversely affects the recruitment and activation of immune cells, respiratory burst and antigen presentation, leading to immune evasion. *Leishmania amazonensis* has been reported to induce histone deacetylase in infected macrophages, which contributes to down regulation of inducible nitric oxide synthase and subsequent parasite survival [8]. *L. donovani* infection causes hypoxic environment within the macrophages by activating hypoxia inducible factor-1α, that in turn up regulates micro RNA-210, while down regulating NF-κB mediated pro-inflammatory immune responses, to establish a safe niche for their survival [9].

Leishmania have evolved stratagems to neutralize macrophage defensive arsenals, the very heart of the immune system's defensive machinery, resulting in replication of the parasites within the phagolysosomal vacuoles of the infected macrophages. Unfolding of these host-pathogen interactions will help in development of effective drug targets that would enable to modulate the host immune system to ameliorate the pathogenesis of infection. Besides the host immune profile and the intrinsic parasite factors that may influence the clinical manifestations of the disease, *Leishmania* virus RNA 1 (LRV1) infecting *Leishmania guyanensis* has been implicated to contribute to immunopathogenesis of American tegumentary leishmaniasis [10]. Studies have also indicated that gut microbiota egested during infected sandfly bites is an important determinant of *Leishmania* dissemination via triggering of inflammasomes, leading to IL-1β production that sustains the neutrophilic infiltrate harboring the parasites [11].

3. Current vaccination and immunotherapeutic approach

A major challenge to mitigation of this endemic disease is to achieve safe, efficacious and low-cost prophylactic or therapeutic vaccines with long-lasting protection. These vaccines should be effective against both stages of the parasite curbing its progression and accompanying pathology that stems from an imbalance between the pathogen and the host immune

system. The plethora of candidate vaccines range from the live non-pathogenic vectors to the recombinant subunit vaccines, alone or together with adjuvants and/or delivery systems for induction of cell-mediated immunity. Some of these include *Leishmania*-activated C-kinase antigen (LACK) [12], *Leishmania* cysteine peptidase A, B in poly-lactic-co-glycolic acid (PLGA) nanoparticles [13], soluble *Leishmania* antigens in nanoliposomes co-delivered with saponin and imiquimod [14], DNA vaccine encoding ornithine decarboxylase [15]. Inclusion of salivary proteins in antileishmanial vaccines has been reported to result in a synergistic protective effect [16]. A live recombinant amastigote 2 antigen vaccine vector using *Trypanosoma cruzi* non-virulent strain, and live attenuated centrin gene–deleted *Leishmania donovani* [17] have been reported to induce strong T cell-mediated protective immune responses against VL and hence could represent promising alternatives for translation to human clinical trials [18]. Recombinant small myristoylated protein-3, a virulence factor has been found to be immunogenic in both mice and humans, with induction of protective immunity against murine VL [19]. In case of CL, intranasal immunization has been found to reduce numbers of $CD4^{+}Foxp3^{+}$ regulatory T cells with increased Th1 response and associated protection [20].

Immunotherapy on the other hand has been found to promote sterilizing cure. However, immunotherapeutic intervention with *L. amazonensis* antigens plus saponin was not found to maintain long-lasting low parasitism in dogs naturally infected with *Leishmania infantum* [21]. Therapy with anti-PDL-1 antibody has been found to promote parasite clearance with concomitant induction of protective immunity against VL by inhibiting autophagy, that is hijacked by *Leishmania* [22]. Immunotherapeutic approach with Th1 stimulating antigens (aldolase, enolase, p45 and triose phosphate isomerase) has also been attempted [23].

An emerging therapeutic modality for CL is photodynamic therapy of zinc porphyrin that results in loss of plasma membrane integrity and hyperpolarization of the mitochondrial membrane potential [24].

4. Therapeutic targets and inhibitors

Identification of new drug targets can contribute towards designing inhibitors and strengthen the pipeline for disease elimination. DNA topoisomerases that control the over- or underwinding of DNA have been reported as deadly targets for topoisomerase inhibitors that may act as potential antileishmanial drugs [25]. Computational tools using *in silico* approaches targeting key enzymes in metabolic pathways of *Leishmania* have led to identification of several potential druggable targets such as cytochrome P450 sterol 14α-demethylase [26], dihydrofolate reductase-thymidylate synthase [27], methylglyoxal degradation superpathway [28], trypanothione reductase [29]. Trypanothione reductase is absent in humans and neutralizes the reactive oxygen species generated inside the infected macrophages. Inhibitors such as chalcones that block the activity of these trypanosomatid enzymes may be effective in treatment of leishmaniasis [29]. β-carbonic anhydrase [30], acid phosphatases [31], uracil DNA glycosylase [32] and Type 2 NADH dehydrogenase [33] are other potential therapeutic targets that are being explored. NLR (NOD-Like Receptor) family member NOD2 has also been implicated as an essential therapeutic target [34].

5. Natural products as source of antileishmanial drugs

In view of looming chemotherapeutic drug resistance, natural products and scaffolds from medicinal plants are being emphasized as leads for drug discovery. Plant-based bioactive compounds have merit over synthetic compounds, considering their unique structural variety, providing an unlimited source of molecules and biological activities [35]. A host of plant extracts or oils and their phytoconstituents (alkaloids, terpenoids, quinones, flavonoids, saponins, phenylpropanoids, flavonoids, lignoids, naphthoquinones, iridoids, and more) have shown promise *in vitro* and/or *in vivo* [36–41]. In some cases, the leishmanicidal effect is potentiated by immunomodulation [3, 42]. Besides plants, secondary metabolites from microorganisms such as fungi [43] and marine organisms have also been reported. Plant defensins have been found to eliminate *Leishmania* parasites via plasma membrane perturbation, mitochondrial membrane collapse, and reactive oxygen species induction [44].

Antimicrobial peptides have been reported to improve the therapeutic outcome of antileishmanial drugs [45]. Synergistic drug-natural product combinations have also been explored [46, 47].

6. Nanomedicines

In recent years, numerous advances in drug discovery have been made for treating leishmaniasis, exploiting nanotechnological approaches to target the immune cell phagolysosomes that harbors the *Leishmania* amastigotes. A plethora of nanoparticles have been reported to elicit protection with modulation of the immune response via reduction in anti-inflammatory cytokine IL-10, and increased nitric oxide production [48]. Recently, antileishmanial activity of sulphonamide nanoemulsions have been reported that target the leishmanial β-carbonic anhydrase [30]. Linalool-loaded gold nanoparticles have also been found to exhibit therapeutic effectiveness against *Leishmania* [49]. A short-course AmBisome regimen has been found to be safe and effective in the treatment of clinically diagnosed PKDL patients in Bangladesh, and may be considered as a viable option for routine programmatic use, contributing towards the VL elimination drive [50]. Biodegradable PLGA microparticles loaded with an antileishmanial nitrochalcone has proved therapeutic effectiveness when administered subcutaneously in BALB/c mice with cutaneous lesions [51].

Green nanoparticles, that is, plant-based synthesis of nanoparticles have an upper edge over the synthetic nanoparticles owing to their biosynthesis being rapid, eco-friendly, non-pathogenic and economical. An array of biogenic nanoparticles from plant extracts has been reported to have antileishmanial activity with boosting of anti-oxidant activity [52, 53].

Miltefosine- and ketoconazole-loaded nanoniosomes with improved antileishmanial activity have also been reported [54]. AmBisome-miltefosine combination therapy for VL-HIV co-infected patients has been reported in Ethiopia with 83.8% cure rate [55].

7. Diagnosis of leishmaniasis

A definitive diagnosis of leishmaniasis is crucial to guide timely and appropriate therapy. The disease is often confused with other co-endemic diseases and HIV co-infections may result in atypical clinical presentation [4]. Differential diagnosis of VL should be considered in patients of endemic areas after organ transplantation [56]. This underscores the need for highly sensitive and specific diagnostic modalities. In this regard, molecular techniques such as real-time polymerase chain reaction (qPCR)-based methods are gaining ground for detection and quantification of *Leishmania* as well as for species identification [57]. However, to rule out false negatives, combination of two PCR techniques is advisable in patients with cutaneous lesions [58]. For VL, serological diagnosis with recombinant antigen rK39-based immunochromatography and direct agglutination test based on the whole parasite antigens have been reported to have high sensitivity and specificity [59]. Nonetheless, amastigote detection in bone marrow aspirates and positive rK39 immunochromatographic test should be further validated by nested PCR [60]. Recently, a loop-mediated isothermal amplification (LAMP) assay based on 18S rDNA and the conserved region of minicircle kDNA has been implicated with high sensitivity for visceral as well as CL diagnostics [61]. Further, *Leishmania* urine antigen has been explored as a probable biomarker for predicting treatment failure and relapse in VL/HIV-coinfected patients [62].

8. Conclusions and future perspectives

To strengthen the leishmaniasis elimination drive, particular emphasis has to be laid on the diagnosis, chemotherapeutics and new targets identification and vaccination strategies for control of this endemic disease. This underscores renewed efforts to combat upcoming challenges in the quest for new drug targets in achieving definitive cure and/or safe, cost-effective prophylactic vaccines with long-lasting immunity against leishmaniasis. An effective therapeutic vaccine may further boost the immunosuppressed state and thus control the visceralizing form of leishmaniasis that is mainly harbored in the South Asian region.

Conflict of interest

The authors declare no conflict of interest.

Author details

Farhat Afrin* and Hassan A. Hemeg

*Address all correspondence to: farhatafrin@gmail.com

Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Taibah University, Al Madinah Al Munawarah, Kingdom of Saudi Arabia

References

[1] Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;**7**(5):e35671

[2] Karunaweera ND, Ferreira MU. Leishmaniasis: Current challenges and prospects for elimination with special focus on the South Asian region. Parasitology. 2018;**145**(4):425-429

[3] Dumetz F, Cuypers B, Imamura H, Zander D, D'Haenens E, Maes I, et al. Molecular preadaptation to antimony resistance in Leishmania donovani on the Indian subcontinent. mSphere. 2018;**3**(2):e00548-e00517

[4] de Lima Henn GA, Júnior ANR, Colares JKB, Mendes LP, Silveira JGC, Lima AAF, et al. Is visceral Leishmaniasis the same in HIV-coinfected adults? The Brazilian Journal of Infectious Diseases. 2018;**22**(2):92-98

[5] Pandey K, Goyal V, Das V, Verma N, Rijal S. PKDL development after combination treatment with miltefosine and paromomycin in a case of visceral leishmaniasis: First ever case report. Journal of Medical Microbiology and Immunology Research. 2018;**2**(1)

[6] do Lago AS, Nascimento M, Carvalho AM, Lago N, Silva J, Queiroz JR, et al. The Elderly Respond to Antimony Therapy for Cutaneous Leishmaniasis Similarly to Young Patients but Have Severe Adverse Reactions. The American Journal of Tropical Medicine and Hygiene. 2018;**98**(5):1317-1324

[7] Siriwardana YD, Deepachandi B, Ranasinghe S, Soysa P, Karunaweera N. Evidence for Seroprevalence in human localized cutaneous leishmaniasis caused by *Leishmania donovani* in Sri Lanka. BioMed Research International. 2018;**2018**:9320367

[8] Calegari-Silva TC, Vivarini ÁC, Pereira RM, Dias-Teixeira KL, Rath CT, Pacheco AS, et al. *Leishmania amazonensis* downregulates macrophage iNOS expression via histone: Deacetylase 1 (HDAC1): A novel parasite evasion mechanism. European Journal of Immunology. 2018;**48**(7):1188-1198

[9] Kumar V, Kumar A, Das S, Kumar A, Abhishek K, Verma S, et al. *Leishmania donovani* activates hypoxia inducible factor-1α and miR-210 for survival in macrophages by downregulation of NF-κB mediated pro-inflammatory immune response. Frontiers in Microbiology. 2018;**9**:385

[10] Borges AF, Gomes RS, Ribeiro-Dias F. Leishmania (Viannia) guyanensis in tegumentary leishmaniasis. Pathogens and Disease. 2018;**76**(4):fty025

[11] Dey R, Joshi AB, Oliveira F, Pereira L, Guimarães-Costa AB, Serafim TD, et al. Gut Microbiota Egested during Bites of Infected Sand Flies Augments Severity of Leishmaniasis Via Inflammasome-Derived IL-1β. 2018

[12] Fernández L, Carrillo E, Sánchez-Sampedro L, Sánchez C, Ibarra-Meneses AV, Jimenez MA, et al. Antigenicity of leishmania-activated C-kinase antigen (LACK) in human peripheral blood mononuclear cells, and protective effect of prime-boost vaccination with pCI-neo-LACK plus attenuated LACK-expressing Vaccinia viruses in hamsters. Frontiers in Immunology. 2018;**9**

[13] Noormehr H, Hosseini AZ, Soudi S, Beyzay F. Enhancement of Th1 immune response against Leishmania cysteine peptidase A, B by PLGA nanoparticle. International Immunopharmacology. 2018;**59**:97-105

[14] Emami T, Rezayat SM, Khamesipour A, Madani R, Habibi G, Hojatizade M, et al. The role of MPL and imiquimod adjuvants in enhancement of immune response and protection in BALB/c mice immunized with soluble Leishmania antigen (SLA) encapsulated in nanoliposome. Artificial Cells, Nanomedicine, and Biotechnology. 2018:1-10

[15] Kumar A, Dikhit MR, Amit A, Zaidi A, Pandey RK, kumar Singh A, et al. Immunomodulation induced through ornithine decarboxylase DNA immunization in Balb/c mice infected with *Leishmania donovani*. Molecular Immunology. 2018;**97**:33-44

[16] Martin-Martin I, Chagas AC, Guimaraes-Costa AB, Amo L, Oliveira F, Moore IN, et al. Immunity to LuloHya and Lundep, the salivary spreading factors from Lutzomyia longipalpis, protects against *Leishmania major* infection. PLoS Pathogens. 2018;**14**(5):e1007006

[17] Banerjee A, Bhattacharya P, Dagur PK, Karmakar S, Ismail N, Joshi AB, et al. Live attenuated *Leishmania donovani* centrin gene–deleted parasites induce IL-23–dependent IL-17–protective immune response against visceral leishmaniasis in a murine model. The Journal of Immunology. 2018;**200**(1):163-176

[18] Almeida APM, Machado LF, Doro D, Nascimento FC, Damasceno L, Gazzinelli RT, et al. New vaccine formulations containing a modified version of the amastigote 2 antigen and the non-virulent *Trypanosoma cruzi* CL-14 strain are highly antigenic and protective against *Leishmania infantum* challenge. Frontiers in Immunology. 2018;**9**:465

[19] Oliveira MP, Martins VT, Santos TT, Lage DP, Ramos FF, Salles B, et al. Small myristoylated protein-3, identified as a potential virulence factor in *Leishmania amazonensis*, proves to be a protective antigen against visceral leishmaniasis. International Journal of Molecular Sciences. 2018;**19**(1):129

[20] Bezerra IPS, Abib MA, Rossi-Bergmann B. Intranasal but not subcutaneous vaccination with LaAg allows rapid expansion of protective immunity against cutaneous leishmaniasis. Vaccine. 2018;**36**(18):2480-2486

[21] Viana KF, Lacerda G, Teixeira NS, Cangussu ASR, Aguiar RWS, Giunchetti RC. Therapeutic vaccine of killed *Leishmania amazonensis* plus saponin reduced parasite burden in dogs naturally infected with *Leishmania infantum*. Veterinary Parasitology. 2018;**254**:98-104

[22] Habib S, El Andaloussi A, Elmasry K, Handoussa A, Azab M, Elsawey A, et al. PDL-1 blockade prevents T cell exhaustion, inhibits autophagy, and promotes clearance of *Leishmania donovani*. Infection and Immunity. 2018;**86**(6):e00019-e00018

[23] Yadav NK, Joshi S, Ratnapriya S, Sahasrabuddhe AA, Dube A. Immunotherapeutic potential of Leishmania (Leishmania) donovani Th1 stimulatory proteins against experimental visceral leishmaniasis. Vaccine. 2018;**36**(17):2293-2299

[24] Andrade C, Figueiredo R, Ribeiro K, Souza L, Sarmento-Neto J, Rebouças J, et al. Photodynamic effect of zinc porphyrin on the promastigote and amastigote forms of *Leishmania braziliensis*. Photochemical & Photobiological Sciences. 2018;**17**(4):482-490

[25] Elmahallaw E, Garcia-Estrada C, Carbajo-Andres R, Balana-Fouce R. DNA topoisomerases of Leishmania parasites; druggable targets for drug discovery. Current Medicinal Chemistry. 2018

[26] Shokri A, Abastabar M, Keighobadi M, Emami S, Fakhar M, Teshnizi SH, et al. Promising antileishmanial activity of novel imidazole antifungal drug Luliconazole against *Leishmania major*: In vitro and in silico studies. Journal of Global Antimicrobial Resistance. 2018. pii: S2213-7165(18)30091-2

[27] Vadloori B, Sharath A, Prabhu NP, Maurya R. Homology modelling, molecular docking, and molecular dynamics simulations reveal the inhibition of *Leishmania donovani* dihydrofolate reductase-thymidylate synthase enzyme by Withaferin-A. BMC Research Notes. 2018;**11**(1):246

[28] Chávez-Fumagalli MA, Schneider MS, Lage DP, Tavares GSV, Mendonça DVC, Santos TTO, et al. A computational approach using bioinformatics to screening drug targets for *Leishmania infantum* species. Evidence-Based Complementary and Alternative Medicine. 2018;**2018**

[29] Ortalli M, Ilari A, Colotti G, De Ionna I, Battista T, Bisi A, et al. Identification of chalcone-based antileishmanial agents targeting trypanothione reductase. European Journal of Medicinal Chemistry. 2018;**2018**:6813467

[30] da Silva Cardoso V, Vermelho AB, Ricci Junior E, Almeida Rodrigues I, Mazotto AM, Supuran CT. Antileishmanial activity of sulphonamide nanoemulsions targeting the β-carbonic anhydrase from Leishmania species. Journal of Enzyme Inhibition and Medicinal Chemistry. 2018;**33**(1):850-857

[31] Dorsey BM, McLauchlan CC, Jones MA. Evidence that speciation of oxovanadium complexes does not solely account for inhibition of Leishmania acid phosphatases. Frontiers in Chemistry. 2018;**6**:109

[32] Mishra A, Khan M, Jha PK, Kumar A, Das S, Das P, et al. Oxidative stress-mediated overexpression of uracil DNA glycosylase in *Leishmania donovani* confers tolerance against antileishmanial drugs. Oxidative Medicine and Cellular Longevity. 2018;**2018**:4074357

[33] Stevanović S, Perdih A, Senćanski M, Glišić S, Duarte M, Tomás AM, et al. In silico discovery of a substituted 6-methoxy-quinalidine with Leishmanicidal activity in *Leishmania infantum*. Molecules. 2018;**23**(4):772

[34] Jawed JJ, Saini P, Majumdar S. Exploring the role of immune-modulators in pathogen recognition receptor NOD2 mediated protection against visceral leishmaniasis. World Academy of Science, Engineering and Technology. International Journal of Medical and Health Sciences. 2018;**5**(3)

[35] Varela M, Fernandes J. Natural products: Key prototypes to drug discovery against neglected diseases caused by Trypanosomatids. Current Medicinal Chemistry. 2018

[36] Simoben CV, Ntie-Kang F, Akone SH, Sippl W. Compounds from African medicinal plants with activities against selected parasitic diseases: Schistosomiasis, trypanosomiasis and leishmaniasis. Natural Products and Bioprospecting. 2018:1-19

[37] de Lima Moreira F, Riul TB, de Lima Moreira M, Pilon AC, Dias-Baruffi M, Araújo MS, et al. Leishmanicidal effects of piperlongumine (Piplartine) and its putative metabolites. Planta Medica. 2018

[38] dos Santos Sales V, Monteiro ÁB, de Araújo Delmondes G, do Nascimento EP. Antiparasitic activity and essential oil chemical analysis of the piper Tuberculatum Jacq fruit. Iranian Journal of Pharmaceutical Research. 2018;**17**(1):268-275

[39] Zafar S, Ur-Rehman F, Shah ZA, Rauf A, Khan A, Humayun Khan M, et al. Potent leishmanicidal and antibacterial metabolites from Olea ferruginea. Journal of Asian Natural Products Research. 2018:1-9

[40] Krstin S, Sobeh M, Braun MS, Wink M. Anti-parasitic activities of *Allium sativum* and *Allium cepa* against *Trypanosoma b. brucei* and *Leishmania tarentolae*. Medicine. 2018;**5**(2):37

[41] Monzote L, Geroldinger G, Tonner M, Scull R, De Sarkar S, Bergmann S, et al. Interaction of ascaridole, carvacrol, and caryophyllene oxide from essential oil of Chenopodium ambrosioides L. with mitochondria in Leishmania and other eukaryotes. Phytotherapy Research. 2018

[42] Domeneghetti L, Demarchi I, Caitano J, Pedroso R, Silveira T, Lonardoni M. Calophyllum brasiliense modulates the immune response and promotes *Leishmania amazonensis* intracellular death. Mediators of Inflammation. 2018;**2018**:6148351

[43] Peretz A, Zabari L, Pastukh N, Avital N, Masaphy S. In vitro antileishmanial activity of a black Morel, *Morchella importuna* (ascomycetes). International Journal of Medicinal Mushrooms. 2018;**20**(1):71-80

[44] Souza GS, de Carvalho LP, de Melo EJT, Gomes VM, AdO C. The toxic effect of Vu-Defr, a defensin from Vigna unguiculata seeds, on *Leishmania amazonensis* is associated with reactive oxygen species production, mitochondrial dysfunction, and plasma membrane perturbation. Canadian Journal of Microbiology. 2018;**64**(999):1-10

[45] Fragiadaki I, Katogiritis A, Calogeropoulou T, Brückner H, Scoulica E. Synergistic combination of alkylphosphocholines with peptaibols in targeting *Leishmania infantum* in vitro. International Journal for Parasitology: Drugs and Drug Resistance. 2018;**8**(2):194-202

[46] Khanra S, Kumar YP, Dash J, Banerjee R. In vitro screening of known drugs identified by scaffold hopping techniques shows promising leishmanicidal activity for suramin and netilmicin. BMC Research Notes. 2018;**11**(1):319

[47] Vieira-Araújo FM, Rondon FCM, Vieira ÍGP, Mendes FNP, de Freitas JCC, de Morais SM. Sinergism between alkaloids piperine and capsaicin with meglumine antimoniate against *Leishmania infantum*. Experimental parasitology. 2018;**188**:79-82

[48] Halder A, Shukla D, Das S, Roy P, Mukherjee A, Saha B. Lactoferrin-modified Betulinic acid-loaded PLGA nanoparticles are strong anti-leishmanials. Cytokine. 2018. pii: S1043-4666(18) 30208-4

[49] Jabir MS, Taha AA, Sahib UI. Linalool loaded on glutathione-modified gold nanoparticles: A drug delivery system for a successful antimicrobial therapy. Artificial Cells, Nanomedicine, and Biotechnology. 2018;**Apr 4**:1-11

[50] den Boer M, Das AK, Akhter F, Burza S, Ramesh V, Ahmed B-N, et al. Safety and effectiveness of short-course AmBisome in the treatment of post-kala-azar dermal leishmaniasis (PKDL): A prospective cohort study in Bangladesh. Clinical Infectious Diseases. 2018

[51] de Jesus Sousa-Batista A, Pacienza-Lima W, Arruda-Costa N, CAB F, Ré MI, Rossi-Bergmann B. Depot subcutaneous injection with chalcone CH8-loaded poly (lactic-co-glycolic acid) microspheres as a single-dose treatment of cutaneous leishmaniasis. Antimicrobial Agents and Chemotherapy. 2018;**62**(3):e01822-e01817

[52] El-khadragy M, Alolayan EM, Metwally DM, El-Din MFS, Alobud SS, Alsultan NI, et al. Clinical efficacy associated with enhanced antioxidant enzyme activities of silver nanoparticles biosynthesized using Moringa oleifera leaf extract, against cutaneous leishmaniasis in a murine model of *Leishmania major*. International Journal of Environmental Research and Public Health. 2018;**15**(5):1037

[53] Ovais M, Khalil AT, Raza A, Islam NU, Ayaz M, Saravanan M, et al. Multifunctional theranostic applications of biocompatible green-synthesized colloidal nanoparticles. Applied Microbiology and Biotechnology. 2018;**102**(10):4393-4408

[54] Nazari-Vanani R, Vais RD, Sharifi F, Sattarahmady N, Karimian K, Motazedian M, et al. Investigation of anti-leishmanial efficacy of miltefosine and ketoconazole loaded on nanoniosomes. Acta Tropica. 2018;**185**:69-76

[55] Abongomera C, Diro E, de Lima Pereira A, Buyze J, Stille K, Ahmed F, et al. The initial effectiveness of liposomal amphotericin B (AmBisome) and miltefosine combination for treatment of visceral leishmaniasis in HIV co-infected patients in Ethiopia: A retrospective cohort study. PLoS Neglected Tropical Diseases. 2018;**12**(5):e0006527

[56] Sánchez FC, Sánchez TV, Díaz MC, Moyano VS, Gallego CJ, Marrero DH, editors. Visceral leishmaniasis in renal transplant recipients: Report of 2 cases. Transplantation Proceedings. 2018;**50**(2):581-582

[57] Galluzzi L, Ceccarelli M, Diotallevi A, Menotta M, Magnani M. Real-time PCR applications for diagnosis of leishmaniasis. Parasites & Vectors. 2018;**11**(1):273

[58] Merino-Espinosa G, Rodríguez-Granger J, Morillas-Márquez F, Tercedor J, Corpas-López V, Chiheb S, et al. Comparison of PCR-based methods for the diagnosis of cutaneous leishmaniasis in two different epidemiological scenarios: Spain and Morocco. Journal of the European Academy of Dermatology and Venereology. 2018

[59] Bangert M, Flores-Chávez MD, Llanes-Acevedo IP, Arcones C, Chicharro C, García E, et al. Validation of rK39 immunochromatographic test and direct agglutination test for

the diagnosis of Mediterranean visceral leishmaniasis in Spain. PLoS Neglected Tropical Diseases. 2018;**12**(3):e0006277

[60] Shrestha M, Pandey BD, Maharjan J, Dumre SP, Tiwari PN, Manandhar KD, et al. Visceral leishmaniasis from a non-endemic Himalayan region of Nepal. Parasitology Research. 2018;**117**(7):2323-2326

[61] Adams ER, Schoone G, Versteeg I, Gomez MA, Diro E, Mori Y, et al. Development and evaluation of a novel LAMP assay for the diagnosis of cutaneous and visceral leishmaniasis. Journal of Clinical Microbiology. 2018;**56**(7). pii: e00386-18

[62] Van Griensven J, Mengesha B, Mekonnen T, Fikre H, Takele Y, Adem E, et al. Leishmania antigenuria to predict initial treatment failure and relapse in visceral leishmaniasis/HIV coinfected patients: An exploratory study nested within a clinical trial in Ethiopia. Frontiers in Cellular and Infection Microbiology. 2018;**8**:94

Leishmaniasis: An Overview

Chapter 2

Visceral Leishmaniasis

Sujit K. Bhattacharya, Ajanta Ghosal,
Sudeshna Ganguly, Sandipan Ganguly,
Sabahat Azim and Shanta Dutta

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75907

Abstract

Clinically, leishmaniasis is of three types—visceral leishmaniasis (VL) or kala-azar, cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL). Post-kala-azar dermal leishmaniasis (PKDL) is considered as a complication of VL. VL is characterized by fever, anemia and splenomegaly in a VL-endemic area (malaria excluded). A subject with such symptoms should be subjected to an rK39 strip test. Confirmation of diagnosis is made by demonstration of the parasite (*Leishmania donovani*) from samples obtained by aspiration of bone marrow or iliac crest puncture. Miltefosine, stibogluconate, amphotericin B, liposomal amphotericin B and paromomycin are effective available anti-leishmaniasis drugs. Vector (*Phleblotomus argentipes*) control for reduction of transmission and early diagnosis and complete treatment are essential elements of case management. There is no effective vaccine against VL. This review on VL aims at providing state-art knowledge on epidemiology, diagnosis and case-management and vaccine development.

Keywords: leishmaniasis, PKDL, rK39 strip test, kala-azar vaccine

1. Introduction

The distinct clinical forms of leishmaniasis are visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL). PKDL is considered to be a complication of VL. Kala-azar is a neglected tropical disease (NTD). It affects the poorest of the poor living in endemic areas. Post-kala-azar dermal leishmaniasis (PKDL) is associated with stigma. Fortunately, it is not difficult to diagnose the disease and several drugs are available for treatment of the disease.

1.1. VL

VL is prevalent in 88 countries, and there is an estimated 2 million new cases per year, of which 5,00,000 are VL and 15,00,000 are CL. The disease burden is calculated at 23,57,000 disability-adjusted life years, a significant ranking among communicable diseases [1]. More than 147 million people living in the Southeast Asia Region are at risk. In this region, the highest disease burden is seen in the northern part of the state of Bihar, India. A large number of VL cases are seen in the international cross-border areas between the countries. In view of this, cross-border collaboration is crucial for elimination of the disease [2]. VL prevails mostly among poor people in marginalized communities. It attacks the internal organs and can be fatal if left untreated as it affects the vital organs of the body. Symptoms include irregular bouts of fever, weight loss, enlargements of the spleen and liver and anemia. All patients diagnosed to be VL require prompt and complete specific medical treatment; otherwise, the patient may die.

1.2. PKDL

PKDL is considered an important long-lasting complication of kala-azar. This is seen in about 1–15% in the Indian subcontinent and in about 60% of treated, partially treated and untreated or active cases of kala-azar in Sudan [3]. The lesions generally appear from 1 to 15–20 years of kala-azar. The typical lesions are most prominently seen in the face (macular, papular and nodular). In contrast to the Indian subcontinent, the nodular lesions generally ulcerate as they grow in Sudan. The macular lesions are sometimes confused with leprosy. Diagnosis is confirmed by demonstration of *Leishmania donovani* from the tissue obtained from the lesions. A polymerase chain reaction (PCR) test to detect the DNA is highly reliable and can be performed in special laboratories. Treatment is long-term use of sodium stibogluconate (sometimes in combination with rifampicin) [4]. A recent study showed that a 12-week course of miltefosine is safe and effective in the treatment of PKDL [5]. Another study showed that miltefosine 50 mg 3 times daily for 60 days or 50 mg twice daily for 90 days has been shown to be effective [6]. In 2008, Berman remarked that 'Miltefosine is effective and can be recommended for visceral disease in India and in Ethiopia, and for cutaneous disease in Colombia and Bolivia. For unusual forms of disease that require long periods of treatment such as diffuse CL, oral miltefosine is probably the treatment of choice'. In 2006, Simon and Engel remarked that 'Miltefosine is active against most *Leishmania* species, including those that cause CL'. It has been demonstrated that in 2015 the efficacy of miltefosine has declined. Paromomycin has been shown to be effective against PKDL [7]. Amphotericin B is effective in the treatment of PKDL and several courses, where gaps between the two courses are required.

1.3. CL

CL and MCL are the most common manifestations of leishmaniasis. This is also known as oriental sore. MCL is seen in the tropics and subtropics. The variety of leishmaniasis causes disfigurement. CL is caused by *L. tropica* and *L. major*. *L. braziliensis* causes MCL transmitted by *Phleblotomus argentipes*, sand fly. A clinical diagnosis should be supported by a PCR test [8].

1.4. Diagnosis

Clinical features like prolonged fever (>14 days), anaemia and splenomegaly (malaria excluded) in a kala-azar-endemic area will constitute a suspect case. This can be supported by an rK39 strip test. Accurate VL diagnosis till 1990 required parasitological confirmation by microscopy or culture of the blood, bone marrow, lymph nodes or spleen. Splenic aspirate was sometimes fatal and this prompted development of rapid diagnostic tests (RDTs). An RK39 test had a sensitivity of 72.1% and a specificity of 76.9% and DAT sensitivity was 62.8% and its specificity was 69.2%, using initial diagnosis (confirmed on clinical and serological basis) as reference in both cases. Both rK39 and DAT have the potential in diagnosing VL using urine [9]. A polymerase chain reaction test (PCR) to detect the DNA is highly reliable and can be performed in special laboratories.

2. Management

2.1. Management of VL

2.1.1. Miltefosine

This is the first ever oral drug developed against VL. Initially the drug was used in the treatment of skin metastases from breast cancer. Subsequently, it was found to be effective in vitro against the *Leishmania donovani* parasite [9]. Phase I–Phase III clinical trials conducted in India demonstrated that the drug was effective against VL to the extent of ~95% [10–12]. Most of the side-effects included nausea, vomiting, abdominal pain, diarrhea and fever [11]. These side-effects occur during the first week of treatment. A Phase IV trial was conducted involving 13 centres in Bihar (India). This pivotal trial clearly demonstrated that the drug can be dispensed in the kala-azar elimination programme in the first condition [13]. A few major side-effects that occurred affected the kidney, liver and bone marrow. The dose of the drug is 50 mg 2 times daily after food to avoid gastric irritation for 28 days. In children, the dose is 2.5 mg/kg daily for 28 days [12]. In summer time, these patients are usually dehydrated and they should be rehydrated with ORS or IV fluids depending upon the degree of dehydration. Anemia is very common in such patients. Blood transfusion may be required and should be given. If these simple measures are taken, the patients tolerate the drug better. Miltefosine is teratogenic and should not be given to pregnant mothers.

2.1.2. Paromomycin

This drug belongs to the group of aminoglycosides. Unlike miltefosine, this drug is administered by intramuscular injections. A full course comprises daily injections (11 mg/kg/day) for 21 days [14, 15]. Although paromomycin is an aminoglycoside, it does not exhibit much ototoxicity or nephrotoxicity. It is safe in pregnancy. The most common adverse effects associated with paromomycin are abdominal cramps, diarrhea, heartburn, nausea and vomiting. Long-term use of paromomycin increases the risk for bacterial or fungal infection. Signs of

overgrowth include white patches in the oral cavities. Other less common adverse events include myasthenia gravis, kidney damage, enterocolitis, malabsorption syndrome, eosinophilia, headache, hearing loss, ringing in the ear, itching, severe dizziness and pancreatitis.

2.1.3. Amphotericin B

This is an anti-fungal drug and has substantial activity against *Leishmania donovani*. When resistance to stibogluconate becomes high, amphotericin B became the first-line drug in many places. The dose is 15 alternate-day infusions of 1 mg/kg over 30 days (total dose, 15 mg/kg) or daily treatment with 1 mg/kg for 20 days (total dose, 20 mg/kg). The most common side-effects are chill, rigor and fever. Injection of antihistamine alleviates the symptoms. The drug exhibits nephrotoxicity and ototoxicity [16–18].

2.1.4. Liposomal amphotericin B

Liposomal amphotericin B is safer than amphotericin B and safest among all anti-VL drugs. It is given by intravenous infusion. The dose is 5 mg/kg $\times$ 3 days or 3 mg/ kg $\times$ 5 days. A single dose of 10 mg/kg has shown a cure rate of more than 95%. Liposomal amphotericin B replaced miltefosine as the first-line drug in the kala-azar elimination programme [19–21]. However, it is felt that this decision to switch over from miltefosine to liposomal amphotericin B could have been delayed as the programme was going on smoothly using miltefosine as the first-line drug.

2.1.5. Sodium stibogluconate

For more than last 6 decades, sodium stibogluconate was the effective drug treatment for visceral leishmaniasis and PKDL. The dose is 20 mg/kg/day given by intramuscular injection for 30 days [22]. Afterwards, the parasites developed resistance to the drug and it became ineffective. In order to overcome the drug resistance, the dose of the drug was increased but the cardiotoxicity of the drug increased. In Bihar, India, currently, the drug resistance is to the tune of 60% [23]. However, the drug is still used in places where the parasites are sensitive to the drug [24]. It can be given by both intramuscular and intravenous routes. The intramuscular injections are painful.

2.1.6. Urea stibamine

Urea stibamine was an effective anti-leishmanial drug. Since the developer did not keep any record of the compound, the drug had its natural death [25].

2.1.7. Anti-fungal agents

Anti-fungal agents like ketoconazole and anti-tuberculosis drugs were not found effective in the treatment of VL. However, both ketoconazole and pentostam were more effective than placebo against *L. braziliensis* panamensis cutaneous leishmaniasis. Oral ketoconazole is comparable in efficacy to this parenteral pentostam regimen and can be recommended as initial treatment for this disease. Sitamaquine is undergoing clinical trial for VL treatment and initial

results are encouraging [26]. Sitamaquine interacts with phospholipids and accumulates rapidly in the *Leishmania*. An advantage of sitamaquine is its short elimination half-life, preventing a rapid emergence of resistance.

2.1.8. Combination therapy

In view of the drug resistance and toxicity [27], it was expected that using the combination of two drugs will prevent or delay appearance of drug resistance, minimize toxicity, enhance efficacy and shorten duration of therapy. A safety and efficacy trial of combinations were conducted in Bangladesh [28].

1. Liposomal amphotericin B alone or a combination of:
2. single dose of Liposomal amphotericin B (day 1) with miltefosine (day 2–8)
3. single dose of liposomal amphotericin B (day 1) with paromomycin (day 2–11)
4. combinations of miltefosine with paromomycin (day 1–10)

All the combinations were non-inferior to the standard treatment with liposomal amphotericin B in usual doses. The combination therapy efficacy of all the regimens was ~95%. It was recommended that combination therapy would be an alternative to liposomal amphotericin B (10 mg/kg) in the context of kala-azar elimination programme in the Indian subcontinent.

3. Vector control

Vector control is of paramount importance in combating VL. When malaria control programme was carried out in India, as a collateral benefit, the incidence of kala-azar came down. In India, generally DDT is used for vector control but in Bangladesh and Nepal, synthetic pyrithroids are used. The exact role of long-lasting net (LLN) or long-lasting impregnated nets (LLIN) is not completely clear [29].

4. Elimination of kala-azar from Southeast Asian region

Keeping in view the high disease burden in India, Nepal and Bangladesh and availability of effective tools to diagnose the disease in the field situation and the effective and safe drug to treat the disease in an outpatient setting, the three countries embarked on eliminating the dreaded disease. In 2005, a memorandum of understanding was signed by India, Nepal and Bangladesh under the auspices of World Health Organization to cooperate and collaborate with each other to eliminate the disease from their respective countries. The target of elimination was less than 1 case per 10,000 people in an endemic area. Three countries of the WHO's Southeast Asian Region —Bangladesh, India and Nepal—are poised to eliminate VL (kala-azar) as a public health problem. The number of cases have reduced by 53%, from a high of 1,82,000 cases during 2005–2008 to 85,000 cases during 2011–2014. The 10,209 new cases reported in 2014 represent a 75%

decrease from 2005 when the kala-azar elimination programme was launched. In fact, Nepal has already achieved elimination and sustained for 2 years. Bangladesh is approaching fast towards elimination and India is expected to catch up [30–32].

5. Kala-azar vaccine

Conceptually, it is ideal to have a vaccine which will provide long-lasting immunity and simultaneously protect against VL and CL. Vaccines against VL and CL should be cost-effective. Currently, there are no effective and safe vaccines against VL but several are in various stages of developments. These candidate vaccines should be able to elicit balanced T_H1- and T_H2-mediated immune response. In view of safety concerns, live-oral vaccines are no longer recommended. The approach now is to insert a suicidal killed vector directly into the leishmania genome.

Research into first-generation vaccines based on whole-cell, killed leishmania parasites demonstrated that killed parasites showed efficacy as both therapeutic and prophylactic vaccines. Numerous preparations of killed parasites were tested. Although they showed good safety profiles, no first-generation vaccine using killed parasites has been demonstrated having sufficient efficacy as a prophylactic vaccine. The second generation of vaccines exploits the subunit, recombinant protein approach utilizing to augment the immune response. Third-generation vaccines derived from antigen-encoding DNA plasmids including heterologous prime-boost *Leishmania* vaccine have been examined for control and prevention of visceral leishmaniasis [33–35]. Vaccines based on recombinant protein and antigen-encoding DNA plasmids have given promising results.

6. HIV/kala-azar

Kala-azar patients may acquire HIV infection. Both the diseases lower the immunity of the person and opportunistic infections supervene. These patients easily acquire cryptosporidial infection and they respond to paromomycin. Tuberculosis is also common in these patients. Treatment of the three diseases requires a large number of drugs and may cause drug-drug interactions [36].

7. Concluding remarks

VL is potentially a life-threatening disease affecting the poorest of the poor in several regions of the world. There was a dearth of drugs, diagnostics and vector control methods until recently. The active collaboration of the scientists of the three countries joined by several other national and international agencies was the culmination of reliable diagnostics, drugs and vector control methods to diagnose and treat the disease. The third arm is vector control which is

essentially to interrupt transmission of the parasite. The WHO HQ and WHO regional office of the Southeast Asian Region extended formidable technical support to the programme. The role played by international agencies is unforgettable.

Author details

Sujit K. Bhattacharya[1]*, Ajanta Ghosal[2], Sudeshna Ganguly[1], Sandipan Ganguly[2], Sabahat Azim[2] and Shanta Dutta[1]

*Address all correspondence to: sujitkbhattacharya@yahoo.com

1 Glocal Healthcare Systems Pvt. Ltd, Kolkata, India

2 National Institute of Cholera and Enteric Diseases, Kolkata, India

References

[1] WHO/Department of Control of Neglected Tropical Diseases. Global leishmaniasis update, 2006–2015: A turning point in leishmaniasis surveillance. Weekly Epidemiological Record. 2017;**92**:557-572

[2] WHO. South-East Asia poised to defeat visceral leishmaniasis (kala-azar). 2015. Available from: http://www.who.int/neglected_diseases/news/SEARO_poised_to_defeat_VL/en /

[3] Desjeux P, Ghosh RS, Dhalaria P, Strub-Wourgaft N, Zijlstra E. Report of the post kala-azar dermal Leishmaniasis (PKDL) consortium meeting, New Delhi, India, 27–29 June 2012. Parasites & Vectors. 2013;**6**:196. DOI: 10.1186/1756-3305-6-196

[4] Musa AM, Khalil EAG, Younis BM, Elfaki MEE, Elamin MY, Adam AOA, Mohamed HAA, Dafalla MMM, Abuzaid AA, El-Hassan AM. Treatment-based strategy for the management of post-kala-azar dermal leishmaniasis patients in the Sudan. Journal of Tropical Medicine. 2013;**2013**:1-5. Article ID: 708391. DOI: 10.1155/2013/708391

[5] Sundar S, Singh A, Chakravarty J, Rai M. Efficacy and safety of Miltefosine in treatment of post-kala-azar dermal Leishmaniasis. The Scientific World Journal. 2015;**2015**:1-6. Article ID: 414378. DOI: 10.1155/2015/414378

[6] Sundar S, Singh A, Tiwari A, Shukla S, Chakravarty J, Rai M. Efficacy and safety of paromomycin in treatment of post-kala-azar dermal Leishmaniasis. ISRN Parasitology. 2014;**2014**:1-4. Article ID: 548010. DOI: 10.1155/2014/548010

[7] Ramesh V, Singh R, Kumar A, Verma A, Deep DK, Verma S, Salotra P, Chatterjee M. Decline in clinical efficacy of oral miltefosine in treatment of post kala-azar dermal leishmaniasis (PKDL) in India. PLoS Neglected Tropical Diseases. 2015;**9**(10):1-12. DOI: 10.1371/journal.pntd.0004093

[8] WHO. Cutaneous leishmaniasis. Available from: http://www.who.int/leishmaniasis/cutaneous_leishmaniasis/en/

[9] Emma van Rij R, Nour BYM, Henk DFH. Schallig evaluation of urine as a diagnostic specimen for visceral leishmaniasis in Sudan. Journal of Tropical Disease. 2013;**1**:106. DOI: 10.4172/2329-891X.1000106

[10] Agrawal VK, Singh Z. Miltefosine: First oral drug for treatment of visceral Leishmaniasis. Medical Journal Armed Forces India. 2006;**62**(1):66-67. DOI: 10.1016/S0377-1237(06)80162-0

[11] Dorlo TPC, Balasegaram M, Beijnen JH, de Vries PJ. Miltefosine: A review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. Journal of Antimicrobial Chemotherapy. 2012;**67**(11):2576-2597. DOI: 10.1093/jac/dks275

[12] Bhattacharya SK, Jha TK, Sundar S, Thakur CP, Engel J, Sindermann H, Junge K, Karbwang J, Bryceson ADM, Berman JD. Efficacy and tolerability of Miltefosine for childhood visceral Leishmaniasis in India. Clinical Infectious Diseases. 2004;**38**(2):217-221. DOI: 10.1086/380638

[13] Bhattacharya SK, Sinha PK, Sundar S, Thakur CP, Jha TK, Pandey K, Das VR, Kumar N, Lal C, Verma N, Singh VP, Ranjan A, Verma RB, Anders G, Sindermann H, Ganguly NK. Phase 4 trial of Miltefosine for the treatment of Indian visceral Leishmaniasis. The Journal of Infectious Diseases. 2007;**196**(4):591-598. DOI: 10.1086/519690

[14] Boelaert M, Sundar S. Recommended treatment regimens for visceral leishmaniasis, ranked by preference. In: Farrar J, Hotez P, Junghanss T, Kang G, Lalloo D, White N, editors. Manson's Tropical Infectious Diseases. 23rd ed. US: Elsevier Saunders; 2014. pp. 631-651. DOI: 10.1016/B978-0-7020-5101-2.00048-0

[15] Sundar S, Jha TK, Thakur CP, Sinha PK, Bhattacharya SK. Injectable paromomycin for visceral leishmaniasis in India. The New England Journal of Medicine. 2007;**356**(25):2571-2581. DOI: 10.1056/NEJMoa066536

[16] Mishra M, Biswas UK, Jha AM, Khan AB. Amphotericin versus sodium stibogluconate in first-line treatment of Indian kala-azar. Lancet. 1994;**344**(8937):1599-1600. DOI: 10.1016/S0140-6736(94)90406-5

[17] Thakur CP, Sinha GP, Pandey AK, Barat D, Sinha PK. Amphotericin B in resistant kala-azar in Bihar. The National Medical Journal of India. 1993;**6**(2):57-60. PMID: 8477209

[18] Sundar S, Chakravarty J, Rai VK, Agrawal N, Singh SP, Chauhan V, Murray HW. Amphotericin B treatment for Indian visceral Leishmaniasis: Response to 15 daily versus alternate-day infusions. Clinical Infectious Diseases. 2007;**45**:556-561. DOI: 10.1086/520665

[19] Sundar S, Chakravarty J. Liposomal amphotericin B and Leishmaniasis: Dose and response. Journal of Global Infectious Diseases. 2010;**2**(2):159-166. DOI: 10.4103/0974-777X.62886

[20] Sundar S, Jha TK, Thakur CP, Mishra M, Singh VP, Buffels R. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: A multicenter study. Clinical Infectious Diseases. 2003;**37**(6):800-804. DOI: 10.1086/377542

[21] Mondal D, Alvar J, Hasnain MG, Hossain MS, Ghosh D, Huda MM, Nabi SG, Sundar S, Matlashewski G, Arana B. Efficacy and safety of single-dose liposomal amphotericin B for visceral leishmaniasis in a rural public hospital in Bangladesh: A feasibility study. The Lancet Global Health. 2014;**2**(1):e51-e57. DOI: 10.1016/S2214-109X (13)70118-9

[22] Sundar S, Chakravarty J. An update on pharmacotherapy for leishmaniasis. Expert Opinion on Pharmacotherapy. 2015;**16**(2):237-252. DOI: 10.1517/14656566.2015.973850

[23] Ibrahim ME, Hag-Ali M, el-Hassan AM, Theander TG, Kharazmi A. Leishmania resistant to sodium stibogluconate: Drug-associated macrophage-dependent killing. Parasitology Research. 1994;**80**(7):569-574

[24] Chakravarty J, Sundar S. Drug resistance in leishmaniasis. Journal of Global Infectious Diseases. 2010;**2**(2):167-176. DOI: 10.4103/0974-777X.62887

[25] Brahmachari UN. Chemotherapy of antimonial compounds in kala-azar infection. Part IV. Further observations on the therapeutic values of urea stibamine. By U.N. Brahmachari, 1922. Indian Journal of Medical Research. 1989;**89**:393-404. PMID: 2644171

[26] Sundar S, Singh A. Recent developments and future prospects in the treatment of visceral leishmaniasis. Therapeutic Advances in Infectious Disease. 2016;**3**(3–4):98-109. DOI: 10.1177/2049936116646063

[27] Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clinical Microbiology Reviews. 2006;**19**(1):111-126. DOI: 10.1128/CMR.19.1.111-126.2006

[28] van Griensven J, Balasegaram M, Meheus F, Alvar J, Lynen L, Boelaert M. Combination therapy for visceral leishmaniasis. Vol 10; March 2010. www.thelancet.com/infection

[29] NVBDCP. Guidelines on vector control in kala-azar elimination. Available from: http://nvbdcp.gov.in/Doc/Guidelines-Vector-Control-KA-Elimination-FINAL.pdf

[30] Bhattacharya SK, Dash AP. Elimination of kala-azar from the Southeast Asia region. The American Journal of Tropical Medicine and Hygiene. 2017;**96**(4):802-804. DOI: 10.4269/ajtmh.16-0279

[31] Bhattacharya SK, Sur D, Sinha PK, Karbwang J. Elimination of leishmaniasis (kala-azar) from the Indian subcontinent is technically feasible & operationally achievable. Indian Journal of Medical Research. 2006;**123**(3):195-6. PMID: 16778303

[32] Bhattacharya SK. Treatment of visceral leishmaniasis: Options and choice. The Lancet Infectious Diseases. 2016;**16**(2):142-143. DOI: 10.1016/S1473-3099(15)00528-9

[33] Gillespie PM, Beaumier CM, Strych U, Hayward T, Hotez PJ, Bottazzi ME. Status of vaccine research and development of vaccines for leishmaniasis. Vaccine. 2016;**34**(26):2992-2995. DOI: 10.1016/j.vaccine.2015.12.071

[34] Osman M, Mistry A, Keding A, Gabe R, Cook E, Forrester S, Wiggins R, Di Marco S, Colloca S, Siani L, Cortese R, Smith DF, Aebischer T, Kaye PM, Lacey CJ. A third generation vaccine for human visceral leishmaniasis and post kala azar dermal leishmaniasis:

First-in-human trial of ChAd63-KH. PLoS Neglected Tropical Diseases. 2017;**11**(5):1-24. DOI: 10.1371/journal.pntd.0005527

[35] Evans KJ, Kedzierski L. Development of vaccines against visceral leishmaniasis. Journal of Tropical Medicine. 2012;**2012**. http://dx.doi.org/10.1155/2012/892817

[36] Burza S, Mahajan R, Sinha PK, van Griensven J, Pandey K, Lima MA, Sanz MG, Sunyoto T, Kumar S, Mitra G, Kumar R, Verma N, Das P. Visceral Leishmaniasis and HIV co-infection in Bihar, India: Long-term effectiveness and treatment outcomes with liposomal amphotericin B (AmBisome). PLOS Neglected Tropical Diseases. 2014;**8**(8): e3053. DOI: 10.1371/journal.pntd.0003053

Chapter 3

Leishmaniases in West Africa: Past and Current

Abdoulaye Kassoum Kone, Mahamadou A. Thera, Babacar Faye and Ogobara K. Doumbo†

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77009

Abstract

Leishmaniases are vector-borne diseases. Cutaneous leishmaniasis (CL) is endemic in West Africa. Sporadic and anecdotal cases of visceral leishmaniasis (VL) have been reported in the past. Recent data showed the changing of epidemiology of leishmaniases in West Africa, with the occurrence of outbreak of CL due to *Leishmania major* in urban and rural areas. CL is transmitted by *Phlebotomus duboscqi.* The role of *Sergentomyia (Spelaeomyia) darlingi* as vector in rural areas has been evoked but not confirmed. Cases of VL due to *Leishmania spp.* have been described in West Africa; however, parasites species were not identified and dogs were suspected to be the reservoir. No humans' case of symptomatic VL due to *L. infantum* has been described in West Africa. Recent data in rural areas of Senegal confirmed dog as reservoir of *L. infantum.* In the same study in Senegal, *Sergentomyia* sandflies were found infected with *L. infantum,* indicating a possible role in leishmaniasis transmission. Coinfection leishmaniases-HIV is reported but rare. In this chapter, we included most recent publications and propose an updated landscape of CL and VL epidemiology in West Africa.

Keywords: leishmaniases, epidemiology, West Africa

1. Introduction

Leishmaniases are anthropozoonoses common in animals and humans. Leishmaniases are endemics in 98 countries. The annual incidence is 0.7–1.2 million cases of cutaneous leishmaniasis (CL) and 0.2–0.4 million of cases of visceral leishmaniasis (VL) causing 20,000–40,000 deaths annually [1]. In sub-Saharan African region, the estimated annual incidence of CL was between 770 and 1500 cases. *Leishmania* parasites are characterized by their enzyme electrophoretic profile that defines zymodemes. *L. major* is the main parasite causing CL and its

zymodemes MON-26, MON-25, MON-17 and MON-117 have been identified in West Africa [2–4]. CL has been cited earlier as endemic in rural areas. However, outbreaks of CL in urban areas have been recently reported, showing a change in CL epidemiology [5]. VL presents a different epidemiological pattern in West Africa. Previous data described VL as a rare condition, with occurrence of sporadic and anecdotal cases in limited areas [6–8]. Recent data on VL in West Africa showed the occurrence of several cases of canine visceral leishmaniasis and asymptomatic human infections [9–11]. We will describe an updated dynamics of CL and VL characteristics in West Africa.

2. Burden and characteristics

2.1. Cutaneous leishmaniasis

The overall burden of CL is poorly characterized, due mainly to paucity of data. The frequency of CL in suspected patients was 78.4% in Mali [12], and Niger it was 66.7% [13]. In Burkina Faso, CL was perceived as a public health problem due to occurrence of outbreaks with an average incidence of 0.1% [14] and hospital frequency of 1.1% [15]. In Senegal, the frequency of CL in hospital based study was 38 cases over 4 years (9.5 cases per year) [16].

In Mali, recent positive LST survey showed a higher prevalence (49.9%) in Diema, Kayes region [17]. LST prevalence in Kayes was 25.7% in 1969 [18]. This difference in LST prevalence in the region of Kayes over a period of more than 45 years is likely to indicate an increasing trend in CL transmission.

L. major is the main species of *Leishmania* reported in Mali and has been identified in humans as a causative agent of CL [19]. The reservoirs of *L. major* in West Africa are rodents in Senegal. *Mastomys erythrolocus*, *Tatera gambiana* and *Arvicanthis niloticus* were found infected by *L. major* [20, 21]. *Phlebotomus duboscqi* previously was cited as vector of CL. *L. major* DNA was identified recently in *P. duboscqi* confirming its role in CL transmission in Mali [22, 23]. *Sergentomyia (Spelaeomyia) darlingi* may also play a role in *L. major* transmission [22]. Other species of *Leishmania* causing CL such as *L. tropica* has been identified in *S. dissimillima, S. ingrami, S. simillima, S. dissimillima, and S. hamoni* in Ghana [24], but this species has been not identified in humans in West Africa.

Recent findings revealed *L. infantum* in a HIV-positive child suffering from CL in Senegal [25]. A new species of *Leishmania* classified as *Leishmania enrietti* complex was found in humans in Ghana [26]. These findings call to strengthen CL diagnosis and stimulate efforts to determine the causative *Leishmania* species in human infections.

The enzymes electrophoresis analysis identified several strains of *L. major* in West Africa. The most frequent strain in Mali was MON-26 and MON-74 was the more frequent in Burkina Faso [2, 4, 27]. Travelers visiting endemic areas in West Africa are at risk of getting infected with *Leishmania* [28].

In West African countries (**Figure 1**), CL outbreaks may occur in rural areas where health care centers personnel are not well trained for diagnosis or case management. Often, an

investigation post-outbreak is conducted to determine the *Leishmania spp*., the vectors and reservoirs involved [5, 29]. In urban areas [30], outbreaks are occurring in larger population. Rapid urbanization is considered as a favoring factor that also makes uneasy outbreak control.

Coinfection CL-HIV is rare in West Africa. In Mali, the frequency of coinfection was 1% [31]. Coinfection CL-HIV has been reported in Burkina Faso and Ghana [32, 33]. In patients with coinfection, diffuse CL, mucosal involvement and bone marrow invasion have been reported [16, 34–36].

2.2. Visceral leishmaniasis

In West Africa, VL has been described, and previous data have shown the scarcity of disease (**Figure 1**). For several years, anecdotal and sporadic cases of VL were reported. Up to today, the data reviewed identified most of the clinical cases of VL in Ivory Coast and Niger [6–8]. In the Gambia, a case of VL has been reported in humans and in dogs [37, 38]. Underreporting of diseases is a known feature of West African health care system. This is also true for VL. The underreporting of VL in West Africa could be favored by the absence of appropriate biological diagnosis and the absence of specificity of VL clinical symptoms. VL cases may be confounded with others frequent parasitic diseases such as malaria or schistosomiasis. It is also assumed that the parasite strains found in West Africa are less virulent than those found elsewhere (Asia and East Africa). The strain identified in Senegal and likely those in West Africa are coming from Mediterranean basin [39].

Figure 1. Modified map of West Africa from map (http://d-maps.com/carte.php?num_car=36688&lang=fr) and status of leishmaniasis endemicity in West Africa (WHO weekly report 2017) [45]. : Cutaneous leishmaniasis endemic; : Cutaneous leishmaniasis previously reported; : Visceral leishmaniasis endemic; : Visceral leishmaniasis previously reported.

Parasite species identified in West Africa is *L. infantum* using serology method in asymptomatic Senegalese [11]. *L. donovani* has not yet been identified in West Africa. Cases of VL encountered in Niger and Ivory Coast had their parasite identified by microscopy [6–8]. It is known that microcopy cannot distinguish between *Leishmania* species. For species, diagnosis serology or molecular biology is required.

Sandflies of the genus *Sergentomyia* (*Se. dubia*, *Se. schwetzi* and *Se. magna*) have been found infected with *L. infantum* in Mont-Roland district in Senegal [40]. This raises the possibility that *Sergentomyia spp.* may be involved in VL transmission in Senegal.

Canine visceral leishmaniasis is well described in West Africa. Recent studies in domestic dogs showed that *L. infantum* was the causal pathogen in Senegal, in Burkina Faso and Nigeria [9, 10, 41].

Geographic diversity: the area of transmission could be wider, therefore underestimating the cases of leishmaniasis. In Senegal, previous studies have shown that the vectors *Phlebotomus* or *Sergentomyia* are found in many areas of the country (Kédougou, South East, Keur Moussa in Dakar region, Ferlo area [42]. Environmental changes are risk factors of explosion.

3. Management and control

VL is rarely encountered in West Africa. Most cases of human Leishmaniasis are cutaneous leishmaniasis. Often, CL cases are under-diagnosed, and their clinical management is poorly done. This is particularly true for cases encountered at peripheral health care centers with personnel poorly trained. For those encountered at referral health care facilities with good capacity for the diagnosis, treatment is available. In Mali, CL cases are referred to National Center for Diseases Control [CNAM acronym in French for Centre National d'Appui à Lutte contre la Maladie]. Treatment is done using either meglumine antimoniate locally or local thermotherapy [43]. In Burkina Faso, meglumine antimoniate is the first line treatment [15]. Treatment outcome is favorable with healing of lesions in 2–4 weeks. Treatment response in HIV coinfected patients is also favorable but hampered by the frequency of relapses [44].

In rural areas where these treatments are not available dermatologists advice to clean skin lesions and apply tetracycline ointment until healing [43]. Meglumine antimoniate and amphotericin B have been used to treat VL in West Africa [6–8].

4. Conclusion

Compared to other endemic parts of the world, leishmaniases are not very common in West Africa. CL is widely distributed in few West African countries such as Burkina Faso, Mali, Nigeria and Senegal. Urbanization is the main risk factor. Human VL human is sporadic in few countries. Also, VL affects more domestic dogs. Our review acknowledged the changing of CL epidemiology with more report of outbreaks and description of new parasite species in West

Africa. A surveillance system based on referral health care centers with training of health care personnel will help to better address clinical and diagnostic challenges imposed by leishmaniasis.

Conflict of interest

No conflict of interest declared.

Author details

Abdoulaye Kassoum Kone[1]*, Mahamadou A. Thera[1], Babacar Faye[2] and Ogobara K. Doumbo[1†]

*Address all correspondence to: fankone@icermali.org

1 Malaria Research and Training Center, Department of Epidemiology of Parasitic Diseases/ Faculty of Medicine, and Dentistry/ UMI-3189/ University of Science, Technique and Technology of Bamako, BP, Bamako, Mali

2 Service de Parasitologie-Mycologie, Faculté de Médecine, Université Cheikh Anta DIOP, Dakar, Sénégal

† Deceased

References

[1] Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;**7**:e35671. DOI: 10.1371/journal.pone.0035671

[2] Izri MA, Doumbo O, Belazzoug S, Pratlong F. Presence of *Leishmania major* MON-26 in Mali. Annales de Parasitologie Humaine et Comparée. 1989;**64**:510-511

[3] Garin JP, Peyramond D, Piens MA, Rioux JA, Godfrey DG, Lanotte G, et al. Presence of *Leishmania major* Yakimoff and Schokhor, 1914 in Mali. Enzymatic identification of a strain of human origin. Annales de Parasitologie Humaine et Comparée. 1985;**60**:93-94

[4] Pratlong F, Lami P, Ravel C, Balard Y, Dereure J, Serres G, et al. Geographical distribution and epidemiological features of old world *Leishmania infantum* and *Leishmania donovani* foci, based on the isoenzyme analysis of 2277 strains. Parasitology. 2013;**140**:423-434

[5] Kone AK, Delaunay P, Djimdé AA, Thera MA, Giudice PD, Coulibaly D, Traoré K, Goita SM, Abathina A, Izri A, Marty P, Doumbo OK. Epidemiology of cutaneous leishmaniasis in five villages of Dogon country, Mali. Bulletin De La Societe De Pathologie Exotique. 2012 Feb;**105**(1):8-15

[6] Eholié SP, Tanon AK, Folquet-Amorissani M, Doukouré B, Adoubryn KD, Yattara A, Bissagnéné E. Three new cases of visceral leishmaniasis in Côte d'Ivoire. Bulletin De La Societe De Pathologie Exotique. 2008 Feb;**101**(1):60-61

[7] Kouassi B, Horo K, Achi VH, Adoubryn KD, Kakou ES, et al. Leishmaniose viscérale à Abidjan à propos de 3 observations. La Medicina Tropical. 2005;**65**:602-603

[8] Djidingar D, Chippaux JP, Gragnic G, Tchani O, Meynard D, Julvez J. Visceral leishmaniasis in Niger: Six new parasitologically confirmed cases. Bulletin De La Societe De Pathologie Exotique. 1997;**90**(1):27-29

[9] Faye B, Bañuls AL, Bucheton B, Dione MM, Bassanganam O, Hide M, Dereure J, Choisy M, Ndiaye JL, Konaté O, Claire M, Senghor MW, Faye MN, Sy I, Niang AA, Molez JF, Victoir K, Marty P, Delaunay P, Knecht R, Mellul S, Diedhiou S, Gaye O. Canine visceral leishmaniasis caused by *Leishmania infantum* in Senegal: Risk of emergence in humans? Microbes and Infection. 2010 Dec;**12**(14-15):1219-1225

[10] Sangaré I, Djibougou Djibougou A, Yaméogo BK, Drabo F, Diabaté A, Banuls AL, Fournet F, Price H, Guiguemdé RT, Dabiré RK. First detection of *Leishmania infantum* in domestic dogs from Burkina Faso (West Africa). Research Journal of Parasitology. 2017;**12**(1):27-32

[11] Faye B, Bucheton B, Bañuls AL, Senghor MW, Niang AA, Diedhiou S, Konaté O, Dione MM, Hide M, Mellul S, Knecht R, Delaunay P, Marty P, Gaye O. Seroprevalence of *Leishmania infantum* in a rural area of Senegal: Analysis of risk factors involved in transmission to humans. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2011 Jun;**105**(6):333-340

[12] Kone AK, Niare DS, Thera MA, Kayentao K, Djimde A, Delaunay P, Kouriba B, Giudice PD, Izri A, Marty P, Doumbo OK. Epidemiology of the outbreak, vectors and reservoirs of cutaneous leishmaniasis in Mali: A systematic review and meta-analysis. Asian Pacific Journal of Tropical Medicine. 2016 Oct;**9**(10):985-990. DOI: 10.1016/j.apjtm.2016.07.025 Epub 2016 Aug 20

[13] Develoux M, Blanc L, Garba S, Mamoudou HD, Warter A, Ravisse P. Cutaneous leishmaniasis in Niger. The American Journal of Tropical Medicine and Hygiene. 1990;**43**: 29-30

[14] Bamba S, Barro-Traoré F, Drabo MK, Gouba A, Traoré A, Guiguemdé TR. Epidemiological profile, clinical and therapeutic cutaneous leishmaniasis in the Department of Dermatology at University Hospital in Ouagadougou, Burkina Faso. Revue Medicale De Bruxelles. 2013 Sep–Oct;**34**(5):392-396

[15] Bamba S, Gouba A, Drabo MK, Nezien D, Bougoum M, Guiguemdé TR. Epidemiological profile of cutaneous leishmaniasis: Retrospective analysis of 7444 cases reported from 1999 to 2005 at Ouagadougou, Burkina Faso. The Pan African Medical Journal. 2013 Mar 19;**14**:108

[16] Diadie S, Diatta BA, Ndiaye M, Seck NB, Diallo S, Niang SO, Dieng MT. Cutaneous leishmaniasis in Senegal: A series of 38 cases at the Aristide Le Dantec University Hospital in Dakar. Medecine Et Sante Tropicales. 2018 Feb 1;**28**(1):106-108. DOI: 10.1684/mst.2017.0722

[17] Traoré B, Oliveira F, Faye O, Dicko A, Coulibaly CA, Sissoko IM, Sibiry S, Sogoba N, Sangare MB, Coulibaly YI, Traore P, Traore SF, Anderson JM, Keita S, Valenzuela JG, Kamhawi S, Doumbia S. Prevalence of cutaneous Leishmaniasis in districts of high and low endemicity in Mali. PLoS Neglected Tropical Diseases. 2016 Nov 29;**10**(11):e0005141

[18] Imperato PJ, Diakité S. Leishmaniasis in the Republic of Mali. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1969;**63**(2):236-241

[19] Paz C, Samake S, Anderson JM, Faye B, Traore P, Tall K, et al. *Leishmania major*, the predominant *Leishmania* species responsible for cutaneous leishmaniasis in Mali. The American Journal of Tropical Medicine and Hygiene. 2013;**88**:583-585

[20] Dedet JP, Derouin F. Isolation of *Leishmania major* from *Mastomys erythroleucus* and *Tatera gambiana* in Senegal (West Africa). Annals of Tropical Medicine and Parasitology. 1979; **73**:433-437

[21] Camerlynck P, Ranque P, Quilici M. Importance of systematic cultures and subcultures in research on natural viral reservoirs in cutaneous leishmaniasis. Apropos of the isolation of 5 strains of *Leishmania* in *Arvicanthis niloticus*. Medecine Tropicale (Mars). 1967;**27**:89-92

[22] Berdjane-Brouk Z, Koné AK, Djimdé AA, Charrel RN, Ravel C, Delaunay P, del Giudice P, Diarra AZ, Doumbo S, Goita S, Thera MA, Depaquit J, Marty P, Doumbo OK, Izri A. First detection of Leishmania major DNA in *Sergentomyia (Spelaeomyia) darlingi* from cutaneous leishmaniasis foci in Mali. PLoS One. 2012;**7**(1):e28266

[23] Anderson JM, Samake S, Jaramillo-Gutierrez G, Sissoko I, Coulibaly CA, Traoré B, Soucko C, Guindo B, Diarra D, Fay MP, Lawyer PG, Doumbia S, Valenzuela JG, Kamhawi S. Seasonality and prevalence of *Leishmania major* infection in Phlebotomus duboscqi Neveu-Lemaire from two neighboring villages in Central Mali. PLoS Neglected Tropical Diseases. 2011 May 10;**5**(5):e1139

[24] Nzelu CO, Kato H, Puplampu N, Desewu K, Odoom S, Wilson MD, Sakurai T, Katakura K, Boakye DA. First detection of *Leishmania* tropica DNA and Trypanosoma species in *Sergentomyia* sand flies (Diptera: Psychodidae) from an outbreak area of cutaneous leishmaniasis in Ghana. PLoS Neglected Tropical Diseases. 2014 Feb 6;**8**(2):e2630

[25] Diatta BA, Diallo M, Diadie S, Faye B, Ndiaye M, Hakim H, Diallo S, Seck B, Niang SO, Kane A, Dieng MT. Cutaneous leishmaniasis due to *Leishmania infantum* associated with HIV. Annales de Dermatologie et de Vénéréologie. 2016 Oct;**143**(10):625-628

[26] Kwakye-Nuako G, Mosore MT, Duplessis C, Bates MD, Puplampu N, Mensah-Attipoe I, Desewu K, Afegbe G, Asmah RH, Jamjoom MB, Ayeh-Kumi PF, Boakye DA, Bates PA. First isolation of a new species of Leishmania responsible for human cutaneous leishmaniasis in Ghana and classification in the *Leishmania enriettii* complex. International Journal for Parasitology. 2015 Sep;**45**(11):679-684. DOI: 10.1016/j.ijpara.2015.05.001. Epub 2015 Jun 19

[27] Guiguemdé TR, Sawadogo NO, Botero S, Traore KL, Nezien D, Nikiema L, et al. *Leishmania major* and HIV coinfection in Burkina Faso. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2003;**97**:168-169

[28] Kelly P, Baudry T, Peyron F. Imported cutaneous leishmaniasis in a short-term traveler returning from Central Mali the role of PCR. Travel Medicine and Infectious Disease. 2012;**10**:97-100

[29] Kweku MA, Odoom S, Puplampu N, Desewu K, Nuako GK, Gyan B, Raczniak G, Kronmann KC, Koram K, Botero S, Boakye D, Akuffo H. An outbreak of suspected cutaneous leishmaniasis in Ghana: Lessons learnt and preparation for future outbreaks. Global Health Action. 2011;**4**:5527. DOI: 10.3402/gha.v4i0.5527

[30] Traore KS, Sawadogo NO, Traoré A, Ouedraogo JB, Traoré KL, Guiguemdé TR. Preliminary study of cutaneous leishmaniasis in the town of Ouagadougou from 1996 to 1998. Bulletin De La Societe De Pathologie Exotique. 2001;**94**:52-55

[31] Mahé A, Bobin P, Coulibaly S, Tounkara A. Skin diseases disclosing human immunodeficiency virus infection in MaliAnnales de Dermatologie et de Vénéréologie. 1997; **124**:144-150

[32] Niamba P, Traoré A, Goumbri-Lompo O, Labrèze C, Traoré-Barro F, Bonkoungou M, et al. Leishmaniose cutanée chez les malades infectés par le VIH. Annales de Dermatologie et de Vénéréologie. 2006;**133**:537-542

[33] Lartey M, Adusei L, Hanson-Nortey L, Addy J. Coinfection of cutaneous Leishmaniasis and HIV infection. Ghana Medical Journal. 2006 Sep;**40**(3):110-112

[34] Niamba P, Goumbri-Lompo O, Traoré A, Barro-Traoré F, Soudré RT. Diffuse cutaneous leishmaniasis in an HIV-positive patient in western Africa. The Australasian Journal of Dermatology. 2007 Feb;**48**(1):32-34

[35] Barro-Traoré F, Preney L, Traoré A, Darie H, Tapsoba P, Bassolé A, Sawadogo S, Niamba P, Grosshans E, Geniaux M. Cutaneous leishmaniasis due to Leishmania major involving the bone marrow in an AIDS patient in Burkina Faso. Annales de Dermatologie et de Vénéréologie. 2008 May;**135**(5):380-383

[36] Ndiaye PB, Develoux M, Dieng MT, Huerre M. Diffuse cutaneous leishmaniasis and acquired immunodeficiency syndrome in a Senegalese patient. Bulletin De La Societe De Pathologie Exotique. 1996;**89**(4):282-286

[37] Desjeux P, Bryan JH, Martin-Saxton P. Leishmaniasis in the Gambia. 2. A study of possible vectors and animal reservoirs, with the first report of a case of canine leishmaniasis in the Gambia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1983;**77**(2):143-148

[38] Walters JH. A case of indigenous Kala-azar in the Gambia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1949 Nov;**43**(3):287-292. pl

[39] Cassan C, Dione MM, Dereure J, Diedhiou S, Bucheton B, Hide M, Kako C, Gaye O, Senghor M, Niang AA, Bañuls AL, Faye B. First insights into the genetic diversity and origin of *Leishmania infantum* in Mont Rolland (Thiès region, Senegal). Microbes and Infection. 2016 Jun;**18**(6):412-420

[40] Senghor MW, Niang AA, Depaquit J, Ferté H, Faye MN, Elguero E, Gaye O, Alten B, Perktas U, Cassan C, Faye B, Bañuls AL. Transmission of *Leishmania infantum* in the canine leishmaniasis focus of Mont-Rolland, Senegal: Ecological, parasitological and molecular evidence for a possible role of *Sergentomyia* sand flies. PLoS Neglected Tropical Diseases. 2016;**2**(10):11, e0004940

[41] Adediran OA, Kolapo TU, Uwalaka EC. Seroprevalence of canine leishmaniasis in Kwara, Oyo and Ogun states of Nigeria. Journal of Parasitic Diseases. 2016 Jun;**40**(2):510-514. DOI: 10.1007/s12639-014-0535-2. Epub 2014 Sep 5

[42] Ba Y, Trouillet J, Thonnon J, Fontenille D. Phlebotomus of Senegal: Survey of the fauna in the region of Kedougou. Isolation of arbovirus. Bulletin De La Societe De Pathologie Exotique. 1999 May;**92**(2):131-135

[43] Tall K. Etude épidémio-clinique et prise en charge de la leishmaniose cutanée à Bamako et dans deux villages endémiques du Mali [thesis]. Faculty of Medicine and Dentistry; 2008. http://indexmedicus.afro.who.int/iah/fulltext/Thesis_Bamako/05P41.PDF

[44] Niamba P, Traoré A, Goumbri-Lompo O, Labrèze C, Traoré-Barro F, Bonkoungou M, Ilboudo L, Gaulier A, Soudré BR. Cutaneous leishmania in HIV patient in Ouagadougou: Clinical and therapeutic aspects. Annales de Dermatologie et de Vénéréologie. 2006 Jun-Jul;**133**(6-7):537-542

[45] WHO. Weekly Epidemiological Record. No. 38; 2017, Vol. 92. pp. 557-572. Available at: http://www.who.int/leishmaniasis/resources/REH_38_TABLEAU_S1_S2_Version_finale.pdf?ua=1

Section 3

Immune Evasion in Leishmaniasis

Chapter 4

Immune Evasion Strategies

M. Magdalena Aguirre-Garcia,
Alma R. Escalona-Montaño,
Arturo A. Wilkins-Rodríguez and
Laila Gutiérrez-Kobeh

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75169

Abstract

Leishmania is the causative protozoan parasite of leishmaniasis. Distinct species provoke localized/diffuse cutaneous leishmaniasis or visceral leishmaniasis. *Leishmania* parasites have developed diverse strategies to evade the host immune response expressed through various cells, especially macrophages, NK cells, and dendritic cells. Participating in some of these strategies are *Leishmania* surface molecules, such as lipophosphoglycan (LPG) and protease gp63, which are thus considered virulence factors. LPG has been shown to modulate proinflammatory responses. For example, *L. major* LPG activates NK cells through toll-like receptor-2 (TLR2), while *L. mexicana* LPG elicits a differential production of cytokines in human dendritic cells and monocytes. Moreover, *L. mexicana* LPG activates MAP kinases in macrophages, which in turn enhance proinflammatory cytokine production through TLRs. Additionally, *Leishmania* exosomes have been found to strongly affect macrophage signaling and functions. Furthermore, proteins secreted by *Leishmania* promastigotes and amastigotes modulate the production of proinflammatory cytokines in human macrophages. Since *Leishmania* is an obligate intracellular parasite, its promastigotes utilize several mechanisms to survive and duplicate inside host cells, including the inhibition of apoptosis. It is now clear that MAPK p38, JNK, ERK 1/2, and PI3K/Akt participate in the inhibition of both natural and induced apoptosis of macrophages, neutrophils, and dendritic cells.

Keywords: *Leishmania*, cytokines, TLR, inflammasome, apoptosis, NO

1. Introduction

Leishmaniasis is a complex of neglected tropical diseases (NTDs) caused by protozoan parasites of the genus *Leishmania*. Epidemiological studies have revealed that 12 million people are

infected worldwide, with 2 million new cases each year. Approximately 350 million people are currently at risk of contracting leishmaniasis, mostly in developing countries.

Leishmania is a dimorphic protozoan parasite that completes its life cycle in two organisms: the sand fly vector and a mammalian host (a rodent, canid, or human). In the vector, the parasite enters the insect as aflagellated amastigotes through a blood meal from a mammal. In the sand fly midgut, this form changes into motile extracellular flagellated promastigotes that divide by binary fission. After being injected into the bloodstream of a mammalian host, the promastigotes are quickly engulfed by macrophages, where they differentiate into aflagellated intracellular amastigotes that can survive in this acidic environment. In macrophages, the amastigotes replicate by binary fission, causing the lysis of the cell and the invasion of other cells [1].

The outcome of a *Leishmania* infection depends on a wide array of factors, especially the species of the parasite and the host immune response. Species such as *L. major*, *L. mexicana* and *L. guyanensis* induce cutaneous leishmaniasis, while *L. amazonensis* and *L. braziliensis* cause the mucocutaneous form. *L. donovani* and *L. chagasi*, on the other hand, cause visceral leishmaniasis (VL) [2].

Whether *Leishmania* parasites manage to establish themselves in mammalian cells depends on their capacity to surpass host defense mechanisms. The survival strategies of the protozoan are based on the manipulation of distinct host cell functions, including modulation of cell signaling pathways through phosphorylation and dephosphorylation mechanisms [3]. The different strategies of *Leishmania* to evade the host immune response during the process of infection involve macrophages, NK cells, and dendritic cells. One of the most successful survival strategies displayed by *Leishmania* is the inhibition of apoptosis of host cells through the activation or silencing of proapoptotic or antiapoptotic signaling pathways [4, 5]. Among the *Leishmania* surface components participating in such evasion strategies is lipophosphoglycan (LPG), an abundant molecule that exerts its activity by binding to TLR2. Although the ability of *Leishmania* to inhibit inflammatory signaling pathways has been proposed as a virulence mechanism, the molecular events underlying this process have still not been fully elucidated [6].

2. Parasite molecules that regulate host cell signaling pathways

Two of the most studied molecules of *Leishmania* spp. are LPG and glycoprotein 63 (gp63), postulated as possible virulence factors for some species. LPG covers the surface of the parasite and the flagellum, forming a glycocalyx. The structure of LPG, which differs between the distinct species of *Leishmania*, is mainly constituted by repeating units of a disaccharide and a phosphate bound to the membrane by glycosylphosphatidylinositol (GPI). This molecule is more abundant in promastigotes than amastigotes [7]. Contrarily, gp63 is more frequently expressed in amastigotes than promastigotes. The absence of LPG in amastigotes emphasizes the relevance of gp63 in protozoan survival, as well as in the regulation of signaling pathways of host cells [8, 9].

Other important molecules for *Leishmania* are glycosylinositolphospholipids (GPILs), a class of glycolipids bound by GPIs and expressed 10 times more frequently than LPG. Their small size keeps them close to the parasite membrane [10, 11].

The term "secretome" was introduced for the first time in the global study of the genome of proteins secreted by *Bacillus subtilis.* The authors defined the secretome as a subset of the proteome consisting of secreted proteins and the components of the cellular machinery involved in protein secretion. They predicted all exported *B. subtilis* proteins by employing computational methods to search for signal peptides and cellular retention signals in protein sequences [12]. In *Plasmodium falciparum*, the secretome refers to all proteins exported to the host erythrocyte and mediated by an endoplasmic reticulum signal sequence, along with one export element of this parasite [13, 14]. Until very recently, there was very little information about the proteins secreted by protozoan parasites. Given the role of these proteins as virulence factors and their capacity to modulate host cells, this scant information represented an important scientific limitation.

Regarding trypanosomatids, the term secretome was introduced by Silverman in a proteomics approach used to identify a large number of extracellular proteins in a culture media conditioned by *L. donovani* [15]. Several studies on trypanosomatids have aimed to identify and characterize excreted/secreted factors due to their potential for the development of vaccines and/or new drugs [16].

Leishmania and other intracellular pathogens have developed strategies to invade and persist within the respective host cell. In some cases, the mechanisms entail the export of virulence factors to the cytosol of this same cell [15]. Almost a decade has passed since the first report of the secretion proteins by *L. donovani*. By means of a stable isotope label of amino acids in a culture called SILAC, the authors identified 151 proteins secreted by this species into the culture media. Interestingly, few of these are secreted through classical mechanisms [15]. Additionally, the bioinformatic analysis in the same study showed that none of the histidine phosphatase proteins result from the classical mechanism of secretion.

The authors found various proteins with several possible functions. For instance, some proteins take part in the vesicular transport process, essential for the survival of the parasite, and may thus be virulence factors. Among the proteins participating in signal transduction are those encoded by the gene LmjF 25.0750 of *L. major*, including a phosphatase serine threonine type phosphoprotein, a metal-dependent phosphatase (PPM) called PP2C [15]. This protein was cloned from the DNA genome of *L. major* and localized in the pocket and flagellum of the parasite through fluorescence microscopy assays and transmission electron microscopy [3].

Since the flagellum of *L. major* represents an important structure for differentiation in trypanosomatids, this location of PP2C suggests a role in a vital biological process of the parasite [3]. The same authors have shown that *L. mexicana* promastigotes and amastigotes secrete proteins with phosphatase activity into the culture medium. Such activity was more pronounced in the promastigote than amastigote secretion medium. Both media stimulated the production of various cytokines in human macrophages: tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-12p70, and IL-10 [17].

2.1. Exosomes secreted by *Leishmania*

Exosomes are organelles (30–100 nm) released by numerous mammalian cells, including reticulocytes, B cells, T cells, dendritic cells, and macrophages [18]. Bioactive exosomes are released

by cells infected with viruses and bacteria as well as some tumor cells [19, 20]. The release of exosomes also constitutes a mechanism for the secretion of proteins by *Leishmania*, and these vesicles allow for communication with the host cell [15]. Among the proteins released through exosomes, the metalloprotease 63 kDa and the elongation factor 1 alpha (EF-1 α) are known to have a substantial role in deregulating certain signaling pathways. These two molecules, contained in microvesicles, are responsible for the activation of tyrosine phosphatases (SHP-1) in the host cell [21]. A study of *L. mexicana* demonstrated a type of vesicle induced at a temperature of 37°C that did not correspond to exosomes, as evidenced by its size.

Other studies confirm a nonclassical route of secretion for *Leishmania* proteins. Two-dimensional electrophoresis displayed 270 secreted protein spots originating from *L. braziliensis*, of which 42 were identified. About 57% of these proteins presented non-classical secretion mechanisms [22].

In *L. infantum*, distinct proteins were observed in exovesicles of the parasites in the different phases of their growth. Ribosomal proteins were detected in the logarithmic phase of growth, thus indicating their crucial role in protein turnover. In the stationary phase, contrarily, there was a specific enrichment of vesicles with properties similar to apoptotic vesicles [23].

Diverse *Leishmania* species have been analyzed to explore the effect exerted by their secreted molecules on the host immune response. For example, in infection by *L. donovani* and interferon (IFN)-γ treatment, the exposure of human monocytes to the exosomes secreted by this parasite led to an alteration in the cytokine response. The resulting inhibition of IL-8 and TNF-α production combined with enhanced levels of IL-10 caused an anti-inflammatory effect. This immune suppression induced during *Leishmania* infection suggests that the secretion of exosomes by *Leishmania* likely plays a major role in the establishment of infection. Indeed, exosomes may be a mechanism of immune modulation used more generally by intracellular and extracellular pathogens [24].

3. *Leishmania* modulates proinflammatory cytokines, inflammasomes, and TLR expression

To detect *Leishmania* infections, the innate immune system utilizes different sets of germline-encoded receptors, including the TLRs found on cell membranes and in the endosome. Other receptors that have only been detected in the cytoplasm, such as the NOD-like receptor (NLR) family, also play a role in defending the host from a *Leishmania* invasion. Encompassing 34 members in all, NLRs sense pathogen- and danger-associated molecular patterns (PAMPs and DAMPs, respectively).

There is a subset of NLRs that assembles large multiprotein complexes known as inflammasomes. The latter trigger inflammatory caspase 1, which in turn promotes the conversion of pro–IL-1β and pro–IL-18 into their bioactive forms. For instance, NLRP3 (the best-characterized inflammasome) consists of the NLRP3 protein, the bipartite adaptor protein ASC, and caspase 1 (in its minimum form). It has been demonstrated that this inflammasome, activated

by bacterial toxins, bacterial RNA, ATP, nigericin, uric acid, and silica crystals, is an essential component of the host immune response against bacterial and viral pathogens [25].

Leishmania parasites have developed diverse strategies to evade the immune response, especially in the form of macrophages, NK cells, and dendritic cells. Becker analyzed the interaction between *Leishmania* LPG and TLR2 receptors on human NK cells, finding that LPG purified from metacyclic and procyclic promastigotes of *L. major* stimulates these host cells. The consequent activation of NK cells leads to an upregulation of TLR2 expression, the nuclear translocation of NF-κB, and an increased production of IFN-γ and TNF-α. Indeed, the activation of NK cells turned out to be greater with the infective metacyclic form than the non-infective procyclic form of LPG [26].

L. mexicana LPG elicits a differential production of proinflammatory cytokines, such as IL-12, TNF-α and IL-10, as well as the nuclear translocation of NF-κB in monocytes and dendritic cells [27]. It also activates ERK and p38 MAP kinase in macrophages and induces proinflammatory cytokine production through TLR2 and TLR4 signaling [28].

After infecting the THP1 human cell line with *L. donovani*, the production of IL-10, TNF-α and IFN-γ was measured, and the expression of TLR2, TLR4, and TLR9 was determined in blood samples and the THP1 cell line. IL-10 levels were higher in controls positive to the leishmanin skin test (LST+) compared to patients with VL. TNF-α was moderately produced, exhibiting no variation between patients, controls, and THP1 cells. TLR4 and TLR9 expression was elevated in patients with VL. *L. donovani* increased the expression of TLR4 and TLR9 in patients with VL, and of TLR2 in THP1 cells, which suggests a link between TLRs and the generation of a mixed cytokine response [29].

Interestingly, other authors analyzed the expression of some components related to the inflammasome pathway in murine macrophages infected with *L. major*. At 6 and 18 h post infection, they evaluated the mRNA expression levels in control and infected macrophages of two NLRs (NLRP3 and NAIP5), the inflammasome adaptor molecule ASC, proinflammatory caspase-1, and proinflammatory cytokines IL-1β and IL-18. The components related to the inflammasome pathway (NLRP3, ASC, IPAF, IL-1β, and IL-18) were upregulated in murine macrophages infected with *L. major*. The activity of caspase-1 was more pronounced in infected than noninfected macrophages. Infected (versus uninfected) macrophages also showed significantly greater caspase-1 activity in harvested cells and a significantly higher concentration of IL-1β in the supernatant of the cultured media [30].

It has been documented, based on *in vitro* (in macrophages) and *in vivo* studies, that a *Leishmania* infection activates the NLRP3 inflammasome and that the latter is key to the inhibition of parasite replication. For example, the capacity of inflammasome-deficient mice to resist infection with *L. amazonensis, L. braziliensis, L. infantum,* and *L. chagasi* was favored by IL-1β production resulting from inflammasome activation. The mechanism involved in such activation was the increase in the level of nitric oxide (NO), which in turn was mediated by the elevated availability of nitric oxide synthase NOS2 resulting from signaling through the IL-1 receptor and MyD88. Lima-Junior et al. previously showed that the NLR3 inflammasome is vital for the host response to *L. amazonensis* infection, having proven to restrict

parasite replication in both isolated macrophages and *in vivo*. As can be appreciated, IL-1β production is involved in the host resistance to infection. The signaling that triggers the production of this cytokine takes place through the IL-1 receptor and MyD88, contributing to elevated levels of NOS2. An increase in the latter enzyme leads to a greater generation of NO, a major host defense mechanism against *Leishmania* spp. [31].

In the case of *L. major*, secreted antigens suppressed the proliferation of BALB/c mice lymphocytes *in vitro*. After semi-purifying these secreted antigens, they were found to suppress 60% of lymphocyte proliferation and prevent the stimulation of lymphocytes. The fractions obtained decreased the production of IFN-γ and increased the level of IL-4 in lymphocytes, whereas they downregulated the formation of NO by activated macrophages. Hence, proteins secreted by *L. major* may function as immunosuppressive factors that downregulate the immune system [32]. On the other hand, the immunomodulatory effect of proteins excreted/secreted by *L. infantum* was described in the context of differentiation and maturation of human dendritic cells [33].

Regarding *L. donovani*, an immunomodulatory role has also been established for leishmanial excretory-secretory antigens (LESAs) released by promastigotes to the culture medium. The separation of fractions from LESAs revealed proteins of different molecular weights. Both fractions were highly immunogenic, as they significantly enhanced the activity of NADPH oxidase and SOD, as well as the production of NO, TNF-α, IFN-γ, and IL-12 in stimulated RAW 264.7 macrophages. These results strongly suggest the potential role of LESAs in the modulation of macrophage effector functions and Th1 responses, which could possibly be used in the development of a potent vaccine for visceral leishmaniasis [34]. Similarly, Kumar reported a potential immunostimulatory effect of soluble exogenous antigens of *L. donovani*, which may be instrumental in developing a subunit vaccine against VL [35].

4. *Leishmania* modulates L-arginine metabolism via NOS2 and arginase-1

Among other strategies developed by *Leishmania* parasites to avoid elimination by the host immune response, regulation of L-arginine metabolism via NOS2 and arginase-1 (ARG-1) enzymes has emerged as a crucial mechanism for parasite survival. Macrophages, the main host cells that battle *Leishmania*, can be instructed to kill or host intracellular amastigote forms of this parasite, depending on their ability to express NOS2 or ARG-1. The expression of these enzymes, which share L-arginine as a substrate, is regulated in macrophages by their perception of the environmental balance of cytokines.

Proinflammatory cytokines (e.g., TNF-α and IFN-γ) induce the classical activation of macrophages, upregulating NOS2 expression. This enzyme catalyzes the conversion of L-arginine into L-citrulline and NO, the latter molecule being considered the most potent leishmanicidal agent for the elimination of intracellular *Leishmania* parasites [36]. On the other hand, anti-inflammatory cytokines (e.g., IL-4, IL-10 and IL-13) elicit an alternative activation of macrophages that upregulates ARG-1 [37], which in turn catalyzes the conversion of L-arginine into

urea and L-ornithine. The latter is a basic source for the synthesis of polyamines, essential nutrients for the growth and surveillance of *Leishmania* [38, 39]. Hence, whether L-arginine metabolism takes place through ARG-1 or NOS2 is decisive for the life or death of *Leishmania* during infection [40].

To establish infection and avoid host surveillance, *Leishmania* parasites have developed different strategies to hijack L-arginine metabolism in order to promote the production of polyamines rather than NO. During its development inside the vector, for instance, the parasite generates a mucin-rich gel that sand flies deliver into the host skin when transmitting *Leishmania* promastigotes [41]. This gel, called promastigote secretory gel, is known to modulate L-arginine metabolism in macrophages [42]. Accordingly, promastigote secretory gel stimulates the recruitment of macrophages and promotes their alternative activation, causing an increased expression of arginase-1 along with its greater capacity to metabolize L-arginine to afford polyamines, which in turn enhance the growth of *Leishmania* [42].

The parasite-induced upregulation of arginase-1 can affect the production of NO through substrate (L-arginine) competition [41]. Additionally, the generation of some polyamines resulting from L-arginine metabolism via arginase-1 (e.g., spermine, spermidine and putrescine) inhibit NOS2 function [38, 39, 43]. The modulation of L-arginine metabolism is relevant not only during the onset of the infection but throughout the course the disease. The success of host immunity or the pathology of leishmaniasis depends mainly on the balance of the immune response. Since the formation of either NOS2 or arginase leads to the inhibition of the other, these two metabolic states are competitive and tightly regulated [44], determining the levels of NO and therefore the outcome of *Leishmania* infection (survival versus elimination) in the host.

Besides the production of polyamines and the resulting enhancement of *Leishmania* intracellular growth in alternatively activated macrophages, recently findings have shown that *Leishmania*-induced L-arginine metabolism via ARG-1 polarization is advantageous to the parasite in yet another way. A substantial accumulation of alternatively activated macrophages causes an elevated demand, consumption, and depletion of L-arginine in the microenvironment [41]. Since T lymphocytes are very sensitive to L-arginine starvation, a greater consumption of this amino acid via ARG-1 limits its availability to T cells, which in turn notably impairs the development and function of these cells that are required for the control of a *Leishmania* infection [45, 46].

5. Inhibition of apoptosis

Leishmania is an obligate intracellular parasite that invades a variety of host cells, but it is in dendritic cells and macrophages where it can survive and replicate inside the phagosome. The condition of being obligate intracellular parasites presupposes the utilization by *Leishmania* of mechanisms to manipulate host cells in order to evade the immune response and survive inside cells. Along the evolutionary history of this parasite, diverse survival strategies have been developed.

Although the inhibition of the phagosome-lysosome fusion comprises one such strategy, one of the most intriguing is the inhibition of apoptosis. The latter process is a type of programmed cell death characterized by a very orderly and immunologically silent dismantling of a cell [47, 48]. The activation or inhibition of several signaling pathways is required for apoptosis to occur [49–53]. Whereas the initiation of apoptosis involves gene activation and transduction pathways, the executioner phase requires the activation of the cellular machinery necessary for the dismantling of the cell.

Apoptosis is a crucial defense mechanism against intracellular pathogens [54]. However, many pathogenic microorganisms such as virus [55], bacteria [56], and protozoan parasites [57, 58] have developed mechanisms to persist within host cells without inducing apoptosis. It has been widely documented that *Leishmania* inhibits apoptosis of different cells such as macrophages [59–61], monocytes [64] and neutrophils [66].

Recently, it has been demonstrated that *L. mexicana* promastigotes and amastigotes also inhibit apoptosis in dendritic cells [62, 63]. In some of these studies, monocytes, dendritic cells, and macrophages were grown under apoptogenic conditions and infected with different species of *Leishmania*, resulting in the inhibition of normal apoptosis.

In particular, infection with *L. donovani* or a stimulus with its LPG inhibits apoptosis in macrophages. Cellular activation caused by infection increases the production of TNF-α, TGF-β, IL-6, and GM-CSF, while decreasing the secretion of M-CSF and IL-1β [60]. Additionally, *L. major* delayed apoptosis by inhibiting the release of mitochondrial cytochrome C in infected macrophages grown in the presence of staurosporine [59]. Studies performed on other cell lines report a similar outcome, such as the inhibition of actinomycin D-induced apoptosis in the monocyte cell line U937 infected with *L. infantum* [64]. In macrophages from the cell line RAW 264.7 infected with *L. major*, apoptosis diminished even in the presence of cycloheximide [65]. Exposing neutrophils to *L. major* led to reduced caspase-3 activity, thus inhibiting spontaneous apoptosis [66]. Moreover, amastigotes and promastigotes of *L. mexicana* inhibited camptothecin-induced apoptosis in monocyte-derived dendritic cells [62, 63]. In the majority of reports, the antiapoptotic effect has been associated with a significant decline in caspase-3 activity in cells.

5.1. Signaling pathways involved in *Leishmania*-induced inhibition of host cell apoptosis

Although *Leishmania* infection is known to inhibit apoptosis in several cells, the mechanism(s) through which this process takes place in infected cells is not fully clear. One signaling pathway involved in apoptosis is that of MAPKs, a family of serine/threonine kinases. Four major pathways have been identified in mammalian cells for signaling by MAPKs: extracellular signal-related kinases (ERK1/2), c-Jun amino–terminal kinases (JNK1/2/3), p38 (α, β, γ, δ), and ERK5 [67–70]. MAPKs respond to a wide variety of stimuli, such as proinflammatory cytokines, environmental stress, DNA damage, and growth factors [50, 71]. The p38 pathway is associated with cytokine production, inflammation, cell growth and differentiation, and cell death. JNK participates in the control of cell death and is encoded by three genes: JNK1/SAPKγ, JNK2/SAPKα, and JNK3/SAPKβ. Contrarily, the signaling pathway of PI3K/AKT has an antiapoptotic role through the phosphorylation of PI3K/AKT that conducts to the downstream activation of multiple signaling pathways related to growth, development, and

cellular survival processes [72]. MAPKs [73, 74] and PI3K [75] are activated during *Leishmania* infections and participate in the apoptosis or survival cells [76–78].

Diverse signaling pathways have been implicated in the inhibition of apoptosis by *Leishmania*, such as NF-κB, PI3K, and p38 MAPK PI3K. However, only the inhibition of PI3K resulted in the abrogation of the antiapoptotic phenotype, whose activation confers apoptosis inhibition in infected macrophages [79] and dendritic cells [5]. ERK1/2 is activated in neutrophils infected with *L. major* and modulates multiple apoptotic pathways [80]. Contrarily, p38 and JNK are deactivated to prevent apoptosis [4, 5]. Survival is mediated by signaling pathways (e.g., PI3K/Akt), as well as by the expression of antiapoptotic proteins of the Bcl-2 family (e.g., Bcl-2, Bcl-xL, MCL-1, and A1). We have demonstrated that *L. mexicana* promastigotes and amastigotes also inhibit apoptosis in dendritic cells [62, 63] through the downregulation of proapoptotic pathways (e.g., MAPK p38 and JNK), as well as the activation of antiapoptotic routes such as PI3K/Akt [4, 5].

6. Conclusion

Leishmania parasites have developed diverse strategies to evade the immune response: elicitation of a differential production of proinflammatory cytokines, upregulation of TLR2 and TLR4 expression, the nuclear translocation of NF-κB, and modulation of inflammasome. These parasites have a dual relation with host cells. Whereas host cells provide them with nutrients and a place to survive and replicate, these same cells exhibit an immune response aimed at the destruction of the parasites. Hence, the latter must display a wide array of strategies to overcome host cells defense mechanisms. One of the most successful strategies utilized by *Leishmania* inside host cells is the inhibition of apoptosis.

Apoptosis is a type of programmed cell death involving a precisely orchestrated series of steps that culminate in the orderly dismantling of the cell. This process encompasses the activation and silencing of a wide variety of signaling pathways, among which a leading role is played by MAPK, PI3K/Akt and proapoptotic/antiapoptotic proteins of the Bcl-2 family. *Leishmania* has the capacity to inhibit apoptosis of different cells, especially macrophages, monocytes, neutrophils, and dendritic cells. Although the precise mechanisms have not been fully elucidated, it is now clear that MAPK p38, JNK, ERK 1/2, and PI3K/Akt participate in the inhibition of apoptosis in macrophages, neutrophils, and dendritic cells. The inhibition of apoptosis is a key strategy for the survival and replication of *Leishmania* in host cells and may have implications for its pathogenesis because of favoring the invasion of the host and the persistence of the parasite in host cells. Further research is needed on the mechanisms of activation and regulation of the inflammasome pathway to provide insights into the pathophysiology of chronic diseases and reveal new therapeutic targets.

Acknowledgements

The authors are especially grateful to Bruce Allan Larsen for proofreading the manuscript.

Financial support

This work was supported by grant IN220816 from DGAPA-PAPIIT to MMAG.

Author details

M. Magdalena Aguirre-Garcia*, Alma R. Escalona-Montaño, Arturo A. Wilkins-Rodríguez and Laila Gutiérrez-Kobeh

*Address all correspondence to: maguirre@unam.mx

División de Investigación, Facultad de Medicina, Instituto Nacional de Cardiología Ignacio Chávez, Unidad Periférica de Investigación UNAM-INC, Ciudad de México, Mexico

References

[1] Killick-Kendrick R. The life-cycle of Leishmania in the sandfly with special reference to the form infective to the vertebrate host. Annales de Parasitologie Humaine et Comparée. 1990;**65**(Suppl 1):37-42. DOI: 10.1051/parasite/1990651037

[2] Faria MS, Reis FC, Lima AP. Toll-like receptors in leishmania infections: Guardians or promoters? Journal of Parasitology Research. 2012;**2012**:930257. DOI: 10.1155/2012/930257

[3] Escalona-Montano AR, Pérez-Montfort R, Cabrera N, Mondragón-Flores R, Vélez-Ramírez DE, Gómez-Sandoval JN, Gutierrez-Kobeh L, Becker I, Aguirre-Garcia MM. Protein phosphatase PP2C, in the flagellum of *Leishmania major*: Cloning and characterization. Parasitology Open. 2017;**3**:1-9. DOI: 10.1017/pao.2017.14

[4] Rodriguez-Gonzalez J, Wilkins-Rodriguez A, Argueta-Donohue J, Aguirre-Garcia M, Gutierrez-Kobeh L. *Leishmania mexicana* promastigotes down regulate JNK and p-38 MAPK activation: Role in the inhibition of camptothecin-induced apoptosis of monocyte-derived dendritic cells. Experimental Parasitology. 2016;**163**:57-67. DOI: 10.1016/j.exppara.2015.12.005

[5] Vazquez-Lopez R, Argueta-Donohue J, Wilkins-Rodriguez A, Escalona-Montano A, Aguirre-Garcia M, Gutierrez-Kobeh L. *Leishmania mexicana* amastigotes inhibit p38 and JNK and activate PI3K/AKT: Role in the inhibition of apoptosis of dendritic cells. Parasite Immunology. 2015;**37**:579-589. DOI: 10.1111/pim.12275

[6] Srivastav S, Kar S, Chande AG, Mukhopadhyaya R, Das PK. *Leishmania donovani* exploits host deubiquitinating enzyme A20, a negative regulator of TLR signaling, to subvert host immune response. Journal of Immunology. 2012;**189**:924-934. DOI: 10.4049/jimmunol.1102845

[7] Olivier M, Gregory DJ, Forget G. Subversion mechanisms by which Leishmania parasites can escape the host immune response: A signaling point of view. Clinical Microbiology Reviews. 2005;**18**:293-305. DOI: 10.1128/CMR.18.2.293-305.2005

[8] McConville MJ, Schnur LF, Jaffe C, Schneider P. Structure of Leishmania lipophosphoglycan: Inter- and intra-specific polymorphism in old World species. The Biochemical Journal. 1995;**310**(Pt 3):807-818

[9] McConville MJ, Turco SJ, Ferguson MA, Sacks DL. Developmental modification of lipophosphoglycan during the differentiation of *Leishmania major* promastigotes to an infectious stage. The EMBO Journal. 1992;**11**:3593-3600

[10] Ferguson MA. The surface glycoconjugates of trypanosomatid parasites. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 1997;**352**: 1295-1302. DOI: 10.1098/rstb.1997.0113

[11] McConville MJ, Ralton JE. Developmentally regulated changes in the cell surface architecture of Leishmania parasites. Behring Institute Mitteilungen. 1997:34-43

[12] Tjalsma H, Bolhuis A, Jongbloed JD, Bron S, van Dijl JM. Signal peptide-dependent protein transport in *Bacillus subtilis*: A genome-based survey of the secretome. Microbiology and Molecular Biology Reviews. 2000;**64**:515-547

[13] Hiller NL, Bhattacharjee S, van Ooij C, Liolios K, Harrison T, Lopez-Estrano C, Haldar K. A host-targeting signal in virulence proteins reveals a secretome in malarial infection. Science. 2004;**306**:1934-1937. DOI: 10.1126/science.1102737

[14] van Ooij C, Tamez P, Bhattacharjee S, Hiller NL, Harrison T, Liolios K, Kooij T, Ramesar J, Balu B, Adams J, Waters AP, Janse CJ, Haldar K. The malaria secretome: From algorithms to essential function in blood stage infection. PLoS Pathogens. 2008;**4**:e1000084. DOI: 10.1371/journal.ppat.1000084

[15] Silverman JM, Chan SK, Robinson DP, Dwyer DM, Nandan D, Foster LJ, Reiner NE. Proteomic analysis of the secretome of *Leishmania donovani*. Genome Biology. 2008;**9**:R35. DOI: 10.1186/gb-2008-9-2-r35

[16] Corrales RM, Sereno D, Mathieu-Daude F. Deciphering the Leishmania exoproteome: What we know and what we can learn. FEMS Immunology and Medical Microbiology. 2010;**58**:27-38. DOI: 10.1111/j.1574-695X.2009.00608.x

[17] Escalona-Montano AR, Ortiz-Lozano DM, Rojas-Bernabe A, Wilkins-Rodriguez AA, Torres-Guerrero H, Mondragon-Flores R, Mondragon-Gonzalez R, Becker I, Gutierrez-Kobeh L, Aguirre-Garcia MM. *Leishmania mexicana*: Promastigotes and amastigotes secrete protein phosphatases and this correlates with the production of inflammatory cytokines in macrophages. Parasitology. 2016;**143**:1409-1420. DOI: 10.1017/S0031182016000949

[18] Wang GJ, Liu Y, Qin A, Shah SV, Deng ZB, Xiang X, Cheng Z, Liu C, Wang J, Zhang L, Grizzle WE, Zhang HG. Thymus exosomes-like particles induce regulatory T cells. Journal of Immunology. 2008;**181**:5242-5248

[19] Graner MW, Alzate O, Dechkovskaia AM, Keene JD, Sampson JH, Mitchell DA, Bigner DD. Proteomic and immunologic analyses of brain tumor exosomes. The FASEB Journal. 2009;**23**:1541-1557. DOI: 10.1096/fj.08-122184

[20] Nguyen DG, Booth A, Gould SJ, Hildreth JE. Evidence that HIV budding in primary macrophages occurs through the exosome release pathway. The Journal of Biological Chemistry. 2003;**278**:52347-52354. DOI: 10.1074/jbc.M309009200

[21] Silverman JM, Reiner NE. Leishmania exosomes deliver preemptive strikes to create an environment permissive for early infection. Frontiers in Cellular and Infection Microbiology. 2011;**1**:26. DOI: 10.3389/fcimb.2011.00026

[22] Cuervo P, De Jesus JB, Saboia-Vahia L, Mendonca-Lima L, Domont GB, Cupolillo E. Proteomic characterization of the released/secreted proteins of *Leishmania (Viannia) braziliensis* promastigotes. Journal of Proteomics. 2009;**73**:79-92. DOI: 10.1016/j.jprot.2009.08.006

[23] Santarem N, Silvestre R, Tavares J, Silva M, Cabral S, Maciel J, Cordeiro-da-Silva A. Immune response regulation by leishmania secreted and nonsecreted antigens. Journal of Biomedicine & Biotechnology. 2007;**2007**:85154. DOI: 10.1155/2007/85154

[24] Silverman JM, Clos J, de'Oliveira CC, Shirvani O, Fang Y, Wang C, Foster LJ, Reiner NE. An exosome-based secretion pathway is responsible for protein export from Leishmania and communication with macrophages. Journal of Cell Science. 2010;**123**:842-852. DOI: 10.1242/jcs.056465

[25] Gurung P, Karki R, Vogel P, Watanabe M, Bix M, Lamkanfi M, Kanneganti TD. An NLRP3 inflammasome-triggered Th2-biased adaptive immune response promotes leishmaniasis. The Journal of Clinical Investigation. 2015;**125**:1329-1338. DOI: 10.1172/JCI79526

[26] Becker I, Salaiza N, Aguirre M, Delgado J, Carrillo-Carrasco N, Kobeh LG, Ruiz A, Cervantes R, Torres AP, Cabrera N, Gonzalez A, Maldonado C, Isibasi A. Leishmania lipophosphoglycan (LPG) activates NK cells through toll-like receptor-2. Molecular and Biochemical Parasitology. 2003;**130**:65-74

[27] Argueta-Donohue J, Carrillo N, Valdes-Reyes L, Zentella A, Aguirre-Garcia M, Becker I, Gutierrez-Kobeh L. *Leishmania mexicana:* Participation of NF-kappaB in the differential production of IL-12 in dendritic cells and monocytes induced by lipophosphoglycan (LPG). Experimental Parasitology. 2008;**120**:1-9. DOI: 10.1016/j.exppara.2008.04.002

[28] Rojas-Bernabe A, Garcia-Hernandez O, Maldonado-Bernal C, Delegado-Dominguez J, Ortega E, Gutierrez-Kobeh L, Becker I, Aguirre-Garcia M. *Leishmania mexicana* lipophosphoglycan activates ERK and p38 MAP kinase and induces production of proinflammatory cytokines in human macrophages through TLR2 and TLR4. Parasitology. 2014;**141**:788-800. DOI: 10.1017/S0031182013002187

[29] Babiker DT, Bakhiet SM, Mukhtar MM. *Leishmania donovani* influenced cytokines and Toll-like receptors expression among Sudanese visceral leishmaniasis patients. Parasite Immunology. 2015;**37**:417-425. DOI: 10.1111/pim.12202

[30] Mahmoudian-Sani M, Hajizade A, Sankian M, Fata A, Mellat M, Hassanpour K, Farnoosh. Evaluation of the expression of the inflammasome pathway related components in *Leishmania major* infected murine macrophaghes. European Journal of Experimental Biology. 2013;**3**(1):104-109

[31] Lima-Junior DS, Costa DL, Carregaro V, Cunha LD, Silva AL, Mineo TW, Gutierrez FR, Bellio M, Bortoluci KR, Flavell RA, Bozza MT, Silva JS, Zamboni DS. Inflammasome-derived IL-1beta production induces nitric oxide-mediated resistance to Leishmania. Nature Medicine. 2013;**19**:909-915. DOI: 10.1038/nm.3221

[32] Tabatabaee PA, Abolhassani M, Mahdavi M, Nahrevanian H, Azadmanesh K. *Leishmania major:* secreted antigens of *Leishmania major* promastigotes shift the immune response of the C57BL/6 mice toward Th2 in vitro. Experimental Parasitology. 2011;**127**:46-51. DOI: 10.1016/j.exppara.2010.06.033

[33] Markikou-Ouni W, Drini S, Bahi-Jaber N, Chenik M, Meddeb-Garnaoui A. Immunomodulatory effects of four *Leishmania infantum* potentially excreted/secreted proteins on human dendritic cells differentiation and maturation. PLoS One. 2015;**10**:e0143063. DOI: 10.1371/journal.pone.0143063

[34] Gour JK, Kumar V, Singh N, Bajpai S, Pandey HP, Singh RK. Identification of Th1-responsive leishmanial excretory-secretory antigens (LESAs). Experimental Parasitology. 2012;**132**:355-361. DOI: 10.1016/j.exppara.2012.04.022

[35] Kumar A, Samant M, Misra P, Khare P, Sundar S, Garg R, Dube A. Immunostimulatory potential and proteome profiling of *Leishmania donovani* soluble exogenous antigens. Parasite Immunology. 2015;**37**:368-375. DOI: 10.1111/pim.12189

[36] Bogdan C. Nitric oxide and the immune response. Nature Immunology. 2001;**2**:907-916. DOI: 10.1038/ni1001-907

[37] Modolell M, Corraliza IM, Link F, Soler G, Eichmann K. Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. European Journal of Immunology. 1995;**25**:1101-1104. DOI: 10.1002/eji.1830250436

[38] Iniesta V, Gomez-Nieto LC, Molano I, Mohedano A, Carcelen J, Miron C, Alonso C, Corraliza I. Arginase I induction in macrophages, triggered by Th2-type cytokines, supports the growth of intracellular Leishmania parasites. Parasite Immunology. 2002; **24**:113-118

[39] Kropf P, Fuentes JM, Fahnrich E, Arpa L, Herath S, Weber V, Soler G, Celada A, Modolell M, Muller I. Arginase and polyamine synthesis are key factors in the regulation of experimental leishmaniasis in vivo. The FASEB Journal. 2005;**19**:1000-1002. DOI: 10.1096/fj.04-3416fje

[40] Wanasen N, Soong L. L-arginine metabolism and its impact on host immunity against Leishmania infection. Immunologic Research. 2008;**41**:15-25. DOI: 10.1007/s12026-007-8012-y

[41] Rogers ME. The role of leishmania proteophosphoglycans in sand fly transmission and infection of the Mammalian host. Frontiers in Microbiology. 2012;**3**:223. DOI: 10.3389/fmicb.2012.00223

[42] Rogers M, Kropf P, Choi BS, Dillon R, Podinovskaia M, Bates P, Muller I. Proteophosophoglycans regurgitated by Leishmania-infected sand flies target the L-arginine metabolism of host macrophages to promote parasite survival. PLoS Pathogens. 2009; **5**:e1000555. DOI: 10.1371/journal.ppat.1000555

[43] Blachier F, Mignon A, Soubrane O. Polyamines inhibit lipopolysaccharide-induced nitric oxide synthase activity in rat liver cytosol. Nitric Oxide. 1997;**1**:268-272. DOI: 10.1006/niox.1997.0127

[44] Munder M, Eichmann K, Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4+ T cells correlates with Th1/Th2 phenotype. Journal of Immunology. 1998; **160**:5347-5354

[45] Choi BS, Martinez-Falero IC, Corset C, Munder M, Modolell M, Muller I, Kropf P. Differential impact of L-arginine deprivation on the activation and effector functions of T cells and macrophages. Journal of Leukocyte Biology. 2009;**85**:268-277. DOI: 10.1189/jlb.0508310

[46] Munder M, Choi BS, Rogers M, Kropf P. L-arginine deprivation impairs *Leishmania major*-specific T-cell responses. European Journal of Immunology. 2009;**39**:2161-2172. DOI: 10.1002/eji.200839041

[47] Danial NN, Korsmeyer SJ. Cell death: Critical control points. Cell. 2004;**116**:205-219

[48] Green DR. Overview: Apoptotic signaling pathways in the immune system. Immunological Reviews. 2003;**193**:5-9

[49] Hoeflich KP, Yeh WC, Yao Z, Mak TW, Woodgett JR. Mediation of TNF receptor-associated factor effector functions by apoptosis signal-regulating kinase-1 (ASK1). Oncogene. 1999;**18**:5814-5820. DOI: 10.1038/sj.onc.1202975

[50] Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiological Reviews. 2001;**81**:807-869. DOI: 10.1152/physrev.2001.81.2.807

[51] Luo HR, Hattori H, Hossain MA, Hester L, Huang Y, Lee-Kwon W, Donowitz M, Nagata E, Snyder SH. Akt as a mediator of cell death. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**:11712-11717. DOI: 10.1073/pnas.1634990100

[52] Macdonald NJ, Delderfield SM, Zhang W, Taglialatela G. Tumour necrosis factor-alpha-vs. growth factor deprivation-promoted cell death: Distinct converging pathways. Aging Cell. 2003;**2**:245-256

[53] Tran TH, Andreka P, Rodrigues CO, Webster KA, Bishopric NH. Jun kinase delays caspase-9 activation by interaction with the apoptosome. The Journal of Biological Chemistry. 2007;**282**:20340-20350. DOI: 10.1074/jbc.M702210200

[54] Barry M, Bleackley RC. Cytotoxic T lymphocytes: All roads lead to death. Nature Reviews. Immunology. 2002;**2**:401-409. DOI: 10.1038/nri819

[55] Roulston A, Marcellus RC, Branton PE. Viruses and apoptosis. Annual Review of Microbiology. 1999;**53**:577-628. DOI: 10.1146/annurev.micro.53.1.577

[56] Gao LY, Kwaik YA. The modulation of host cell apoptosis by intracellular bacterial pathogens. Trends in Microbiology. 2000;**8**:306-313

[57] Schaumburg F, Hippe D, Vutova P, Luder CG. Pro- and anti-apoptotic activities of protozoan parasites. Parasitology. 2006;**132**(Suppl):S69-S85. DOI: 10.1017/S0031182006000874

[58] Sinai AP, Payne TM, Carmen JC, Hardi L, Watson SJ, Molestina RE. Mechanisms underlying the manipulation of host apoptotic pathways by *Toxoplasma gondii*. International Journal for Parasitology. 2004;**34**:381-391. DOI: 10.1016/j.ijpara.2003.11.009

[59] Akarid K, Arnoult D, Micic-Polianski J, Sif J, Estaquier J, Ameisen JC. *Leishmania major*-mediated prevention of programmed cell death induction in infected macrophages is associated with the repression of mitochondrial release of cytochrome c. Journal of Leukocyte Biology. 2004;**76**:95-103. DOI: 10.1189/jlb.1001877

[60] Moore KJ, Matlashewski G. Intracellular infection by *Leishmania donovani* inhibits macrophage apoptosis. Journal of Immunology. 1994;**152**:2930-2937

[61] Moore KJ, Turco SJ, Matlashewski G. *Leishmania donovani* infection enhances macrophage viability in the absence of exogenous growth factor. Journal of Leukocyte Biology. 1994;**55**:91-98

[62] Gutierrez-Kobeh L, de Oyarzabal E, Argueta J, Wilkins A, Salaiza N, Fernandez E, Lopez O, Aguirre M, Becker I. Inhibition of dendritic cell apoptosis by *Leishmania mexicana* amastigotes. Parasitology Research. 2013;**112**:1755-1762. DOI: 10.1007/s00436-013-3334-2

[63] Gutierrez-Kobeh L, de Oyarzabal E, Argueta J, Wilkins A, Salaiza N, Fernandez E, Lopez O, Aguirre M, Becker I. Gutierrez-Kobeh L. *Leishmania mexicana*: Inhibition of camptothecin-induced apoptosis of monocyte-derived dendritic cells. Experimental Parasitology. 2009;**121**:199-207. DOI: 10.1016/j.exppara.2008.10.020

[64] Lisi S, Sisto M, Acquafredda A, Spinelli R, Schiavone M, Mitolo V, Brandonisio O, Panaro M. Infection with *Leishmania infantum* inhibits actinomycin D-induced apoptosis of human monocytic cell line U-937. The Journal of Eukaryotic Microbiology. 2005;**52**:211-217. DOI: 10.1111/j.1550-7408.2005.00026.x

[65] Donovan MJ, Maciuba BZ, Mahan CE, McDowell MA. Leishmania infection inhibits cycloheximide-induced macrophage apoptosis in a strain-dependent manner. Experimental Parasitology. 2009;**123**:58-64. DOI: 10.1016/j.exppara.2009.05.012

[66] Aga E, Katschinski DM, van Zandbergen G, Laufs H, Hansen B, Muller K, Solbach W, Laskay T. Inhibition of the spontaneous apoptosis of neutrophil granulocytes by the intracellular parasite *Leishmania major*. Journal of Immunology. 2002;**169**:898-905

[67] Jiang Y, Chen C, Li Z, Guo W, Gegner JA, Lin S, Han J. Characterization of the structure and function of a new mitogen-activated protein kinase (p38beta). The Journal of Biological Chemistry. 1996;**271**:17920-17926

[68] Jiang Y, Gram H, Zhao M, New L, Gu J, Feng L, Di Padova F, Ulevitch RJ, Han J. Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38delta. The Journal of Biological Chemistry. 1997; **272**:30122-30128

[69] Pimienta G, Pascual J. Canonical and alternative MAPK signaling. Cell Cycle. 2007;**6**: 2628-2632. DOI: 10.4161/cc.6.21.4930

[70] Pulverer BJ, Kyriakis JM, Avruch J, Nikolakaki E, Woodgett JR. Phosphorylation of c-jun mediated by MAP kinases. Nature. 1991;**353**:670-674. DOI: 10.1038/353670a0

[71] Raman M, Chen W, Cobb MH. Differential regulation and properties of MAPKs. Oncogene. 2007;**26**:3100-3112. DOI: 10.1038/sj.onc.1210392

[72] Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nature Reviews. Cancer. 2002;**2**:489-501. DOI: 10.1038/nrc839

[73] Agallou M, Dotsika E, Frydas S, Karagouni E. Toll-like receptor 4 promotes control of *Leishmania infantum* infection through inducement of leishmanicidal activity in host macrophages: Role of mitogen activated kinases. Journal of Biological Regulators and Homeostatic Agents. 2014;**28**:41-52

[74] Boggiatto PM, Martinez PA, Pullikuth A, Jones DE, Bellaire B, Catling A, Petersen C. Targeted extracellular signal-regulated kinase activation mediated by *Leishmania amazonensis* requires MP1 scaffold. Microbes and Infection. 2014;**16**:328-336. DOI: 10.1016/j.micinf.2013.12.006

[75] Ruhland A, Kima PE. Activation of PI3K/Akt signaling has a dominant negative effect on IL-12 production by macrophages infected with *Leishmania amazonensis* promastigotes. Experimental Parasitology. 2009;**122**:28-36. DOI: 10.1016/j.exppara.2008.12.010

[76] Handley ME, Thakker M, Pollara G, Chain BM, Katz DR. JNK activation limits dendritic cell maturation in response to reactive oxygen species by the induction of apoptosis. Free Radical Biology & Medicine. 2005;**38**:1637-1652. DOI: 10.1016/j.freeradbiomed.2005.02.022

[77] Nakagawa S, Ohtani T, Mizuashi M, Mollah ZU, Ito Y, Tagami H, Aiba S. p38 mitogen-activated protein kinase mediates dual role of ultraviolet B radiation in induction of maturation and apoptosis of monocyte-derived dendritic cells. The Journal of Investigative Dermatology. 2004;**123**:361-370. DOI: 10.1111/j.0022-202X.2004.23238.x

[78] Xie J, Qian J, Yang J, Wang S, Freeman ME 3rd, Yi Q. Critical roles of Raf/MEK/ERK and PI3K/AKT signaling and inactivation of p38 MAP kinase in the differentiation and survival of monocyte-derived immature dendritic cells. Experimental Hematology. 2005;**33**:564-572. DOI: 10.1016/j.exphem.2005.03.001

[79] Ruhland A, Leal N, Kima PE. Leishmania promastigotes activate PI3K/Akt signalling to confer host cell resistance to apoptosis. Cellular Microbiology. 2007;**9**:84-96. DOI: 10.1111/j.1462-5822.2006.00769.x

[80] Sarkar A, Aga E, Bussmeyer U, Bhattacharyya A, Moller S, Hellberg L, Behnen M, Solbach W, Laskay T. Infection of neutrophil granulocytes with *Leishmania major* activates ERK 1/2 and modulates multiple apoptotic pathways to inhibit apoptosis. Medical Microbiology and Immunology. 2013;**202**:25-35. DOI: 10.1007/s00430-012-0246-1

Section 4

Vaccine Candidates: Past, Present and Future

Chapter 5

Vaccines for Human Leishmaniasis: Where Do We Stand and What Is Still Missing?

Pedro Cecílio, Fabiano Oliveira and
Anabela Cordeiro da Silva

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75000

Abstract

Responsible for up to 30,000 deaths annually, leishmaniasis is a complex spectrum of diseases endemic in 97 countries around the globe. Disease control relies heavily on the early diagnosis and treatment of the active cases (relevant for anthroponotic disease), although it is widely accepted that a prophylactic vaccine for human leishmaniasis is the way to achieve the successful elimination of human disease (taking in consideration the vast list of non-human reservoirs that enable the perpetuation of parasites all around the globe). The notion that infection leads to strong and long-lasting immunity against leishmaniasis supports vaccination as an achievable goal. However, and in spite of the different candidates tested along the years, till date, we still do not have an approved vaccine for humans. In this chapter, we will explore the last advances made in the field of vaccines against *Leishmania* without forgetting the historical perspective, essential to the understanding of the road already undergone. We will then discuss the correlates of disease and protection, still neither consensual nor definitive, as well as the issue of pre-clinical to clinical translation. The complete understanding of these issues will be essential for the approval of a successful vaccine for human leishmaniasis.

Keywords: leishmaniasis, human vaccines, correlates of protection, cellular immunity, cross-protection

1. Introduction

Vaccination is undoubtedly one of the greatest achievements of modern medicine, responsible, together with the use of antimicrobials and access to clean water and sanitation, for the global human demographics transformation in the past two centuries [1–3]. The apparently

insignificant proportion of the world population, whose lives are spared annually, thanks to vaccines (0.04 or 0.1%, if we include deaths avoided by smallpox eradication), is equivalent to up to 3 (or 8) million lives spared per year and a cumulative of more than half a billion deaths avoided just in the twentieth century [4, 5]. Nevertheless, and notwithstanding the significant and successful global efforts toward the goal of universal health protection/promotion, the picture could be much better. On the one hand, just by improving global vaccination coverage, an additional 1.5 million deaths could be avoided yearly [5]. On the other hand, there are still many deadly infectious diseases, whose prevention through vaccination is theoretically possible but for which there are no vaccines approved [6–8]. There are different compatible explanations/hypothesis that together justify it. The first one has to do with legal and ethical reasons: to test/approve/administer a pharmaceutical product nowadays is harder than it was 100 years ago [9]. Also in a chronologic point of view, it is not surprising that there are still no vaccines available for emerging diseases (e.g., Zika or MERS-CoV) [10, 11]. Other reasons have to do directly with the convergence of the nature of the pathogens with the evolution of vaccine technologies [12]: (i) almost all vaccines available till date are humoral based, which is not the best option against intracellular pathogens (e.g., *Leishmania* spp., *Trypanosoma cruzi*) [13, 14] and (ii) there are pathogens with immune-evasion strategies dependent on high antigenic variability that poses a challenge in vaccine development [6, 15]. Lastly, but not least important, there are diseases more relevant from an economic standpoint than others: many diseases for which there are still no vaccines available affect almost exclusively the poorest of the poor (neglected tropical diseases—NTDs) [8, 16, 17].

Fortunately, with the arrival of the new millennium, WHO/UN initiatives such as the Millennium Development Goals (Goal 6, Target 3) and more recently the Sustainable Development Goals (Goal 3, Target 3.3) contributed to an increase in the awareness on the NTDs and consequently the investment on strategies to control them [18, 19]. The best example of concrete measures undertaken to "end the neglect" is given by the London Declaration on NTDs, signed in 2012 by 20 parties (including governmental organizations, non-profits and pharmaceutical companies) and endorsed thereafter by many others, that proposes to meet the goals set by the WHO Roadmap to overcome the global burden of NTDs (2012–2020), that include the elimination of five diseases and the control of five others. One of the potential short-term controllable NTDs is the fatal form of leishmaniasis [20].

Endemic in 97 countries around the globe, leishmaniasis is a complex spectrum of diseases [21, 22]. The first layer of complexity is given by its vector-borne nature, which introduces an extra variable (the phlebotomine vector) to the binomium host pathogen. The second one is given by the 20 *Leishmania* species known to cause human diseases (usually in a species/disease-manifestation-specific fashion), which is mostly but not exclusively of zoonotic origin (there are no animal reservoirs recognized for *L. donovani*) [23]. The third one relates to the infectious process, which frequently does not lead to an overt disease but instead to a chronic and "benign" asymptomatic state [24]. These are some of the main challenges to consider within the topic of disease control, which relies heavily on the early diagnosis and treatment of the active cases (whose influence in the diminishment of disease incidence should be relevant in anthroponotic *versus* zoonotic leishmaniasis) [25, 26]. Although till date there is no vaccine available against human leishmaniasis, not only is it widely accepted that the development of an effective vaccine is possible but also it is recognized that vaccination is the only viable option to achieve zoonotic disease elimination [25].

With this chapter, we propose to explore the broad anti-*Leishmania* vaccines field, with humans as the focus population. Starting from a historical perspective, we will clarify where we stand today by discussing the different candidates and approaches followed along the years, situating them in the vaccine development pipeline. Additionally, we will debate what is missing (focusing mainly, but not only, on the correlates of protection and the disease models) as a way to substantiate why currently there are no vaccines against leishmaniasis approved for humans.

2. Vaccines for human leishmaniasis: where do we stand?

2.1. Leishmanization as the proof of principle of vaccines against leishmaniasis

The close relation of the human host and *Leishmania* parasites is quite ancient: there is evidence of parasite genetic material (identified retrospectively) in mummies from the year 2000 B.C. [23, 27]. However, the major breakthroughs in the leishmaniasis field were only achieved starting from the beginning of the twentieth century, with the identification of the causative agent(s), the incrimination of the vector(s), and consequently the understanding of parasite(s) life cycle and the distinct physiopathologic mechanisms that characterize each of the leishmaniasis forms [23, 28]. The definitive allocation of leishmaniasis within the infectious (or communicable) diseases, in convergence with the "success of variolation" and the birth of vaccination [29], boosted the investigation of the anti-*Leishmania* immune response envisioning the development of an effective prophylactic approach. The first reports date from early 1900 and are based on either contemporary common "medical practices" from Old World Cutaneous Leishmaniasis (CL) endemic countries or directly on evidence produced in human clinical trial-like studies [30, 31]. The general conclusions of these pioneer "vaccine studies" that used as inoculum either material from CL patient's ulcers or live parasites collected from *in vitro* cultures (*L. tropica*) were (i) only the individuals that developed a lesion and then self-healed were resistant to reinfection and (ii) reinoculation of immune individuals led to what is nowadays known as Type I delayed type hypersensitivity (DTH) reaction [30, 31]. Such studies established the dogma accepted today by the scientific community—"previous infection leads to robust immunity against *Leishmania*"—and were the proof of principle of the only prophylactic approach clinically used against leishmaniasis known as leishmanization.

Leishmanization was no more than the controlled induction of the cutaneous disease to prevent the consequences of natural infection, such as the scarification of exposed body parts (particularly the face) and the consequent life-long psychosocial impact and simultaneously to decrease the disease incidence in hyperendemic areas [32, 33]. In the 1970s and 1980s, several trials were performed using live virulent *L. major* parasites with promising results (up to 80% efficacy, **Table 1**) [32, 34–36]. This vaccine approach was accepted in countries such as the former Soviet Union, Iran, Israel, and Uzbekistan [32, 36, 37]. However, it was generally abandoned (with the exception of Uzbekistan, where it is still a licensed approach according to the most recent reviews on the field [34, 36, 38]) due to a number of concerns such as: (i) some individuals (1–2/10,000 inoculations) developed non-healing lesions, hard to resolve with chemotherapy [32, 39]; (ii) live vaccines (even the attenuated) are contraindicated to immuno-suppressed individuals [40] (whose worldwide prevalence has increased in the modern days, due not only to the HIV pandemic but also, for instance, to the increase of organ transplantation

procedures [41]); (iii) batch-to-batch variability issues of such complex immunogens raise reproducibility concerns [36, 42]; and (iv) complex logistics are usually associated with live vaccines [42].

2.2. An overview of the vaccine candidates against human leishmaniasis explored since leishmanization until the present day

The knowledge produced by leishmanization trials and campaigns conducted at the end of the last century is the most important evidence that the development of a vaccine against leishmaniasis is quite far from being impossible. The quest for such an essential pharmaceutical, indispensable for the achievement of global disease control, has been continuous (in a scale proportional to the funding for NTD research) and fruitful if we consider the number of candidates and different approaches tested. Here we will separate them into five major groups: live vaccines ("leishmanization like"), first-, second-, and third-generation vaccines, and vector-derived vaccines. **Table 1** compiles the information to be discussed in the next subheadings, presenting not only the different candidates/approaches tested along the years but also the disease form they were destined to prevent, their placement in the vaccine development pipeline, and the main findings reported.

2.2.1. Live vaccine candidates

The success of leishmanization is still used to support the investigation of vaccine approaches based on live parasites (called by some as leishmanization revisitation [38]), that according to the authors have the advantage of at least partially reproducing the normal infectious process (and consequently induce a "close-to-natural" anti-*Leishmania* memory) [43]. This includes for some candidates the long-term parasite persistence in the site of inoculation that will continuously boost the immune system and prevent the loss of immunity to reinfection [44–46]. The first two approaches explored, relying on parasite persistence as the key to effectiveness, are readaptations of leishmanization directed toward the prevention of visceral disease and proposed the controlled infection with either virulent *L. major* parasites or with a virulent but dermotropic *L. donovani* strain to promote heterologous or homologous protection against visceral disease caused by viscerotropic *L. infantum* or *L. donovani* strains, respectively [38, 47–49]. Still, both approaches, although shown effective in the pre-clinical context, will unlikely proceed in the vaccine development pipeline, mainly due to the safety concerns always raised by the use of virulent pathogens. As a way to partially overcome this barrier, different live vaccine approaches proposed the use of attenuated parasites, that would still mimic the natural infection (although in a sub-clinical form) and induce anti-*Leishmania* memory but in most of the cases would then be completely eliminated. In the pre-genomic era (but not only) chemically and physically attenuated parasites were shown to be effective, in pre-clinical trials, against CL, muco-cutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) [50–52]. These attenuation approaches that did not assure a homogeneous parasite population (with an unpredictable potential of reversion to the virulent form) were almost completely replaced by the genetically modified parasites in the post-genomic era. Two main groups of genetically modified *Leishmania* parasites were used in the pre-clinical context: loss-of-function mutants

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
	Inoculation of live, virulent *L. major* parasites: Leishmanization	CL	Effective clinical use in the former Soviet Union, Israel and Middle East* (discontinued)	About 80% efficacy	[31, 34, 36]
	Heterologous protection mediated by inoculation of live, virulent *L. major*	VL	Pre-clinical studies in mice	No effect in BALB/c mice; protection in C57Bl/6 mice	[46–48]
Live vaccines	Inoculation of a dermotropic *L. donovani*	VL	Pre-clinical studies in mice	Protection against challenge with viscerotropic *L. donovani* in BALB/c mice	[37]
	Physically attenuated parasites	CL/ MCL/ VL	Pre-clinical studies in mice and hamsters	Homologous protection for *L. major*, *L. tropica*, *L. amazonensis*, *L. donovani* and *L. braziliensis*; no effect for *L. Infantum*	[49–51]
Non-defined composition; live, attenuated and/ or drug-sensitive parasites (through culture, chemical, radiation or genetic manipulation)	Chemically attenuated parasites (N-nitrosamines/ antibiotic pressure)	CL/ MCL/ VL	Pre-clinical studies in mice and dogs	Homologous protection for *L. major* and *L. mexicana* in BALB/c mice; promising results for *L. infantum* in dogs	[51]
	Genetically attenuated parasites (**Lmaj**dhfr-ts, **Lmex**CystProt, **Lmaj**LPG2, **Lmaj**PPM, **Ld**Cen1, **Li**HSP70-II, **Ld**p27, **Ld**ALO and **Ld**BT1 null mutants; **Li**SIR2 sKO)	CL/ MCL/ VL	Pre-clinical studies in mice/hamsters/dogs/ macaques	Homologous protection for *L. major* in mice, but not monkeys; *L. mexicana* in mice and hamsters; *L. infantum* in mice and *L. donovani* in mice, hamsters and dogs; heterologous protection for *L. major* in mice, *L. braziliensis* in mice and hamsters and *L. infantum* in dogs (mediated by *L. donovani* KO parasites)	[51–55]
	Genetically modified parasites (gain of function)—suicide mutants: *L. major* tk-cd+/+ (susceptible to Ganciclovir and 5-flurocytosine), *L. amazonensis* alad-pbgd+/+ (used in the context of photodynamic vaccination)	CL/VL	Pre-clinical studies in mice and hamsters	Homologous long-term protection (lesion free) in mice for *L. major*; heterologous protection against *L. donovani* mediated by *L. amazonensis*: 99% reduction in parasite loads and suppression of disease	[50, 56, 57]

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
	Immunization with non-pathogenic *L. tarentolae* (wild type or genetically modified strains producing LPG3, **Ld**A2 or **Ld**A2/CPA/CPB)	VL	Pre-clinical studies in mice and dogs	Promising results in mice and dogs	[50, 60, 61]
First generation vaccines	ALM adjuvanted with BCG	CL/VL	Pre-clinical and human clinical studies	Protection in macaques against *L. donovani* challenge; poor efficacy in humans. Protection in mice against *L. major* infection; clinical studies with disappointing results	[54, 65, 67, 68]
	Alum-ALM adjuvanted with BCG	CL/VL	Pre-clinical and human clinical studies	Immunogenic and safe in humans; protective (single dose) in macaques challenged with *L. donovani*; moderate efficacy against canine visceral leishmaniasis; protection in BALB/c mice against challenge with *L. major*	[54, 56, 69]
	Autoclaved *L. donovani*	VL	Pre-clinical studies in mice	Significant levels of homologous protection	[70]
	Phenol or Heat inactivated *L. guyanensis*, *L. braziliensis* and *L. amazonensis* adjuvanted with BCG	CL/ MCL	Human clinical studies	52% Efficacy in endemic area (phenol inactivation); no protection against *L. amazonensis* infection (heat inactivation)	[65, 67]
	Merthiolate-killed *L. amazonensis* (with/without BCG)	CL	Pre-clinical and Human clinical studies	Protection in mice not reproduced in humans	[65, 73, 74]
Non-defined composition; whole killed parasites or parasite fractions	Sonicated *L. donovani* (whole cell or soluble antigens) adjuvanted (MPL-A, BCG, liposomes)	VL	Pre-clinical studies in mice hamsters and monkeys	Good homologous protection in all species; liposomal formulation elicits the best protection in mice	[36, 54, 71, 72]
	Liposomal *L. major* soluble antigen adjuvanted with CpG	CL	Pre-clinical studies in mice	Significant levels of homologous protection	[36]

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
	Fucose-Manose ligand adjuvanted with saponin	VL	Pre-clinical studies in mice and hamsters; "clinical" studies in dogs	Protection in mice and hamsters challenged with *L. donovani* (homologous); effective heterologous protection (against *L. Infantum*) in dogs; transmission blocking potential; commercialized as a canine anti-*Leishmania* vaccine with the name Leishmune in Brazil (commercialization license suspended in 2014)	[66, 75]
	L. infantum or *L. amazonensis* excreted-secreted antigens adjuvanted with saponin	VL	Pre-clinical/"Clinical" studies in dogs	Significant, long-lasting protection against canine VL in a field trial in an endemic area (**Li**); promising results in terms of heterologous protection against *Leishmania infantum* (**La**); commercialized as a canine anti-*Leishmania* vaccine with the name CaniLeish in Europe (**Li**)	[54, 66, 76, 77]
Second generation vaccines	Membrane proteins: native **Ld**Dp-72, gp63 and PSA-2 and recombinant **Li**LCR1, **Ld**HASPB1, KMP-11 and gp63; adjuvanted (BCG, CpG-ODN, MPL-SE, IL-12, saponin, cationic nanoparticles, liposomes)	CL/VL	Pre-clinical studies in mice, dogs and macaques	Promising results regarding homologous protection in mice; dubious protection in monkeys against *L. major* challenge (gp63)	[50, 54, 71, 83–85]
	"Soluble proteins": recombinant **Ld**A2, **Li**PHB, **Ld**F14, **Ld**p27, **Ld**pSP **Ld**P45, **Ld**PDI, **Ld**TPI, **Ld**TPR, **Li**P0, **Lmaj**STM1, **Li**TDR-1, **Lb**Hyp, EiF5a, eIF-2, NH, CPA and CPB, SMT, PEPCK, Histone H1, Heat shock proteins (HSP), **Li**Ribosomal proteins, **Li**Hypothetical amastigote-specific protein, cysteine proteinases, LACK; adjuvanted (BCG, ALD, *P. acnes*, CpG-ODN, MPL-SE, IL-12, saponin, cationic nanoparticles)	CL/ MCL/ VL	Pre-clinical studies in mice, hamsters and dogs; *ex vivo* human studies	Promising results in mice and hamsters; a major limitation is that most of the antigens were not tested in superior models; positive response in *ex vivo* human studies for **Ld**eIF-2; partial homologous and heterologous (*L. infantum*) protection in dogs and heterologous protection in mice challenged with *L. infantum* and *L. amazonensis* (**Ld**A2: licensed veterinary product in Brazil—LeishTec)	[36, 50, 54, 67, 84, 85, 87–90]

http://dx.doi.org/10.5772/intechopen.75000

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
Defined antigens: (native) or produced through DNA recombinant technology (more frequent)	Peptides: CPA, GP63, LmSTI1, LiKMP-11, PEPCK; often associated DC-based vaccination or nano-sized vaccine-delivery systems; adjuvanted (MPLA, CpG-ODN)	CL/VL	Pre-clinical studies in mice	Partial protection for *L. infantum*; differential protection for *L. major*	[71, 89, 93, 94]
	Fusion protein/polyprotein: Q protein, Leish-F1 (Leish 110-f), Leish-F2 (Leish 110-f), Leish-F3, Leish-F3+, KSAC, 8E + p21 + SMT, KMP-11 + LJL-143 + Leish-F3 + (in virosomes), rLiHyp1 + rLiHyp6 + rLiHyV+rHRF multiepitope; adjuvanted (BCG, Saponin, CpG-ODN, GLA-SE, MPLA, ALD and MPL-SE)	CL/VL	Pre-clinical studies in mice, hamsters, dogs and macaques; human clinical studies	Promising results in mice (CL and VL) and hamsters; protection conferred to dogs against challenge with *L. infantum* (Q protein, Leish 110-f, Leish-F1, KSAC); protection of macaques challenge by *L. major* (Leish-F1); vaccines safe and immunogenic in humans (Leish-F1, Leish-F2 and Leish-F3); licensed veterinary product in Europe (Q protein—Letifend)	[54, 84, 85, 95–105]
Third generation vaccines	DNA plasmidic vaccines (usually self adjuvanted): LdPDI, tuzin, HbR, A2, Histones+p36, LACK, TSA + LmSTI1, gp63, KMP-11, CPB, ORFF, NH36, TRYP, PSA-2, γGCS, PEPCK, LeIF, GP63 + HSP70, LeIF+/orTSA; MIDGE-Th1 vectors encoding conserved T-cell epitopes from KMP11, TSA, CPA, CPB, and P74	CL/VL	Pre-clinical studies in mice, hamsters, dogs and macaques; *ex vivo* human studies	Generally good protective responses in mice and hamsters correlated with the induction of Th1 immunity; partial (Histones+p36) and good (LACK, cysteine proteinase) protection in dogs; protection in macaques (TSA + LmSTI1); effective in mice in immuno-chemotherapeutic approaches (MIDGE Th1); strong possibility of human immunogenicity (MIDGE Th1)	[36, 50, 54, 84, 85, 89, 110–117]
DNA vaccination and/or modified expression systems	Recombinant viral vectors: recombinant/modified vaccinia virus expressing TRYP, LACK, KMP-11; recombinant Influenza virus expressing LACK; (non-replicative) recombinant adenovirus expressing A2, Leish-F3 or KMP-11-HASPB; recombinant lentivirus expressing KMP11-HASPB	CL/VL/ PKDL	Pre-clinical studies in mice, dogs and macaques; human clinical studies	Promising results obtained in all animal models; vaccine safe and immunogenic in humans (replication defective adenovirus coding for KMP-11-HASPB)	[50, 83, 119–123]

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
	Live recombinant bacterial vectors: *Lactococcus lactis* expressing A2 and LACK+IL-12; recombinant *S. typhimurium* vaccine strains expressing gp63, LinJ08.1190 and LinJ23.0410; recombinant *L. monocytogenes* (attenuated) expressing LACK	CL/VL	Pre-clinical studies in mice	Different results obtained, varying from disease exacerbation (A2 *L. lactis*), to limitation of pathology (LACK *L. monocytogenes*) or protection	[51, 124]
Vector-derived vaccines	Th1 immunity inducing sand fly salivary proteins: recombinant or DNA encoding LJM-19 (SALO), PdSP-15, PpSP15 (also *L. tarentolae* based), PpSP-44, LJM-143, LJM-17, LJM-11 (also *L. monocytogenes* based); alone, or in combination with common anti-*Leishmania* vaccine	CL/ MCL/ VL	Pre-clinical studies in mice, hamsters, dogs and macaques	Evidences or described effect in protection from (natural) infection in all animal models, except with PpSP-44 which leads to exacerbation of cutaneous disease	[128–135]
Recombinant or DNA coding for sand fly derived proteins (including heterologous expression systems)	Insect-based transmission blocking vaccine: anti *P. papatasi* galectin (sand fly midgut protein) antibody	CL	*In vitro* and *in vivo* insect studies (artificial feeding)	86% reduction of sand fly-midgut *L. major* infection; impairment of metacyclogenesis	[136–137]

A2, amastigote specific protein 2; ALM, autoclaved *L. major*; ALO, arabino-1,4-lactone oxidase; Cen, centrine; BCG, Bacillus Calmette–Guérin; CL, cutaneous Leishmaniasis; CPA/B, cysteine peptidase A/B; CystProt, cysteine proteinase; dhfr-ts, dihydrofolate reductase-thymidylate synthase; elF, elongation factor; GCS, glutamylcysteine synthetase; GLA, glucopyranosyl lipid A; gp, glycoprotein; HASP, hydrophilic acylated surface protein; HbR, hemoglobin receptor; HSP, heat shock protein; IL, interleukin; KMP, kinetoplastid membrane protein; LACK, *Leishmania* homolog of receptors for activated c-kinase; Ld, *L. donovani*; Li, *L. infantum*; LJL, *Lutzomyia longipalpis* Jacobina large; LJM, *Lutzomyia longipalpis* Jacobina medium; Lmaj, *L. major*; Lmex, *L. mexicana*; LPG, lipophosphoglycan; MIDGE, minimalistic immunogenically defined gene expression; MCL, mucocutaneous leishmaniasis; MPL, monophosphoryl lipid A; NH, nucleoside hydrolase; ODN, oligodeoxynucleotides; ORFF, open reading frame fragment; P0, acidic ribosomal protein P0; PDI, protein disulphide-isomerase; PdSP, *Phlebotomus duboscqi* salivary protein; PEPCK, phosphoenolpyruvate carboxykinase; PHB, prohibitin; PKDL, post kala-azar dermal Leishmaniasis; PPM, phosphomannomutase; PpSP, *Phlebotomus papatasi* salivary protein; PSA, promastigote surface antigen; SE, stable emulsion; SIR, silent information regulator; SMT, sterol 24-c-methyltransferase; TDR, thiol-dependent reductase; TPI, triose phosphate isomerase; TPR, trypanothione reductase; TRYP, tryparedoxin peroxidase; TSA, thiol-specific antioxidant; VL, visceral leishmaniasis.*Supposedly still used in some extent in Uzbekistan [56].

Table 1. Different anti-*Leishmania* vaccine candidates explored in the last century.

(knock-out) and gain-of-function mutants (knock-in). In respect of the first group, nine null mutants [*L. major* dihydrofolate reductase-thymidylate synthase (dhfr-ts$^{-/-}$), *L. mexicana* Cysteine proteases (CPA/CPB$^{-/-}$), *L. major* lipophosphoglycan 2 (LPG2$^{-/-}$), *L. major* phosphomannomutase (PPM$^{-/-}$), *L. donovani* Centrin (Cen$^{-/-}$), *L. infantum* heat shock protein 70 type II (HSP70-II$^{-/-}$), *L. donovani* amastigote specific protein p27 (p27$^{-/-}$), *L. donovani* arabino-1,4-lactone oxidase (ALO$^{-/-}$) and *L. donovani* biopterin transporter 1 (BT1$^{-/-}$)], and one single knock-out [*L. infantum* silent information regulatory protein 2 (SIR2$^{+/-}$)] were proven, in most cases, as effective vaccine candidates (CL, MCL, and VL) [52–56]. Concerning the second group, two gain-of-function mutants were shown effective as vaccines for CL and VL. Both trials relied on the generation of "suicidal mutants" that would be completely eliminated from the immunized host either by the action of chemotherapeutics [L. major thymidine kinase (herpes simplex virus), cytosine deaminase (Saccharomyces cerevisiae) knock-in: tk-cd$^{+/+}$], or by photodynamic therapy (*L. amazonensis* δ-aminolevulinate dehydratase, porphobilinogen deaminase knock-in: alad-pbgd$^{+/+}$) [51, 57, 58]. Yet, although safer in theory than both live virulent and pre-genomic attenuated vaccine candidates, post-genomic live attenuated vaccines still raise safety concerns, both due to the potential for reversion to virulence (higher for gain-of-function parasites but not negligible for knock-out parasites as was reported [59]) and due to the potential risk to the immunosuppressed (that was not explored in most of the trials). The last tested live vaccine approaches we will discuss here propose the use of closely related non-pathogenic parasites as a way to overcome all the live vaccine safety-related red flags. *Leishmania tarentolae* parasites infect reptiles but are unable to generate a sustained infection in humans (although able to enter into human phagocytic cells, there is no evidence of efficient intracellular replication) [60]. Importantly, they share >90% of the gene content with the other *Leishmania* species [60] which makes these parasite species an innocuous source of native *Leishmania* antigens (although some of the important virulence factors of pathogenic parasites that may be essential to the induction of a protective prophylactic response are missing). Using this premise, both wild-type and genetically modified (LPG3, amastigote-specific protein (A2), or A2/CPA/CPB knock-in) non-pathogenic parasites were reported, in the pre-clinical context, as promising vaccine candidates for VL [52, 61, 62].

2.2.2. First-generation vaccine candidates

Together with live attenuated vaccines, killed whole pathogens or fractions of them (inactivated and fraction vaccines) comprise a large proportion of the approved vaccines for humans today [63]. In line with what happened chronologically in modern vaccinology, killed/fractionated vaccines against leishmaniasis were developed both contemporarily and posteriorly to the "leishmanization era," to answer to the safety concerns associated with live virulent/attenuated vaccines. The main advantage of first-generation vaccines in relation to the live vaccine counterparts is consequently their innocuity: the pool of antigens in its native form will still be "delivered" and elicit a specific memory response (diversity in antigenic *repertoire* given by live parasites will be at least partially maintained), while no pathology is expected, even in immunocompromised individuals (no infection = no disease) [64]. This, however, may as well be a disadvantage: while regarding live vaccines, the antigen delivery will be sustained; that will not be true for killed vaccines that may require more than one administration (prime homologous boosts immunization schemes) and/or the co-administration of an immune response enhancer or adjuvant (usually not required in live vaccine approaches) [65], which may or may not be enough to generate long-lasting protection. Additionally, all of the manufacturing and logistics

issues discussed earlier for leishmanization (and live vaccines in general) are also applicable to killed/fractionated vaccines. Notwithstanding, first generation vaccines for leishmaniasis are the better studied ones in the clinical context (the only leishmaniasis vaccine candidates which have undergone phase 3 clinical trials) [66], by itself very relevant for the anti-Leishmania vaccine development field, and are available today in the market as approved vaccines for canine VL (Leishmune® in Brazil and CaniLeish® in Europe) [67]. The better recognized vaccine candidate within this sub-topic is the autoclaved *L. major* (ALM) adjuvanted with BCG, tested in the pre-clinical and clinical contexts, with promising results in the first that were not confirmed in the second [55, 66, 68, 69]. This candidate was then optimized by adsorption of the antigenic fraction to alum (alum-ALM + BCG) and retested once again in both pre-clinical and clinical contexts (CL and VL), with reported different degrees of efficacy in animal models and good immunogenic and safety profiles in humans [55, 66, 70]. A similar parasite-killing approach was used with *L. donovani* parasites, tested in a vaccine pre-clinical trial for VL (mice) that revealed significant homologous protective potential [71]. In parallel, a different inactivation strategy (sonication) was used also with *L. donovani*, and the obtained total or soluble antigens were used together with MPL-A, BCG, or liposomes as vaccine candidates for VL in pre-clinical trials with promising results in all models tested (mice hamsters and monkeys) [37, 55, 72, 73]. Only two other candidates were tested in the clinical context, this time in the New World as CL and/or MCL vaccines. One of them was a trivalent formulation of phenol or heat-inactivated *L. guyanensis*, *L. braziliensis*, and *L. amazonensis* adjuvanted with BCG [66, 68], while the other consisted of merthiolate-killed *L. amazonensis* (with/without BCG) [66, 74, 75]. Curiously, in line with what was verified in the Old World with ALM-BCG, although effective in the pre-clinical context, both candidates generally failed as human vaccines [66, 75]. Apart from crude extracts, parasite fractions have been tested. Liposomal *L. major* soluble antigens adjuvanted with CpG were tested as a vaccine candidate for CL with significant levels of homologous protection observed in mice [37]. A glycoproteic fraction of *L. donovani* parasites (fucose-mannose ligand—FML) adjuvanted with saponin [67, 76] and *L. infantum* (or *L. amazonensis*) excreted-secreted proteins (ESP) also adjuvanted with saponin [55, 67, 77, 78] were tested as vaccine candidates for VL in canines, whose determined efficacy, and safety profiles, was sufficient to warrant their registration as veterinary vaccines (*L. donovani* FML as Leishmune®—out of the market nowadays—and *L. infantum* ESP as CaniLeish®). Nevertheless, they were never tested in the human clinical context, which may be due to different reasons, all connected to the notion that the requirements needed for the approval of a human pharmaceutical are much more strict than the ones required in the veterinary context: (i) the heterogeneous antigen formulation, harder to standardize, may have been considered an obstacle or (ii) the data obtained in the pre-clinical context may not have been sufficient (vaccines conferred only partial protection [67]).

Although it is a topic we do not explore in this chapter, it is important to stress that killed vaccines, different from what was observed in the prophylactic context, have shown great promise in a therapeutic context (revised in [79]).

2.2.3. Second-generation vaccine candidates

The birth and evolution of the molecular biology field contributed immensely to the rhythm of science in general. Today, the production of a single antigen is usually easily achievable,

as it is the possibility of scaling-up the process to an industrial level. Second-generation vaccines are a consequence of this scientific evolution (although some are native proteins, most of them are recombinant antigens) and consist of defined antigens, generally together with an immune response enhancer. They are usually accepted by the scientific community, as well as by the regulatory entities that so far have approved three vaccines for human use (including the hepatitis B recombinant vaccine that replaced the traditional plasma-derived one [80]). The main advantage of these vaccines in relation to the ones earlier discussed is the defined composition that allows an easier standardization. Another advantage we can think of is the elimination of immuno-dominance events that invariably occur if a complex antigen mixture is used as a vaccine and may hinder the potential of good vaccine candidates [81]. As disadvantages, the following should be considered: (i) the limited duration of antigen availability might impact the memory pool and limit the "protection window" [82] (more complex immunization schemes have to be used) and (ii) recombinant proteins, usually expressed in heterologous systems, may be slightly different from native proteins (particularly concerning post-translational modifications [83]) which might impact their immunogenic potential (more relevant for humoral responses, considering conformational epitopes).

Second-generation vaccines against leishmaniasis are the group with higher representativeness. Here, for the sake of clarity, we separate them into four different groups: membrane and soluble proteins (full single recombinants), peptides, and polyproteins (multivalent), whose main candidates are enumerated in **Table 1**. The studies from fractionated parasites postulated that parasite membrane proteins had a good vaccine potential. Because of that, and also due to their relative abundance, relevant in terms of antigen presentation, many membrane proteins were explored as vaccine candidates in the pre-clinical context for both CL and VL with promising results [51, 55, 72, 84–86]. Among these is the well-known, and extensively studied in the context of anti-*Leishmania* vaccination, kinetoplastid membrane protein-11 (KMP-11) [87]. Importantly, most of these proteins were identified by classical immuno-proteomic approaches considering always the amastigote parasite form as the most relevant in the human infectious process and are known virulence factors. This fact is also true for most of the non-membrane proteins (we name here "soluble proteins") also tested in the last decades as vaccines against leishmaniasis, although most of them only in rodent models of CL, MCL, and VL (translatability to humans is not assured) [37, 51, 55, 68, 85, 86, 88–91]. Ribosomal proteins (e.g., P0), metabolic enzymes (e.g., TPI), stress-related proteins (e.g., HSP), antioxidant-machinery components (e.g., TPR), and even hypothetical proteins (**Table 1**) are found among them. One of these candidates, *L. donovani* A2, is today a licensed veterinary vaccine against leishmaniasis in Brazil—LeishTec® (that needs however to be optimized, according to a recent efficacy field trial performed in an endemic area with high transmission rates [92]). In the past few years, the development of vaccine candidates against leishmaniasis became more refined and rationale based, following the trends of twenty-first-century vaccinology [93]. New studies are now usually based in an initial *in-silico* prediction of immunogenicity, validated later ideally through *ex vivo* studies using samples from exposed human individuals, all performed before the design of any clinical trial. Furthermore, the antigens/antigen portions should be "broad spectrum"—conserved in all the pathogenic *Leishmania* spp.—and very different from human "self-antigens." From the application of such approaches and selection criteria, promising new candidates were proposed. Among them, peptide vaccines, chosen from immunogenic portions of known vaccine candidates such as KMP-11, were tested pre-clinically, often associated with DC-based vaccination

strategies or nanosized vaccine-delivery systems [72, 94, 95]. Interestingly, a recently published work proposes a peptide vaccine candidate (from *Leishmania* phosphoenolpyruvate carboxykinase—PEPCK) that may be effective for both VL and CL should the results obtained in the pre-clinical context translate into the clinical one [90]. However some argue that to use a single antigen, or a peptide as vaccine, may be less than optimal, considering that there will be a limitation in terms of epitope diversity. To answer to this, some propose the use of defined polyantigen vaccines (fusion proteins or mixed recombinants), also rationale based, as a way to generate "first-generation-like" second-generation vaccines, increasing epitope diversity and consequently in theory enhancing recognition by human T cells (**Table 1**) [55, 85, 86, 96–101]. Some of these candidates are among the second-generation vaccines that went further in the vaccine pipeline. Q protein (a fusion protein containing portions of *L. infantum* p2a, p2b, and P0 ribosomal proteins and histone H2A) that was demonstrated effective in a pre-clinical trial in dogs infected with *L. infantum* is today the newest approved vaccine for veterinary use—Letifend® [102, 103]. Leish-F1 [fusion protein containing epitopes from *Leishmania* elongation initiation factor (LeIF), thiol-specific antioxidant (TSA), and *Leishmania major* stress-inducible protein 1 (LmSTI1)], Leish-F2 (same immunogenic portions as Leish-F1 but his tagged), and Leish-F3 (fusion protein containing portions of *Leishmania* nucleoside hydrolase and sterol 24-c-methyltransferase), which revealed promising and safe candidates in the pre-clinical context (for both CL and VL), were tested in phase I/II clinical trials that confirmed the translatability of results obtained with animal models to humans [97, 104–106]. Nevertheless, the researchers involved in these clinical trials think that there is still space for improvements and recently presented an improved version of Leish-F3 (with cysteine protease B as an extra fused antigen—Leish-F3^{+}) that is going through the pre-clinical phase of the vaccine development pipeline, with promising results, either alone [99] or in combination with KMP-11 and the vector-derived antigen LJL-143, within a virosomal formulation [96].

We cannot end this sub-section without stressing that generally these second-generation vaccine candidates require the co-administration of adjuvants to warrant their efficacies as vaccines for leishmaniasis. **Table 2** resumes the relevant information on the topic, extensively covered by two recently published reviews [107, 108].

2.2.4. Third-generation vaccine candidates

The notion that intradermal or intramuscular injection of a plasmid into an animal model would be enough to generate antigen-specific immune responses was responsible for the creation of a new arm in the vaccine research field. Although Initially DNA vaccines were not as well accepted as first- and second-generation vaccines, not only due to potential ethical implications (injection of foreign genetic material into humans that could, for instance, integrate within the human genome) but also due to safety concerns such as the possible generation of autoimmune pathologies initiated by the generation of anti-DNA immune responses [109]. However, these potential issues of DNA vaccines were, with time, shown to be irrelevant, both through extensive pre-clinical research and through several clinical trials performed that confirmed DNA vaccines as safe and immunogenic in humans (although for some candidates, the immunogenicity data was not as promising as expected) [109, 110]. Yet, contrary to the other vaccine approaches discussed earlier, we still have no data from phase IV studies of DNA vaccination, since till date there is no third-generation vaccine approved for human use (although there are already four

Adjuvant	Class	Mechanism of action	Type of immune response	Licensed for use in human vaccines
Aluminum mineral salts	Particulate formulation; antigen depot	NALP3, ITAM, antigen delivery, IL-1 secretion, necrosis, inflammasome	Antibody, Th2	✓ (adjuvant of different commercially available vaccines)
Simple or emulsified Lipid A analogues (e.g., GLA, MPL)	Immuno-modulatory molecule	TLR-4 agonists	Antibody, Th1	✓ (in combination with Alum in HBV and HPV vaccines)
Imidazoquinolines (e.g., Imiquimod, R848)		TLR-7, TLR-8 agonists	Antibody, Th1	X (clinically tested in cancer immuno-therapy)
CpG-ODN		TLR-9 agonists	Antibody, Th1, Th2, CD8+ T cells	X (clinically tested in HBV, malaria, influenza and anthrax vaccines and in cancer immuno-therapy)
Saponins (e.g., QuilA, QS21)		Unknown	/	X (clinically tested in combination with cholesterol in HCV, influenza and HPV vaccines and in cancer immuno-therapy)
Nanoparticles (e.g., Virosomes*, Liposomes)	Particulate formulation	Antigen delivery; cross-presentation enhancer*	Antibody, Th1, Th2	✓ (HAV and *Influenza* vaccines)

Adapted from [106, 107].

* The asterisk means that only virosomes are cross presentation enhancers (asterisk in both)

Table 2. Main adjuvants used in anti-*Leishmania* vaccines development.

approaches approved for veterinary use) [109, 110]. However, considering that third-generation vaccines are the most recent approaches (studies started in the 1990s), it is likely a matter of time until the approval of the first DNA vaccines considering some advantages attributed to them: (i) they are easy to design, produce, and scale up (potentially more cost-effective); (ii) they are quite stable, which minimize distribution and logistics-related complications; and (iii) they can induce both humoral and cellular immune responses (including $CD8^+$-mediated cytotoxicity) [110]. Here, we categorize third-generation vaccines in three clusters: DNA vaccines, viral heterologous expression systems, and live bacterial expression systems. DNA vaccines are the more expressive in respect of the number of candidates explored, containing the simplest vaccine candidates: consist of usually non-adjuvanted plasmids (the "real DNA vaccines"). Similar to what was described for second-generation vaccines, both membrane (e.g., KMP-11 and gp63) and non-membrane antigens (e.g., NH, CPB, HSP70, and A2) were explored in the context of plasmid vaccine candidates (**Table 1**), pre-clinically, using animal models for both CL and VL [37, 51, 55, 85, 86, 90, 111–118]. Interestingly, many of the candidates tested as second-generation vaccines (and particularly those that have shown some degree of promise) were retested as DNA vaccines, either individually or in "multi-antigen" approaches (e.g., KMP-11, A2, LACK, and TSA+LmSTI1), showing the adoption of a rationale-based vaccine development [114]. The general reproduction of the results obtained with second-generation vaccines, after immunization with their DNA counterparts (CL and VL models, including mice, hamsters, dogs, and macaques), validated these approaches as potentially effective agents in

the context of anti-*Leishmania* vaccine prophylaxis [51, 55, 85, 86, 90, 114]. In this sub-group, we would like to highlight the LEISHDNAVAX approach, recently proposed for VL. Completely based in a modern vaccine development approach (rationale based), this vaccine candidate, shown to protect mice from an intravenous challenge with *L. donovani*, is composed of five individual plasmid (MIDGE-Th1 vectors) coding for five *Leishmania* antigenic determinants, chosen based on inter-species conservation, "pan-immunogenic" potential (in different human populations), and content of T-cell-restricted epitopes (KMP11, TSA, CPA, CPB, and P74) [112]. This approach, which, according to the authors, is a candidate for clinical trials, has as the main advantage the modular nature: the vaccine is multivalent, but the antigens are not fused together, allowing the rapid modification and adaptation of the vaccine (exchange, addition, or elimination of antigens) [112]. Still within third-generation vaccines, more complex candidates were explored as well, in the form of heterologous expression systems. Among them are viral vectors, referred to as an improvement of classical DNA vaccines, once in one way allow *in situ* antigen expression, and also have an intrinsic adjuvant activity (mediated by pathogen-associated molecular patterns (PAMPs) immune recognition) [119]. One important prerequisite of such vectors is their relative innocuity, being in most cases either human-approved vaccine strains (which have the same counter-indications for immuno-compromised individuals) or replicative-deficient strains. Till date, more than 5 viral-recombinant vaccines (using as viral platforms modified vaccinia virus, influenza virus, non-replicative adenovirus and lentivirus) coding for *de facto* effective antigens such as KMP-11, LACK, Leish-F3 and HASPB, were tested in the pre-clinical context for both CL and VL, with promising results obtained in all animal models used (mice, dogs and macaques) [51, 84, 120–123]. Remarkably, one of them was the first third-generation anti-*Leishmania* vaccine candidate to undergo human clinical trials. The adenoviral-based vaccine (non-replicative strain) expressing a self-cleaving polyprotein (L. donovani KMP-11+HASPB) was shown safe and immunogenic in humans, inducing particularly specific $CD8^+$ T cell responses, and importantly is being proposed as, more than a prophylactic vaccine, a therapeutic vaccine destined to aid in the control of post-kala-azar dermal leishmaniasis—PKDL ("the neglected form of leishmaniasis" in respect of the anti-*Leishmania* vaccine studies) [124]. Last but not the least, some bacterial-based heterologous systems were proposed as anti-*Leishmania* vaccines although with disappointing results in some cases [52]. Also, to these ones, because they are live organisms, the disadvantages discussed earlier for live attenuated vaccines apply (such as counter-indication to immuno-suppressed) with the exception of the use of non-pathogenic organisms, such as *Lactococcus lactis*. From these candidates, we highlight the recombinant *Salmonella typhimurium* vaccine strains and the attenuated *Listeria monocytogenes* expressing different *Leishmania* antigens (e.g., gp63 and LACK), the ones that have shown the most promising results, although only in rodent models of CL/VL [52, 125].

2.2.5. Vector-derived vaccine candidates

It has become clear that to consider the sand fly vector only from the perspective of vector-control strategies would be not only reductive but also contribute to a major delay in the achievement of the disease elimination objective. The anti-*Leishmania* vaccine field became more complex from the moment Kamhawi, Belkaid, and colleagues showed that a previous exposure to uninfected sand fly bites (or to sand fly saliva) would be enough to confer protection against CL [126, 127]. Curiously, the anti-saliva-generated DTH responses were shown to be sufficient to negatively impact *Leishmania* parasites (indirectly). And importantly, such responses are apparently not

influenced by constant saliva exposure that could induce tolerization [128]. Such pieces of evidence supported the exploitation of defined sand fly salivary proteins as anti-*Leishmania* vaccine candidates (either as single recombinant proteins or DNA vaccines—both plasmids and heterologous systems—alone or in multivalent approaches together with *Leishmania*-derived antigens) [129–136]. Several antigens, derived from different sand fly species from both New [129–131, 136] and Old [133–135] Worlds, were explored in the pre-clinical context in models of CL, MCL, and VL, most of them with promising results. The most relevant candidate is PdSP15, which was shown to be protective against cutaneous disease in different models, including in non-human primates (DNA protein prime-boost approach) [133–135]. Another candidate that deserves to be highlighted is LJM-19 (or SALO), which was demonstrated simultaneously as a good candidate against visceral ("homologous protection") and mucocutaneous ("heterologous protection") disease [131, 136]. Still within vector-based anti-*Leishmania* vaccine approaches, and although it is an option which is exploited very little, we believe that transmission blocking vaccines deserve to be mentioned. Such vaccines will act by impacting parasites' development within the vector, impeding, therefore, their transmission to a new host [137]. For their engineering, however, the insect midgut proteins that allow parasite attachment during development (assuming such a process is dependent on specific interactions) have to be identified, which was described only for *Phlebotomus papatasi* (galectin—PpGalec) [138]. Interestingly, this study that shows that flies pre-fed with PpGalec murine pre-immune serum and posteriorly infected with *L. major* parasites were reproducibly less infected than the controls (an 86% decrease in the number of parasites retained in the midgut after blood meal excretion which led to at least a 5-fold reduction in the frequencies of mature infection development) is a proof of principle of *Leishmania* transmission blocking vaccines that may be used, for instance (but not only), in animal reservoirs and still impact human disease incidence [137, 138].

2.3. Questions that deserve to be answered

As a connecting point between the current and subsequent sections, we raise some questions for which we still do not have a clear answer today. The first one is if the development of a pan-*Leishmania* vaccine *sensum latum* (both prophylactic and therapeutic; for endemic and non-endemic individuals; against all disease forms) is something over-ambitious. And such a question makes sense, not only because of the time and investment that are expected to be involved—for the case of leishmaniasis, the non-existence of prophylactic agents implies the "faster is better" motto. For instance, in our recent work, we show that the pre-administration of a salivary antigen, followed by a boost with the same salivary antigen together with two other parasite-derived proteins, has a direct impact in the immunogenicity of the latest [96], which may suggest that vaccines for endemic individuals may not work equally in non-endemic ones and *vice versa*. This point is particularly relevant if we use vector- and parasite-derived components in the vaccines against leishmaniasis, which is related with the second question we pose: should vaccines for leishmaniasis always contemplate both parasite- and vector-derived components? Studies that show the improvement of parasite-derived vaccine candidates when co-administered with vector-derived antigens support this hypothesis [54, 64]. However, there are still some issues that have to be addressed, such as the possibility of tolerance induction, that is known to be dependent on the amount of antigen [139] (expected to be higher in a defined antigen-based vaccination approach, compared with a sand fly bite). Furthermore, another question relates to clinical research. How can we test the effectiveness of safe and immunogenic vaccine candidates? The last phase III

clinical trials were performed more than half a century ago and against the cutaneous disease. But, contrary to other deathly parasitic diseases, such as malaria [140], to perform controlled infections with *L. infantum*, *L. donovani* or even *L. braziliensis* or *L. guyanensis* would be unethical, to say the least. Therefore, such trials would have either to evaluate cross-protection to cutaneous disease (controlled infection with *L. major* that still raises ethical issues) and extrapolate results to the mucocutaneous/visceral forms or be designed and conducted directly as phase IV clinical trials (although to use a placebo in this context would probably also not be admissible).

3. Vaccines for human leishmaniasis: what is still missing?

So far, and consciously, we described the different vaccine candidates explored till the present days as vaccines against leishmaniasis, highlighting only their effectiveness in a qualitative way (effective/non-effective, promising or not) and not discussing the immune mechanisms linked to those results: first, because **Table 1** contemplates vaccine candidates developed for the different leishmaniasis forms, whose pathogenic mechanisms are distinct (and not completely understood) [141] and additionally, because the correlates of protection (that may also be distinct, depending on the disease form) are still far from being well established (they are neither consensual nor definitive). Such facts may have different justifications, as (i) we are still missing key insights concerning vector-parasite-host interactions (both in disease and in health states); (ii) the translation value of the animal models used is limited; or (iii) the models used are not adequate.

3.1. From "mice to man": the issue of animal models, correlates of protection, and translation

Being *Leishmania* parasites obligatory intracellular pathogens (in the mammalian host), it is not surprising that humoral-based responses will be less important than cellular-based ones. Indeed, in animal models of VL, the absence of B-cells contributed to decreased susceptibility to infection [142, 143]. Additionally, it has been shown that antibody-opsonized parasites are more efficiently taken by phagocytes that will become "permissive hosts" due to the high IL-10 and low IL-12 secretion phenotype induced by antibody Fc-receptor (FcγR) interactions [144–147]. Importantly, one of the hallmarks of human disease, is hypergammaglobulinemia (that correlates with disease severity), resultant from a polyclonal B-cell activation, being consequently most of the circulating antibodies non-parasite specific [148–150]. Still, and because the development and role of humoral responses in leishmaniasis is controversial and not completely understood, they may be important [151, 152]. For instance, the type and functionality of the antibodies may be relevant from the standpoint of a vaccine approach, considering lytic functions [e.g., antibody-dependent cell-mediated cytotoxicity (ADCC)] or even "Th1-inducing" FcγR ligation [153]. Yet, even for the proper mounting of effective antigen-specific humoral responses, cell-mediated immunity is of paramount importance [154].

What is known today regarding cellular immune responses to *Leishmania* infection was built on top of the Th1/Th2 paradigm defined on the basis of susceptibility *versus* resistance to *L. major* infection (one of the known CL etiologic agents) [155]. Indeed, the IL-12-mediated IFN-γ production by *Leishmania*-specific $CD4^+$ T cells is essential to promote the switch on of the oxidative cell-parasiticidal machinery, important for infection control both in animal models and in human

disease [156–158] (although in mucocutaneous forms, inflammation is also the cause of pathology [159]). However, while in cutaneous disease a general correlation between Th1 *versus* Th2 responses and immunity *versus* susceptibility is observed, in VL, where the major source of the regulatory cytokine IL-10 is *Leishmania*-specific Th1 cells (Tr1), that is not observed. This mechanism of self-regulation (to prevent inflammation-mediated tissue damage) contributes to parasite persistence [160]. Yet, most of the anti-*Leishmania* vaccine studies rely on the quantification of the levels of IL-10 and IFN-γ-secreted *ex vivo* in response to either the vaccine antigen or to parasite total proteins and use the Th1/Th2 paradigm as a justification for the candidate potential. Others use multi-parametric flow cytometry (or ELISPOT) that allows the characterization of individual cell populations and the disclosure of which cytokines they are producing (most of the times after an *ex vivo* stimulation step): often IFN-γ *versus* IL-10 (individually) or more recently multi-functional T cells [161]. Still, one may claim that results based on such approaches may have a limited validity due to the artificiality of the system: (i) the type and amount of antigen used in the recall and (ii) exclusion of parasite immuno-modulatory potential. To measure directly cytokine expression in the target organ (in an efficacy pre-clinical trial), as is sometimes done in CL models, is a way to bypass the potential limitations of *ex vivo* stimulation approaches. Importantly, the correlates of protection proposed and used should always correlate well with parasite burdens. Another issue that deserves to be emphasized is the cell type(s) we need to look at. Although $CD4^+$ T cells are important in anti-*Leishmania* immunity, so are $CD8^+$ T cells (important from both therapeutic and prophylactic standpoints) that are however many times almost not accounted for in vaccine studies [162]. These cells are nowadays known to be important for resistance to *Leishmania* infection and cure, either by production of IFN-γ (that will activate the microbicidal machinery) or by secretion of cytotoxic mediators that will directly kill infected cells [70, 163, 164]. And because of this, usually, the secretion/expression of IFN-γ or granzyme-B by *ex vivo*-recalled $CD8^+$ cells is used to qualify their responsiveness and considered as potential correlates of protection. Having in consideration what was referred above for $CD4^+$ T cells, an additional problem of translation must be considered. It is known that human $CD8^+$ T cells (and other cytotoxic subsets) produce an antimicrobial peptide (granulysin) with direct parasite-cytotoxic effect, while murine cells do not. Curiously, the infection of a humanized mouse model (granulysin knock-in) with *T. gondi* and *T. cruzi* was less severe than in WT animals, as probably will be reproduced with *Leishmania* spp. [165]. Another important factor to be considered in vaccine effectiveness evaluation is the relevance of the local *versus* systemic responses. Although in CL models, most of the times "specific-systemic responses" are investigated (e.g., recall experiments using splenocytes), it was recently shown that *Leishmania*-specific skin-resident $CD4^+$ T cells are able to confer protection to cutaneous diseases, independently of the central/effector memory pool [166]. However, the immune response in the skin is often not accounted for. Although natural infection (independently of the disease form) always begins by the deposition of parasites in the host dermis (excluding vertical transmission and accidental "human-made" infections resulting from, e.g., blood transfusions), most of the animal models used today in vaccine studies, particularly if we consider VL, completely bypass the skin (controlled infections are performed most of the times either intravenously or intraperitoneally). Therefore, in one way, we may be losing information on the contribution of skin immunity to the protective potential of a given vaccine candidate but on the other hand we may be "overloading the system" and induce responses quite different than the normal ones (too many parasites = excessive inflammation or immuno-modulation)

[167]. Additionally, most of the times in experimental infections, and in this case not only in VL models, the vector is completely disregarded. Importantly, vector saliva was shown to exacerbate infection in different disease models [126, 168, 169]. Also, we have to consider the vector microbiome as a potential infection modulator, as it has been hypothesized [170]. Additionally, parasite-excreted-secreted virulence factors (e.g., promastigote secretory gel and extracellular vesicles/exosomes), or death parasites, all expected to be part of the natural infectious inoculum, were also shown to promote infection [171–173]. Probably, one or the combination of all of these components was the factor responsible for the data published by Peters et al. [174] that have shown the loss of efficacy of the ALM vaccine candidate when tested in the sand fly *versus* needle challenge contexts ("reproducing" the results obtained in the clinical trials of a similar vaccine candidate—ALM + BCG). All of the above discussed point to the use of pre-clinical models to test vaccines that should be as close to what is observed in nature (bearing in mind that even a laboratory-based sand fly transmission model will not be indistinguishable from the natural one, considering the expected differences in the microbiomes [175] and the heterogeneity in vectorial capacities [176]. To improve the chances of translatability (even if the correlates of protection were concrete, the use of an inadequate model would "invalidate" the results), the minimum requirements of vaccine development pre-clinical infection model should be the co-inoculation of parasites together with vector saliva (particularly if the vaccine candidate consists [partially] of vector-derived antigens) in the host dermis, naturally or artificially, by needle injection. No model is perfect and pre-clinical investigation shall ever replace clinical research. However, the system simplification, which is generally used in scientific research as a way to eliminate noise, can also be the reason of loss of translatability. Most of the models used in vaccine development studies have a defined and identical genetic background—they are inbred [177]. Interestingly, vaccine candidates show contradictory results concerning efficacy, depending on the inbred murine model used [178]. We need to have in mind that humans, the target population of the vaccines, are quite a heterogeneous population, with more than 7000 HLA alleles identified so far to which we have to add heterozygosity favored by natural selection [179]. To address this issue, we can start by the vaccine engineering phase that should be more and more rational (using reverse vaccinology approaches [180]) and predict the immunogenicity in different human populations, as a proof of principle that is expected to be validated first pre-clinically and then clinically.

In respect of these three subjects (correlates of protection, animal models, and translatability of pre-clinical studies to humans) that have major overlaps and cannot be separated, there are still too many shades of gray to account for. As a way to eliminate the fogginess, it will be important to identify the divergent and common points of many anti-*Leishmania* vaccine pre-clinical and/or translational studies performed so far. The field would gain a lot from the elaboration and publication of bibliographic statistic studies such as meta-analysis or systematic reviews. Additionally, as suggested by Gannavaram and colleagues, the leishmaniasis research field needs to turn to more complex approaches, such as systems vaccinology, to be able to answer the questions that the community posed a while ago but still remain *quasi*-unanswered [181].

3.2. From "man to mice": the insufficiency of prospective studies

Leishmaniasis animal models have been undoubtedly an extremely useful tool to understand better the host–parasite interactions that influence either resistance to infection or disease

development [157, 182]. This is true for both cutaneous and visceral diseases, although much more relevant in the latest. It would be both unethical and dangerous to biopsy diseased individuals spleen, liver, or bone marrow (target organs of the viscerotropic *L. infantum* and *L. donovani*) just to better understand the infectious process. However, an animal model, even when it combines both conceptual and facial validities, is still just a model; in other words, translation to human health and disease may not always be achievable. In other diseases, prospective studies in human populations have produced valuable information not only from epidemiological and pathological standpoints but also applicable to the vaccine development field [183, 184]. On the other way, till the present days, most of the prospective studies performed in leishmaniasis had an epidemiological character (as invaluable in what respects the common goal of the community, which is disease control) [24, 44]. The development of such studies, focusing on systemic immune responses (particularly cellular based), would be of paramount importance to better understand both disease and resistance in leishmaniasis. For that, there are two target populations that deserve to be studied longitudinally: cured individuals and asymptomatics. While the following of the first population would help to answer the questions related to long-lasting immunity, the following of the second would help to define the potential host factors that determine susceptibility *versus* resistance. Yet, we have to consider as a possible limitation of studies with asymptomatics the less-than-clear and consensual definition of these individuals [24]. Nevertheless, the information generated by such studies would be then possibly "translated back" to animal models, used to better define the correlates of protection to improve vaccine design.

4. Conclusion

Today we still do not have a vaccine approved for human leishmaniasis (regardless of the disease form). Many candidates were tested in the last century, and up to nowadays only vector-derived vaccines were not tested in the clinical context; for all the other parasite-derived candidates, regardless of the vaccine generation they are part of, we have proof of principle of at least immunogenicity and safety (in human healthy individuals) and therefore a precedent is open. Yet, the efficacy clinical trials performed so far (the last more than 50 years ago), excluding leishmanization, were overall disappointing. Such information is however as valuable as any positive result and should be used from a perspective of "learning from our mistakes." There are still many questions to be answered in the anti-*Leishmania* vaccine development field, such as which parameters should be used as correlates of protection and how we should test our vaccine candidates in a way that warrants translation to the clinical context. Additionally, to define the vaccine effectiveness in the clinical context in a controlled way is essential. To address all of these issues, the vaccine development should be more and more rationale based, taking advantage of the modern and of the ancient. Observational studies of target human populations associated with systems biology may for instance help once and for all to disclose the health *versus* disease determinants and contribute to the final establishment of flawless correlates of protection. Additionally, immuno-informatic tools may help to design or refine (through a reverse vaccinology approach) the future vaccine(s) for human leishmaniasis.

Acknowledgements

This work was funded by the project NORTE-01-0145-FEDER-000012, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). Additionally, this work was funded in part by the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases. PC was supported by Foundation for Science and Technology (FCT), Portugal, through the individual grant SFRH/BD/121252/2016.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author details

Pedro Cecílio[1,2,3], Fabiano Oliveira[4] and Anabela Cordeiro da Silva[1,2,3]*

*Address all correspondence to: cordeiro@ibmc.up.pt

1 Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

2 Parasite Disease Group, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

3 Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal

4 Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, NIAID, NIH, Rockville, MD, USA

References

[1] CDC on Infectious Diseases in the United States: 1900-99. Population and Development Review. 1999;**25**(3):635-640

[2] Bonanni P. Demographic impact of vaccination: A review. Vaccine. 1999;**17**(Suppl 3): S120-S125

[3] Rappuoli R, Pizza M, Del Giudice G, De Gregorio E. Vaccines, new opportunities for a new society. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(34):12288-12293

[4] UNICEF. The progress of nations 1996—Health: Vaccines bring 7 diseases under control. Available from: https://www.unicef.org/pon96/hevaccin.htm [Accessed November 16, 2017]

[5] WHO. Immunization Coverage—Fact Sheet 2017. Available from: http://www.who.int/mediacentre/factsheets/fs378/en/ [Accessed November 16, 2017]

[6] Centlivre M, Combadiere B. New challenges in modern vaccinology. BMC Immunology. 2015;**16**:18

[7] Delany I, Rappuoli R, De Gregorio E. Vaccines for the 21st century. EMBO Molecular Medicine. 2014;**6**(6):708-720

[8] WHO. Immuniaztion, Vaccines and Biologicals—Vaccines and Diseases. Available from: http://www.who.int/immunization/diseases/en/ [Accessed November 16, 2017]

[9] Junod S. FDA and clinical drug trials: A short history. In: Davies/Keriman, Editor. A Quick Guide to Clinical Trials. Washington: Bioplan, Inc. 2008; pp. 25-55

[10] Bloom DE, Black S, Rappuoli R. Emerging infectious diseases: A proactive approach. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**(16):4055-4059

[11] Maslow JN. Vaccines for emerging infectious diseases: Lessons from MERS coronavirus and Zika virus. Human Vaccines and Immunotherapeutics. 2017;**13**(12):2918-2930

[12] Nabel GJ. Designing tomorrow's vaccines. The New England Journal of Medicine. 2013;**368**(6):551-560

[13] Griffiths KL, Khader SA. Novel vaccine approaches for protection against intracellular pathogens. Current Opinion in Immunology. 2014;**28**:58-63

[14] Robinson HL, Amara RR. T cell vaccines for microbial infections. Nature Medicine. 2005;**11**(4 Suppl):S25-S32

[15] Servin-Blanco R, Zamora-Alvarado R, Gevorkian G, Manoutcharian K. Antigenic variability: Obstacles on the road to vaccines against traditionally difficult targets. Human Vaccines and Immunotherapeutics. 2016;**12**(10):2640-2648

[16] Bethony JM, Cole RN, Guo X, Kamhawi S, Lightowlers MW, Loukas A, et al. Vaccines to combat the neglected tropical diseases. Immunological Reviews. 2011;**239**(1):237-270

[17] Chirac P, Torreele E. Global framework on essential health R&D. Lancet. 2006;**367** (9522):1560-1561

[18] di Procolo P, Jommi C. Current pipelines for neglected diseases. PLoS Neglected Tropical Diseases. 2014;**8**(9):e3092

[19] UN. Sustainable Development Knowledge Platform—Sustainable Development Goals. Available from: https://sustainabledevelopment.un.org/topics/sustainabledevelopment-goals [Accessed November 17, 2017]

[20] United to Combat Neglected Tropical Diseases. Available from: http://unitingtocombatntds.org/ [Accessed November 17, 2017]

[21] Cecilio P, Perez-Cabezas B, Santarem N, Maciel J, Rodrigues V, Cordeiro da Silva A. Deception and manipulation: The arms of leishmania, a successful parasite. Frontiers in Immunology. 2014;**5**:480

[22] WHO. Global Health Observatory data: Leishmaniasis—Situation and trends: Global Health Observatory. Available from: http://www.who.int/gho/neglected_diseases/leishmaniasis/en/ [Accessed November 17, 2017]

[23] Akhoundi M, Kuhls K, Cannet A, Votypka J, Marty P, Delaunay P, et al. A historical overview of the classification, evolution, and dispersion of Leishmania parasites and sandflies. PLoS Neglected Tropical Diseases. 2016;**10**(3):e0004349

[24] Singh OP, Hasker E, Sacks D, Boelaert M, Sundar S. Asymptomatic Leishmania infection: A new challenge for Leishmania control. Clinical Infectious Diseases. 2014;**58**(10):1424-1429

[25] Kamhawi S. The yin and yang of leishmaniasis control. PLoS Neglected Tropical Diseases. 2017;**11**(4):e0005529

[26] WHO. Control of the leishmaniases—Technical report series 949. Available from: http://apps.who.int/iris/bitstream/10665/44412/1/WHO_TRS_949_eng.pdf [Accessed December 21, 2017]

[27] Steverding D. The history of leishmaniasis. Parasites and Vectors. 2017;**10**(1):82

[28] Novo SP, Leles D, Bianucci R, Araujo A. The process of leishmania infection—Disease and new perspectives of paleoparasitology. Revista do Instituto de Medicina Tropical de São Paulo. 2016;**58**:45

[29] Brimnes N. Variolation, vaccination and popular resistance in early colonial South India. Medical History. 2004;**48**(2):199-228

[30] Marzinowsky EI, Schurenkova A. Oriental sore and immunity against it. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1924;**18**:67-69

[31] Senekji HA, Beattie CP. Artificial infection and immunization of man with cultures of Leishmania tropica. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1941;**34**:415-419

[32] Nadim A, Javadian E, Tahvildar-Bidruni G, Ghorbani M. Effectiveness of leishmanization in the control of cutaneous leishmaniasis. Bulletin de la Société de Pathologie Exotique. 1983;**76**(4):377-383

[33] Okwor I, Uzonna J. Social and economic burden of human Leishmaniasis. The American Journal of Tropical Medicine and Hygiene. 2016;**94**(3):489-493

[34] Khamesipour A, Dowlati Y, Asilian A, Hashemi-Fesharki R, Javadi A, Noazin S, et al. Leishmanization: Use of an old method for evaluation of candidate vaccines against leishmaniasis. Vaccine. 2005;**23**(28):3642-3648

[35] McDowell MA, Rafati S, editors. Neglected Tropical Diseases—Middle East and North Africa. Wien: Springer; 2014

[36] Mutiso JM, Macharia JC, Kiio MN, Ichagichu JM, Rikoi H, Gicheru MM. Development of Leishmania vaccines: Predicting the future from past and present experience. Journal of Biomedical Research. 2013;**27**(2):85-102

[37] Kumar R, Engwerda C. Vaccines to prevent leishmaniasis. Clinical and Translational Immunology. 2014;**3**(3):e13

[38] McCall LI, Zhang WW, Ranasinghe S, Matlashewski G. Leishmanization revisited: Immunization with a naturally attenuated cutaneous *Leishmania donovani* isolate from Sri Lanka protects against visceral leishmaniasis. Vaccine. 2013;**31**(10):1420-1425

[39] Khamesipour A, Abbasi A, Firooz A, Mohammadi AM, Eskandari SE, Jaafari MR. Treatment of cutaneous lesion of 20 years' duration caused by leishmanization. Indian Journal of Dermatology. 2012;**57**(2):123-125

[40] CDC. Recommendations of the Advisory Committee on Immunization Practices (ACIP): Use of Vaccines and Immune Globulins in Persons with Altered Immunocompetence. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/00023141.htm [Accessed November 22, 2017]

[41] Harpaz R, Dahl RM, Dooling KL. Prevalence of immunosuppression among US Adults, 2013. Journal of the American Medical Association. 2016;**316**(23):2547-2548

[42] Smith J, Lipsitch M, Almond JW. Vaccine production, distribution, access, and uptake. Lancet. 2011;**378**(9789):428-438

[43] Sundar S, Singh B. Identifying vaccine targets for anti-leishmanial vaccine development. Expert Review of Vaccines. 2014;**13**(4):489-505

[44] Martinez-Valencia AJ, Daza-Rivera CF, Rosales-Chilama M, Cossio A, Casadiego Rincon EJ, Desai MM, et al. Clinical and parasitological factors in parasite persistence after treatment and clinical cure of cutaneous leishmaniasis. PLoS Neglected Tropical Diseases. 2017;**11**(7):e0005713

[45] Mendonca MG, de Brito ME, Rodrigues EH, Bandeira V, Jardim ML, Abath FG. Persistence of leishmania parasites in scars after clinical cure of American cutaneous leishmaniasis: Is there a sterile cure? The Journal of Infectious Diseases. 2004;**189**(6):1018-1023

[46] Mendonca SC. Differences in immune responses against Leishmania induced by infection and by immunization with killed parasite antigen: Implications for vaccine discovery. Parasites and Vectors. 2016;**9**:492

[47] Nation CS, Dondji B, Stryker GA. Previous exposure to a low infectious dose of *Leishmania major* exacerbates infection with *Leishmania infantum* in the susceptible BALB/c mouse. Parasitology Research. 2012;**111**(3):1407-1415

[48] Romano A, Doria NA, Mendez J, Sacks DL, Peters NC. Cutaneous infection with *Leishmania major* mediates heterologous protection against visceral infection with *Leishmania infantum*. Journal of Immunology. 2015;**195**(8):3816-3827

[49] Streit JA, Recker TJ, Filho FG, Beverley SM, Wilson ME. Protective immunity against the protozoan *Leishmania chagasi* is induced by subclinical cutaneous infection with virulent but not avirulent organisms. Journal of Immunology. 2001;**166**(3):1921-1929

[50] Das A, Ali N. Vaccine prospects of killed but metabolically active Leishmania against visceral leishmaniasis. Expert Review of Vaccines. 2012;**11**(7):783-785

[51] Jain K, Jain NK. Vaccines for visceral leishmaniasis: A review. Journal of Immunological Methods. 2015;**422**:1-12

[52] Saljoughian N, Taheri T, Rafati S. Live vaccination tactics: Possible approaches for controlling visceral leishmaniasis. Frontiers in Immunology. 2014;**5**:134

[53] Anand S, Madhubala R. Genetically engineered ascorbic acid-deficient live mutants of *Leishmania donovani* induce long lasting protective immunity against visceral Leishmaniasis. Scientific Reports. 2015;**5**:10706

[54] Fiuza JA, Gannavaram S, Santiago Hda C, Selvapandiyan A, Souza DM, Passos LS, et al. Vaccination using live attenuated *Leishmania donovani* centrin deleted parasites induces protection in dogs against *Leishmania infantum*. Vaccine. 2015;**33**(2):280-288

[55] Joshi S, Rawat K, Yadav NK, Kumar V, Siddiqi MI, Dube A. Visceral Leishmaniasis: Advancements in vaccine development via classical and molecular approaches. Frontiers in Immunology. 2014;**5**:380

[56] Silvestre R, Cordeiro-Da-Silva A, Santarem N, Vergnes B, Sereno D, Ouaissi A. SIR2-deficient *Leishmania infantum* induces a defined IFN-gamma/IL-10 pattern that correlates with protection. Journal of Immunology. 2007;**179**(5):3161-3170

[57] Davoudi N, Khamesipour A, Mahboudi F, McMaster WR. A dual drug sensitive *L. major* induces protection without lesion in C57BL/6 mice. PLoS Neglected Tropical Diseases. 2014;**8**(5):e2785

[58] Kumari S, Samant M, Khare P, Misra P, Dutta S, Kolli BK, et al. Photodynamic vaccination of hamsters with inducible suicidal mutants of *Leishmania amazonensis* elicits immunity against visceral leishmaniasis. European Journal of Immunology. 2009;**39**(1):178-191

[59] Spath GF, Lye LF, Segawa H, Turco SJ, Beverley SM. Identification of a compensatory mutant (lpg2-REV) of *Leishmania major* able to survive as amastigotes within macrophages without LPG2-dependent glycoconjugates and its significance to virulence and immunization strategies. Infection and Immunity. 2004;**72**(6):3622-3627

[60] Raymond F, Boisvert S, Roy G, Ritt JF, Legare D, Isnard A, et al. Genome sequencing of the lizard parasite *Leishmania tarentolae* reveals loss of genes associated to the intracellular stage of human pathogenic species. Nucleic Acids Research. 2012;**40**(3):1131-1147

[61] Pirdel L, Farajnia S. A non-pathogenic recombinant Leishmania expressing Lipophosphoglycan 3 against experimental infection with *Leishmania infantum*. Scandinavian Journal of Immunology. 2017;**86**(1):15-22

[62] Shahbazi M, Zahedifard F, Taheri T, Taslimi Y, Jamshidi S, Shirian S, et al. Evaluation of live recombinant nonpathogenic *Leishmania tarentolae* expressing cysteine proteinase and A2 genes as a candidate vaccine against experimental canine visceral Leishmaniasis. PLoS One. 2015;**10**(7):e0132794

[63] FDA. Vaccines Licensed for Use in the United States. Available from: https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm [Accessed: November 27, 2017]

[64] Giunchetti RC, Correa-Oliveira R, Martins-Filho OA, Teixeira-Carvalho A, Roatt BM, de Oliveira Aguiar-Soares RD, et al. A killed Leishmania vaccine with sand fly saliva extract and saponin adjuvant displays immunogenicity in dogs. Vaccine. 2008;**26**(5):623-638

[65] Petrovsky N, Aguilar JC. Vaccine adjuvants: Current state and future trends. Immunology and Cell Biology. 2004;**82**(5):488-496

[66] Noazin S, Modabber F, Khamesipour A, Smith PG, Moulton LH, Nasseri K, et al. First generation leishmaniasis vaccines: A review of field efficacy trials. Vaccine. 2008;**26**(52):6759-6767

[67] Gradoni L. Canine Leishmania vaccines: Still a long way to go. Veterinary Parasitology. 2015;**208**(1-2):94-100

[68] Okwor I, Mou Z, Liu D, Uzonna J. Protective immunity and vaccination against cutaneous leishmaniasis. Frontiers in Immunology. 2012;**3**:128

[69] Soudi S, Hosseini AZ, Hashemi SM. Co-administration of rectal BCG and autoclaved *Leishmania major* induce protection in susceptible BALB/c mice. Parasite Immunology. 2011;**33**(10):561-571

[70] Nateghi Rostami M, Keshavarz H, Khamesipour A. Immune response of BALB/c mice against an experimental vaccine of alum precipitated autoclaved *Leishmania major* (alum-ALM) mixed with BCG or mycobacterium vaccae. Tropical Biomedicine. 2010;**27**(1):89-102

[71] Nagill R, Mahajan R, Sharma M, Kaur S. Induction of cellular and humoral responses by autoclaved and heat-killed antigen of *Leishmania donovani* in experimental visceral leishmaniasis. Parasitology International. 2009;**58**(4):359-366

[72] Gholami E, Zahedifard F, Rafati S. Delivery systems for Leishmania vaccine development. Expert Review of Vaccines. 2016;**15**(7):879-895

[73] Ravindran R, Bhowmick S, Das A, Ali N. Comparison of BCG, MPL and cationic liposome adjuvant systems in leishmanial antigen vaccine formulations against murine visceral leishmaniasis. BMC Microbiology. 2010;**10**:181

[74] Carneiro MB, de Andrade e Sousa LM, Vaz LG, dos Santos LM, Vilela L, de Souza CC, et al. short-term protection conferred by Leishvacin(R) against experimental *Leishmania amazonensis* infection in C57BL/6 mice. Parasitology International. 2014;**63**(6):826-834

[75] Velez ID, Gilchrist K, Arbelaez MP, Rojas CA, Puerta JA, Antunes CM, et al. Failure of a killed *Leishmania amazonensis* vaccine against American cutaneous leishmaniasis in Colombia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2005;**99**(8):593-598

[76] Saraiva EM, de Figueiredo Barbosa A, Santos FN, Borja-Cabrera GP, Nico D, Souza LO, et al. The FML-vaccine (Leishmune) against canine visceral leishmaniasis: A transmission blocking vaccine. Vaccine. 2006;**24**(13):2423-2431

[77] Oliva G, Nieto J, Foglia Manzillo V, Cappiello S, Fiorentino E, Di Muccio T, et al. A randomised, double-blind, controlled efficacy trial of the LiESP/QA-21 vaccine in naive

dogs exposed to two *Leishmania infantum* transmission seasons. PLoS Neglected Tropical Diseases. 2014;**8**(10):e3213

[78] Petitdidier E, Pagniez J, Papierok G, Vincendeau P, Lemesre JL, Bras-Goncalves R. Recombinant forms of *Leishmania amazonensis* excreted/secreted promastigote surface antigen (PSA) induce protective immune responses in dogs. PLoS Neglected Tropical Diseases. 2016;**10**(5):e0004614

[79] Khamesipour A. Therapeutic vaccines for leishmaniasis. Expert Opinion on Biological Therapy. 2014;**14**(11):1641-1649

[80] WHO. Biologicals & Vaccines: Hepatitis B. Available from: http://www.who.int/biologicals/vaccines/Hepatitis_B/en/ [Accessed: November 30, 2017]

[81] Orr MT, Ireton GC, Beebe EA, Huang PW, Reese VA, Argilla D, et al. Immune subdominant antigens as vaccine candidates against *Mycobacterium tuberculosis*. Journal of Immunology. 2014;**193**(6):2911-2918

[82] Blair DA, Turner DL, Bose TO, Pham QM, Bouchard KR, Williams KJ, et al. Duration of antigen availability influences the expansion and memory differentiation of T cells. Journal of Immunology. 2011;**187**(5):2310-2321

[83] Jenkins N, Murphy L, Tyther R. Post-translational modifications of recombinant proteins: Significance for biopharmaceuticals. Molecular Biotechnology. 2008;**39**(2):113-118

[84] Grimaldi G Jr, Teva A, Porrozzi R, Pinto MA, Marchevsky RS, Rocha MG, et al. Clinical and parasitological protection in a *Leishmania infantum*-macaque model vaccinated with adenovirus and the recombinant A2 antigen. PLoS Neglected Tropical Diseases. 2014;**8**(6):e2853

[85] Kedzierski L. Leishmaniasis vaccine: Where are we today? Journal of Global Infectious Diseases. 2010;**2**(2):177-185

[86] Okwor I, Uzonna J. Vaccines and vaccination strategies against human cutaneous leishmaniasis. Human Vaccines. 2009;**5**(5):291-301

[87] de Mendonca SC, Cysne-Finkelstein L, Matos DC. Kinetoplastid membrane Protein-11 as a vaccine candidate and a virulence factor in Leishmania. Frontiers in Immunology. 2015;**6**:524

[88] Dias DS, Ribeiro PAF, Martins VT, Lage DP, Ramos FF, Dias ALT, et al. Recombinant prohibitin protein of *Leishmania infantum* acts as a vaccine candidate and diagnostic marker against visceral leishmaniasis. Cellular Immunology. 2017;**323**:59-69

[89] Duarte MC, Lage DP, Martins VT, Costa LE, Carvalho AM, Ludolf F, et al. A vaccine composed of a hypothetical protein and the eukaryotic initiation factor 5a from *Leishmania braziliensis* cross-protection against *Leishmania amazonensis* infection. Immunobiology. 2017;**222**(2):251-260

[90] Mou Z, Li J, Boussoffara T, Kishi H, Hamana H, Ezzati P, et al. Identification of broadly conserved cross-species protective Leishmania antigen and its responding CD4+ T cells. Science Translational Medicine. 2015;**7**(310):310ra167

[91] Silva AM, Tavares J, Silvestre R, Ouaissi A, Coombs GH, Cordeiro-da-Silva A. Characterization of *Leishmania infantum* thiol-dependent reductase 1 and evaluation of its potential to induce immune protection. Parasite Immunology. 2012;**34**(6):345-350

[92] Grimaldi G Jr, Teva A, Dos-Santos CB, Santos FN, Pinto ID, Fux B, et al. Field trial of efficacy of the Leish-tec(R) vaccine against canine leishmaniasis caused by *Leishmania infantum* in an endemic area with high transmission rates. PLoS One. 2017;**12**(9):e0185438

[93] Rueckert C, Guzman CA. Vaccines: From empirical development to rational design. PLoS Pathogens. 2012;**8**(11):e1003001

[94] Agallou M, Margaroni M, Athanasiou E, Toubanaki DK, Kontonikola K, Karidi K, et al. Identification of BALB/c immune markers correlated with a partial protection to *Leishmania infantum* after vaccination with a rationally designed multi-epitope cysteine protease a peptide-based Nanovaccine. PLoS Neglected Tropical Diseases. 2017;**11**(1):e0005311

[95] Bagirova M, Allahverdiyev AM, Abamor ES, Ullah I, Cosar G, Aydogdu M, et al. Overview of dendritic cell-based vaccine development for leishmaniasis. Parasite Immunology. 2016;**38**(11):651-662

[96] Cecilio P, Perez-Cabezas B, Fernandez L, Moreno J, Carrillo E, Requena JM, et al. Preclinical antigenicity studies of an innovative multivalent vaccine for human visceral leishmaniasis. PLoS Neglected Tropical Diseases. 2017;**11**(11):e0005951

[97] Coler RN, Duthie MS, Hofmeyer KA, Guderian J, Jayashankar L, Vergara J, et al. From mouse to man: Safety, immunogenicity and efficacy of a candidate leishmaniasis vaccine LEISH-F3+GLA-SE. Clinical & Translational Immunology. 2015;**4**(4):e35

[98] Duthie MS, Favila M, Hofmeyer KA, Tutterrow YL, Reed SJ, Laurance JD, et al. Strategic evaluation of vaccine candidate antigens for the prevention of visceral Leishmaniasis. Vaccine. 2016;**34**(25):2779-2786

[99] Duthie MS, Pereira L, Favila M, Hofmeyer KA, Reed SJ, Metangmo S, et al. A defined subunit vaccine that protects against vector-borne visceral leishmaniasis. npj Vaccines. 2017;**2**(1):23

[100] Goto Y, Bhatia A, Raman VS, Liang H, Mohamath R, Picone AF, et al. KSAC, the first defined polyprotein vaccine candidate for visceral leishmaniasis. Clinical and Vaccine Immunology. 2011;**18**(7):1118-1124

[101] Martins VT, Lage DP, Duarte MC, Carvalho AM, Costa LE, Mendes TA, et al. A recombinant fusion protein displaying murine and human MHC class I- and II-specific epitopes protects against *Leishmania amazonensis* infection. Cellular Immunology. 2017;**313**:32-42

[102] Letifend—product technical sheet. Available from: https://ec.europa.eu/health/documents/community-register/2016/20160420134483/anx_134483_es.pdf [Accessed December 3, 2017]

[103] Carcelen J, Iniesta V, Fernandez-Cotrina J, Serrano F, Parejo JC, Corraliza I, et al. The chimerical multi-component Q protein from Leishmania in the absence of adjuvant protects dogs against an experimental *Leishmania infantum* infection. Vaccine. 2009;**27**(43):5964-5973

[104] Chakravarty J, Kumar S, Trivedi S, Rai VK, Singh A, Ashman JA, et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+MPL-SE vaccine for use in the prevention of visceral leishmaniasis. Vaccine. 2011;**29**(19):3531-3537

[105] Coler RN, Reed SG. Second-generation vaccines against leishmaniasis. Trends in Parasitology. 2005;**21**(5):244-249

[106] Duthie MS, Raman VS, Piazza FM, Reed SG. The development and clinical evaluation of second-generation leishmaniasis vaccines. Vaccine. 2012;**30**(2):134-141

[107] Maisonneuve C, Bertholet S, Philpott DJ, De Gregorio E. Unleashing the potential of NOD- and Toll-like agonists as vaccine adjuvants. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(34):12294-12299

[108] Reed SG, Coler RN, Mondal D, Kamhawi S, Valenzuela JG. Leishmania vaccine development: Exploiting the host-vector-parasite interface. Expert Review of Vaccines. 2016;**15**(1):81-90

[109] Ferraro B, Morrow MP, Hutnick NA, Shin TH, Lucke CE, Weiner DB. Clinical applications of DNA vaccines: Current progress. Clinical Infectious Diseases. 2011;**53**(3):296-302

[110] Rosa DS, Apostólico JDS, Boscardin SB. DNA vaccines: How much have we accomplished in the last 25 years? Journal of Vaccines and Vaccination. 2015;**6**:283

[111] Amit A, Vijayamahantesh, Dikhit MR, Singh AK, Kumar V, Suman SS, et al. Immunization with *Leishmania donovani* protein disulfide isomerase DNA construct induces Th1 and Th17 dependent immune response and protection against experimental visceral leishmaniasis in Balb/c mice. Molecular Immunology. 2017;**82**:104-113

[112] Das S, Freier A, Boussoffara T, Das S, Oswald D, Losch FO, et al. Modular multiantigen T cell epitope-enriched DNA vaccine against human leishmaniasis. Science Translational Medicine. 2014;**6**(234):234ra56

[113] Dominguez-Bernal G, Horcajo P, Orden JA, Ruiz-Santa-Quiteria JA, De La Fuente R, Ordonez-Gutierrez L, et al. HisAK70: Progress towards a vaccine against different forms of leishmaniosis. Parasites and Vectors. 2015;**8**:629

[114] Kumar A, Samant M. DNA vaccine against visceral leishmaniasis: A promising approach for prevention and control. Parasite Immunology. 2016;**38**(5):273-281

[115] Maspi N, Ghaffarifar F, Sharifi Z, Dalimi A. Codelivery of DNA vaccination encoding LeIF gene and IL-12 increases protection against *Leishmania major* infection in BALB/c mice. Parasite Immunology. 2016;**38**(4):228-235

[116] Maspi N, Ghaffarifar F, Sharifi Z, Dalimi A, Dayer MS. Immunogenicity and efficacy of a bivalent DNA vaccine containing LeIF and TSA genes against murine cutaneous leishmaniasis. Acta Pathologica, Microbiologica et Immunologica Scandinavica. 2017;**125**(3):249-258

[117] Riede O, Seifert K, Oswald D, Endmann A, Hock C, Winkler A, et al. Preclinical safety and tolerability of a repeatedly administered human leishmaniasis DNA vaccine. Gene Therapy. 2015;**22**(8):628-635

[118] Seifert K, Juhls C, Salguero FJ, Croft SL. Sequential chemoimmunotherapy of experimental visceral leishmaniasis using a single low dose of liposomal amphotericin B and a novel DNA vaccine candidate. Antimicrobial Agents and Chemotherapy. 2015;**59**(9):5819-5823

[119] Cawood R, Hills T, Wong SL, Alamoudi AA, Beadle S, Fisher KD, et al. Recombinant viral vaccines for cancer. Trends in Molecular Medicine. 2012;**18**(9):564-574

[120] Hofmeyer KA, Duthie MS, Laurance JD, Favila MA, Van Hoeven N, Coler RN, et al. Optimizing immunization strategies for the induction of antigen-specific CD4 and CD8 T cell responses for protection against intracellular parasites. Clinical and Vaccine Immunology. 2016;**23**(9):785-794

[121] Kedzierska K, Curtis JM, Valkenburg SA, Hatton LA, Kiu H, Doherty PC, et al. Induction of protective CD4+ T cell-mediated immunity by a Leishmania peptide delivered in recombinant influenza viruses. PLoS One. 2012;**7**(3):e33161

[122] Mortazavidehkordi N, Farjadfar A, Khanahmad H, Ghayour Najafabadi Z, Hashemi N, Fallah A, et al. Evaluation of a novel lentiviral vaccine expressing KMP11-HASPB fusion protein against *Leishmania infantum* in BALB/c mice. Parasite Immunology. 2016;**38**(11):670-677

[123] Resende DM, Caetano BC, Dutra MS, Penido ML, Abrantes CF, Verly RM, et al. Epitope mapping and protective immunity elicited by adenovirus expressing the *Leishmania amastigote* specific A2 antigen: Correlation with IFN-gamma and cytolytic activity by CD8+ T cells. Vaccine. 2008;**26**(35):4585-4593

[124] Osman M, Mistry A, Keding A, Gabe R, Cook E, Forrester S, et al. A third generation vaccine for human visceral leishmaniasis and post kala azar dermal leishmaniasis: First-in-human trial of ChAd63-KH. PLoS Neglected Tropical Diseases. 2017;**11**(5):e0005527

[125] Soussi N, Milon G, Colle JH, Mougneau E, Glaichenhaus N, Goossens PL. Listeria monocytogenes as a short-lived delivery system for the induction of type 1 cell-mediated immunity against the p36/LACK antigen of *Leishmania major*. Infection and Immunity. 2000;**68**(3):1498-1506

[126] Belkaid Y, Kamhawi S, Modi G, Valenzuela J, Noben-Trauth N, Rowton E, et al. Development of a natural model of cutaneous leishmaniasis: Powerful effects of vector saliva and saliva preexposure on the long-term outcome of *Leishmania major* infection in the mouse ear dermis. The Journal of Experimental Medicine. 1998;**188**(10):1941-1953

[127] Kamhawi S, Belkaid Y, Modi G, Rowton E, Sacks D. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. Science. 2000;**290**(5495):1351-1354

[128] Oliveira F, Traore B, Gomes R, Faye O, Gilmore DC, Keita S, et al. Delayed-type hypersensitivity to sand fly saliva in humans from a leishmaniasis-endemic area of Mali is Th1-mediated and persists to midlife. The Journal of Investigative Dermatology. 2013;**133**(2):452-459

[129] Abi Abdallah DS, Pavinski Bitar A, Oliveira F, Meneses C, Park JJ, Mendez S, et al. A listeria monocytogenes-based vaccine that secretes sand fly salivary protein LJM11

confers long-term protection against vector-transmitted *Leishmania major*. Infection and Immunity. 2014;**82**(7):2736-2745

[130] Gomes R, Oliveira F, Teixeira C, Meneses C, Gilmore DC, Elnaiem DE, et al. Immunity to sand fly salivary protein LJM11 modulates host response to vector-transmitted leishmania conferring ulcer-free protection. The Journal of Investigative Dermatology. 2012;**132**(12):2735-2743

[131] Gomes R, Teixeira C, Teixeira MJ, Oliveira F, Menezes MJ, Silva C, et al. Immunity to a salivary protein of a sand fly vector protects against the fatal outcome of visceral leishmaniasis in a hamster model. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(22):7845-7850

[132] Kamhawi S, Aslan H, Valenzuela JG. Vector saliva in vaccines for visceral leishmaniasis: A brief encounter of high consequence? Frontiers in Public Health. 2014;**2**:99

[133] Katebi A, Gholami E, Taheri T, Zahedifard F, Habibzadeh S, Taslimi Y, et al. *Leishmania tarentolae* secreting the sand fly salivary antigen PpSP15 confers protection against *Leishmania major* infection in a susceptible BALB/c mice model. Molecular Immunology. 2015;**67**(2 Pt B):501-511

[134] Oliveira F, Lawyer PG, Kamhawi S, Valenzuela JG. Immunity to distinct sand fly salivary proteins primes the anti-Leishmania immune response towards protection or exacerbation of disease. PLoS Neglected Tropical Diseases. 2008;**2**(4):e226

[135] Oliveira F, Rowton E, Aslan H, Gomes R, Castrovinci PA, Alvarenga PH, et al. A sand fly salivary protein vaccine shows efficacy against vector-transmitted cutaneous leishmaniasis in nonhuman primates. Science Translational Medicine. 2015;**7**(290):290ra90

[136] Tavares NM, Silva RA, Costa DJ, Pitombo MA, Fukutani KF, Miranda JC, et al. Lutzomyia longipalpis saliva or salivary protein LJM19 protects against *Leishmania braziliensis* and the saliva of its vector, Lutzomyia intermedia. PLoS Neglected Tropical Diseases. 2011;**5**(5):e1169

[137] Coutinho-Abreu IV, Ramalho-Ortigao M. Transmission blocking vaccines to control insect-borne diseases: A review. Memórias do Instituto Oswaldo Cruz. 2010;**105**(1):1-12

[138] Kamhawi S, Ramalho-Ortigao M, Pham VM, Kumar S, Lawyer PG, Turco SJ, et al. A role for insect galectins in parasite survival. Cell. 2004;**119**(3):329-341

[139] Starzl TE, Zinkernagel RM. Antigen localization and migration in immunity and tolerance. The New England Journal of Medicine. 1998;**339**(26):1905-1913

[140] Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nature Reviews. Immunology. 2011;**11**(1):57-64

[141] Colmenares M, Kar S, Goldsmith-Pestana K, McMahon-Pratt D. Mechanisms of pathogenesis: Differences amongst Leishmania species. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2002;**96**(Suppl 1):S3-S7

[142] Deak E, Jayakumar A, Cho KW, Goldsmith-Pestana K, Dondji B, Lambris JD, et al. Murine visceral leishmaniasis: IgM and polyclonal B-cell activation lead to disease exacerbation. European Journal of Immunology. 2010;**40**(5):1355-1368

[143] Smelt SC, Cotterell SE, Engwerda CR, Kaye PM. B cell-deficient mice are highly resistant to *Leishmania donovani* infection, but develop neutrophil-mediated tissue pathology. Journal of Immunology. 2000;**164**(7):3681-3688

[144] Buxbaum LU. A detrimental role for IgG and FcgammaR in *Leishmania mexicana* infection. Immunologic Research. 2008;**42**(1-3):197-209

[145] Hurrell BP, Beaumann M, Heyde S, Regli IB, Muller AJ, Tacchini-Cottier F. Frontline science: *Leishmania mexicana* amastigotes can replicate within neutrophils. Journal of Leukocyte Biology. 2017;**102**(5):1187-1198

[146] Miles SA, Conrad SM, Alves RG, Jeronimo SM, Mosser DM. A role for IgG immune complexes during infection with the intracellular pathogen Leishmania. The Journal of Experimental Medicine. 2005;**201**(5):747-754

[147] Thomas BN, Buxbaum LU. FcgammaRIII mediates immunoglobulin G-induced interleukin-10 and is required for chronic *Leishmania mexicana* lesions. Infection and Immunity. 2008;**76**(2):623-631

[148] Cooper GR, Rein CR, Beard JW. Electrophoretic analysis of kala-azar human serum; hypergammaglobulinemia associated with seronegative reactions for syphilis. Proceedings of the Society for Experimental Biology and Medicine. 1946;**61**:179-183

[149] Evans TG, Krug EC, Wilson ME, Vasconcelos AW, de Alencar JE, Pearson RD. Evaluation of antibody responses in American visceral leishmaniasis by ELISA and immunoblot. Memórias do Instituto Oswaldo Cruz. 1989;**84**(2):157-166

[150] Ryan JR, Smithyman AM, Rajasekariah GH, Hochberg L, Stiteler JM, Martin SK. Enzyme-linked immunosorbent assay based on soluble promastigote antigen detects immunoglobulin M (IgM) and IgG antibodies in sera from cases of visceral and cutaneous leishmaniasis. Journal of Clinical Microbiology. 2002;**40**(3):1037-1043

[151] Gardinassi LG, Dotz V, Hipgrave Ederveen A, de Almeida RP, Nery Costa CH, Costa DL, et al. Clinical severity of visceral leishmaniasis is associated with changes in immunoglobulin g fc N-glycosylation. MBio. 2014;**5**(6):e01844

[152] Rodrigues V, Cordeiro-da-Silva A, Laforge M, Silvestre R, Estaquier J. Regulation of immunity during visceral Leishmania infection. Parasites and Vectors. 2016;**9**:118

[153] Arnold KB, Chung AW. Prospects from systems serology research. Immunology. 2017; **153**(3):279-289

[154] Sarkander J, Hojyo S, Tokoyoda K. Vaccination to gain humoral immune memory. Clinical and Translational Immunology. 2016;**5**(12):e120

[155] Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to *Leishmania major* in mice. Nature Reviews. Immunology. 2002;**2**(11):845-858

[156] Murray HW. Tissue granuloma structure-function in experimental visceral leishmaniasis. International Journal of Experimental Pathology. 2001;**82**(5):249-267

[157] Stanley AC, Engwerda CR. Balancing immunity and pathology in visceral leishmaniasis. Immunology and Cell Biology. 2007;**85**(2):138-147

[158] Tripathi P, Singh V, Naik S. Immune response to leishmania: Paradox rather than paradigm. FEMS Immunology and Medical Microbiology. 2007;**51**(2):229-242

[159] Silveira FT, Lainson R, Corbett CE. Clinical and immunopathological spectrum of American cutaneous leishmaniasis with special reference to the disease in Amazonian Brazil: A review. Memórias do Instituto Oswaldo Cruz. 2004;**99**(3):239-251

[160] Montes de Oca M, Kumar R, de Labastida Rivera F, Amante FH, Sheel M, Faleiro RJ, et al. Correction: Blimp-1-dependent IL-10 production by Tr1 cells regulates TNF-mediated tissue pathology. PLoS Pathogens. 2016;**12**(2):e1005460

[161] Darrah PA, Patel DT, De Luca PM, Lindsay RW, Davey DF, Flynn BJ, et al. Multifunctional TH1 cells define a correlate of vaccine-mediated protection against *Leishmania major*. Nature Medicine. 2007;**13**(7):843-850

[162] Stager S, Rafati S. CD8(+) T cells in leishmania infections: Friends or foes? Frontiers in Immunology. 2012;**3**:5

[163] Herath S, Kropf P, Muller I. Cross-talk between CD8(+) and CD4(+) T cells in experimental cutaneous leishmaniasis: CD8(+) T cells are required for optimal IFN-gamma production by CD4(+) T cells. Parasite Immunology. 2003;**25**(11-12):559-567

[164] Kaushal H, Bras-Goncalves R, Negi NS, Lemesre JL, Papierok G, Salotra P. Role of CD8(+) T cells in protection against *Leishmania donovani* infection in healed visceral Leishmaniasis individuals. BMC Infectious Diseases. 2014;**14**:653

[165] Dotiwala F, Mulik S, Polidoro RB, Ansara JA, Burleigh BA, Walch M, et al. Killer lymphocytes use granulysin, perforin and granzymes to kill intracellular parasites. Nature Medicine. 2016;**22**(2):210-216

[166] Glennie ND, Volk SW, Scott P. Skin-resident CD4+ T cells protect against *Leishmania major* by recruiting and activating inflammatory monocytes. PLoS Pathogens. 2017;**13**(4):e1006349

[167] Ribeiro-Gomes FL, Roma EH, Carneiro MB, Doria NA, Sacks DL, Peters NC. Site-dependent recruitment of inflammatory cells determines the effective dose of *Leishmania major*. Infection and Immunity. 2014;**82**(7):2713-2727

[168] Norsworthy NB, Sun J, Elnaiem D, Lanzaro G, Soong L. Sand fly saliva enhances *Leishmania amazonensis* infection by modulating interleukin-10 production. Infection and Immunity. 2004;**72**(3):1240-1247

[169] Ockenfels B, Michael E, McDowell MA. Meta-analysis of the effects of insect vector saliva on host immune responses and infection of vector-transmitted pathogens: A focus on leishmaniasis. PLoS Neglected Tropical Diseases. 2014;**8**(10):e3197

[170] Finney CA, Kamhawi S, Wasmuth JD. Does the arthropod microbiota impact the establishment of vector-borne diseases in mammalian hosts? PLoS Pathogens. 2015;**11**(4):e1004646

[171] Atayde VD, Aslan H, Townsend S, Hassani K, Kamhawi S, Olivier M. Exosome secretion by the parasitic protozoan Leishmania within the sand fly Midgut. Cell Reports. 2015;**13**(5):957-967

[172] Rogers M, Kropf P, Choi BS, Dillon R, Podinovskaia M, Bates P, et al. Proteophosphoglycans regurgitated by Leishmania-infected sand flies target the L-arginine metabolism of host macrophages to promote parasite survival. PLoS Pathogens. 2009;**5**(8):e1000555

[173] van Zandbergen G, Bollinger A, Wenzel A, Kamhawi S, Voll R, Klinger M, et al. Leishmania disease development depends on the presence of apoptotic promastigotes in the virulent inoculum. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(37):13837-13842

[174] Peters NC, Kimblin N, Secundino N, Kamhawi S, Lawyer P, Sacks DL. Vector transmission of leishmania abrogates vaccine-induced protective immunity. PLoS Pathogens. 2009;**5**(6):e1000484

[175] Gall CA, Scoles GA, Magori K, Mason KL, Brayton KA. Laboratory colonization stabilizes the naturally dynamic microbiome composition of field collected Dermacentor andersoni ticks. Microbiome. 2017;**5**(1):133

[176] Courtenay O, Peters NC, Rogers ME, Bern C. Combining epidemiology with basic biology of sand flies, parasites, and hosts to inform leishmaniasis transmission dynamics and control. PLoS Pathogens. 2017;**13**(10):e1006571

[177] Loria-Cervera EN, Andrade-Narvaez FJ. Animal models for the study of leishmaniasis immunology. Revista do Instituto de Medicina Tropical de São Paulo. 2014;**56**(1):1-11

[178] Benhnini F, Chenik M, Laouini D, Louzir H, Cazenave PA, Dellagi K. Comparative evaluation of two vaccine candidates against experimental leishmaniasis due to *Leishmania major* infection in four inbred mouse strains. Clinical and Vaccine Immunology. 2009;**16**(11):1529-1537

[179] Markov PV, Pybus OG. Evolution and diversity of the human leukocyte antigen (HLA). Evolution, Medicine, and Public Health. 2015;**2015**(1):1

[180] Kaye PM, Aebischer T. Visceral leishmaniasis: Immunology and prospects for a vaccine. Clinical Microbiology and Infection. 2011;**17**(10):1462-1470

[181] Gannavaram S, Dey R, Avishek K, Selvapandiyan A, Salotra P, Nakhasi HL. Biomarkers of safety and immune protection for genetically modified live attenuated leishmania vaccines against visceral leishmaniasis—Discovery and implications. Frontiers in Immunology. 2014;**5**:241

[182] Scott P, Novais FO. Cutaneous leishmaniasis: Immune responses in protection and pathogenesis. Nature Reviews. Immunology. 2016;**16**(9):581-592

[183] Endy TP. Human immune responses to dengue virus infection: Lessons learned from prospective cohort studies. Frontiers in Immunology. 2014;**5**:183

[184] Escolano A, Dosenovic P, Nussenzweig MC. Progress toward active or passive HIV-1 vaccination. The Journal of Experimental Medicine. 2017;**214**(1):3-16

Chapter 6

Vaccines for Visceral Leishmaniasis: Hopes and Hurdles

Eltahir Awad Gasim Khalil

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75184

Abstract

The leishmaniases are vector-borne parasitic diseases with multiple disease phenotypes that range from self-healing cutaneous ulcers to disfiguring post-kala-azar dermal leishmaniasis and fatal visceral leishmaniasis (VL). Infected individuals can develop subclinical infections or overt disease. Current treatments are toxic and expensive. The only successful control measure is case detection and drug treatment. Resistance to antileishmanial drugs are increasing with few drugs in the pipeline. The *Leishmania* parasites are good candidates for vaccine development, with no change in its antigenic coat and extensive cross-reactivity between species. First-generation vaccines are safe, immunogenic with inconclusive efficiency. These vaccines presented the leishmanin skin test (LST) as a potentially good surrogate marker of immunogenicity/protection that can help in future vaccine studies. First-generation vaccines are the only leishmaniasis vaccines that progressed to phase III. Second-generation vaccines are safe and immunogenic, but none progressed to phase III. Third-generation vaccines recently entered human testing. Alternative approaches include in silico prediction of immunogenic *Leishmania* epitopes with *in vitro* immunogenicity testing. New adjuvants can help in the quest to develop efficacious leishmaniasis vaccines. Failure of second- and third-generation vaccines to reach phase III, rising drug resistance and continued VL pandemics make it a necessity to revisit first-generation vaccines.

Keywords: visceral leishmaniasis, first/second/third-generation vaccines, adjuvants

1. Introduction

The leishmaniases are vector-borne, widely prevalent parasitic diseases that are transmitted by phlebotomine sand flies. The transmission is either zoonotic or anthroponotic. Together with malaria they constitute the most commonly prevalent neglected parasitic diseases. The leishmaniases are among the most commonly neglected tropical diseases. The parasite is a

unicellular organism that leads to multiple disease phenotypes that range from benign self-healing cutaneous ulcers to a markedly disfiguring diffuse cutaneous/post-kala-azar dermal leishmaniasis and fatal visceral leishmaniasis (VL, kala-azar). Cutaneous leishmaniasis (CL) is caused by *L. tropica*, *L. aethiopica*, and *L. major* in the Old World and *L. mexicana*, *L. guyanensis*, *L. amazonensis*, and *L. braziliensis* in the New World. Visceral leishmaniasis is a fatal form of the leishmaniasis if not treated. VL is a major health problem and is caused by *L. donovani* and *L. infantum* that are particularity prevalent in East Africa, the Indian subcontinent, Mediterranean Basin, and Latin America [1–9]. The HIV pandemic aggravated further the leishmaniasis morbidity and mortality. Drug treatment with sodium stibogluconate/paromomycin, miltefosine, or liposomal amphotericin B is expensive and carries major risks of toxicities. Current control measures that include case detection, drug treatment, and insecticide-impregnated bed nets are failing as evidenced by repeated epidemics especially in East Africa. In addition, increasing drug resistance and geographical expansion of the leishmaniases due to global warming and wars make the search for vaccines for the leishmaniases a necessity [2, 10–15].

1.1. Immunity against visceral leishmaniasis

Visceral leishmaniasis is characterized by immune suppression manifesting as pancytopenia and anergy to some antigens like *Leishmania* antigens and purified protein derivative (PPD). Following successful drug treatment, a state of immune reconstitution ensues which is characterized by a dermatosis affecting most Sudanese patients known as post-kala-azar dermal leishmaniasis (PKDL). Macrophages, $CD4^+$ T cells, $CD8^+$, NK cells, and dendritic cells are known to be involved in the immune responses against *Leishmania* infections. Infection with *L. donovani* parasite can follow two different scenarios: susceptible individuals develop overt disease with dissemination of *Leishmania* parasites through infected macrophages with secretion of IL-4 and IL-10, and nonspecific stimulation of B cells and secretion of large amounts of antileishmanial antibodies [Th_2 immune response]. Alternatively, individuals can develop protective immune responses [subclinical infection] with secretion of parasite antigen-specific IFN-γ, TNF-α by stimulated $CD4^+$ T cells [Th_1 immune response], and conversion in the leishmanin skin test (LST). Eliciting an exact immune response is an important VL vaccine requirement that should simulate those induced by natural infection. An important feature of an efficacious *Leishmania* vaccine should be to induce a parasite-specific Th_1 immune response with sufficient amounts of IFN-γ and LST reactivity that should last for life. Induction of antileishmanial antibodies by a vaccine should be taken against it, taking into consideration that patients with overt diseases secrete large amounts of non-neutralizing antibodies. These antibodies have been shown to facilitate the internalization of the *Leishmania* parasites into macrophages [2, 16–19].

1.2. Feasibility of vaccines for the leishmaniases

A vaccine against the *Leishmania* parasite is a real feasibility, because unlike the plasmodium and other parasites, *Leishmania* rarely changes its antigenic coat. *Leishmania* infections induce lifelong immunity with extensive cross-reactivity between different species of leishmania. Therefore, a single vaccine can be potentially effective against many forms of the leishmaniases.

Although the exchange of genetic material between distant *Leishmania* strains [*L. major* and *L. donovani*] has recently been raised, this may have some implications for drug treatment, but not leishmaniasis vaccine development [20, 21].

1.3. Vaccine biomarkers of immunogenicity, susceptibility, and protection

The ability of vaccines to induce antibody production, Th_1 (IFN-γ) or Th_2 (IL-4, IL-10) immune response, can be objectively measured for phase II studies. Based on published data, we believe that the leishmanin skin test (LST) can be used as a surrogate marker for induction of cell-mediated immunity/protection against visceral leishmaniasis in phase II/III studies [22–25].

1.4. The leishmanin skin test (LST)

LST is an in vivo skin test that marks *Leishmania* antigen-specific T-cell responses. The brand of LST reagent used in East Africa is an *L. major* suspension that is manufactured under GMP conditions in Pasteur Institute, Iran. The LST has been shown to be a potentially good diagnostic aide for the diagnosis of African visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) in all age groups in endemic areas. LST reactivity indicates sustained cell-mediated immunity, which is nonreactive in patients with VL and becomes reactive 6 months after cure. In addition, LST reactivity is a lifelong phenomenon [2, 11, 26]. Individuals with LST reactivity do not develop VL as was shown in a two decades follow-up period among the large numbers of LST reactive individuals in VL endemic areas in Sudan [2, 27] (Khalil et al., personal communication). Evaluation of LST reactivity in endemic areas in East Africa and India as reported previously included small sample size and did not specify the duration between cure and LST testing. Bern et al. [28] in Bangladesh demonstrated that the frequency of LST reactivity increased with increasing duration following cure using *L. infantum* antigen. The Bangladesh study mentioned loss of LST reactivity, but did not show any data about population movement that we specifically look at when evaluating LST reactivity from year to year. The questions of LST standardization, sensitivity, potency, stability of the *Leishmania* antigens, and longevity of the skin reaction were addressed satisfactory by Weigle and colleagues in 1991 [29]. Combination of different *Leishmania* strains can markedly improve the specificity of the LST as was shown previously [28–32]. In conclusion, different *Leishmania* strains in the LST reagent, the inadequate technique (subcutaneous rather than intradermal injection), and the time of test reading can greatly affect the outcome and interpretation of results of LST. LST is a potentially good surrogate marker of immunogenicity/protection that can be useful in future VL vaccine studies.

2. Leishmanization

Leishmanization is a true predecessor of leishmaniasis vaccines; the procedure was practiced in Central Asia and the Middle East for times deep in history. Although leishmanization is still practiced in some areas, it is considered unsafe and cannot be standardized. Recently, it

has been used as a method of evaluation for candidate vaccines [33]. Leishmanization gave way to killed or live-attenuated first-generation *Leishmania* vaccines. Leishmanization like first-generation and third-generation vaccines that use genetically modified *Leishmania* parasites or use bacteria and viruses that carry *Leishmania* genes is daunted by the issue of standardization [34].

3. First-generation vaccines

First-generation vaccines as whole parasite killed/attenuated were tested in animals and humans for cutaneous and visceral leishmaniases. Human studies have to be commended despite raised points of standardization and licensure purposes. First-generation vaccines are less costly and easy to manufacture. In addition, first-generation vaccines are the only human prophylactic VL vaccines that went on to phase III. Khalil and colleagues conducted the first human phase III VL vaccine study that was followed by a number of extended phase II studies on vaccines against visceral leishmaniasis [27]. Although the vaccine was not efficaciously different from BCG, important conclusions came out of this study: firstly, the leishmanin skin test (LST) is a first potentially good surrogate marker for immunogenicity/protection in humans. Secondly, modulation of whole parasite vaccines with strong adjuvants like alum markedly improved the immunogenicity of whole parasite vaccine as shown in phase II/extended phase II studies. Lack of funds under the pretense of poor standardization and lack of licensure potentials prevented progression of alum-precipitated *Leishmania* vaccines to phase III [22–25, 35–37].

The future of VL control is bleak based on frequent VL pandemics that kill thousands of people in developing countries, increasing drug resistance, lack of new antileishmanial drugs in the pipeline, and failure of second-generation vaccines to make it to phase III. In view of all the above and the current regulations that prohibit the wide use of whole parasites/antigen vaccines, standardization of whole parasite/antigens has to be addressed objectively.

Important points have to be highlighted when revisiting first-generation vaccines: the *Vaccinia* [smallpox] vacine which is the first vaccine that helped to eradicate small pox has been a whole virus. Furthermore, the control and near elimination of poliomyelitis is successful due to the blessing of an attenuated whole virus. Since the above vaccines are considered fit for human use, whole parasite vaccines have to be given a similar standing especially in the era of existing strong adjuvants. Furthermore, the success of immunochemotherapy of post-kala-azar dermal leishmaniasis using alum-precipitated autoclaved *L. major* vaccine further supports giving a second chance for first-generation VL vaccines. The inconclusive results that were obtained from first-generation vaccine meta-analysis and put it into disrepute are probably due to the fact that the analysis included studies for cutaneous as well as visceral diseases in the same basket. It has to be clearly stated that these disease phenotypes are different with different immune responses and different endpoints of evaluation of efficacy [12, 13, 23, 25, 27, 35, 36, 38–41].

4. Second-generation vaccines

Second-generation vaccines are recombinant *Leishmania* antigens (single peptides/polypeptides) that are highly purified, amenable to standardization/large-scale production, reproducibility, and cost-effective production. Safety and immunogenicity have been assured in phase I and II studies. But, it is clear that strong adjuvants are needed for these subunit vaccines to be satisfactorily immunogenic. Recently, our group tried an alternative cheaper way where an in silico approach was employed to predict immunogenic epitopes/peptides of *Leishmania* parasite antigenic coat. The predicted peptides were manufactured commercially and tested in an in vitro whole blood system and were shown to be immunogenic [IFN-γ production; no IL-10 production]. It was concluded that these peptides can be taken further for prophylactic leishmanin vaccine development. Further studies are underway to combine these peptides with known and potential adjuvants to increase their immunogenicity [42–49]. In conclusion, second-generation VL vaccines will succeed when the mechanisms by which macrophages select the most suitable epitopes to induce the appropriate immune response are known.

5. Third-generation vaccines

DNA vaccines came into existence with advances in molecular biology and biotechnology, and the injection of small circle of DNA encoding potentially immunogenic proteins became a reality [50]. A number of experimental third-generation vaccines have been studied with demonstrated immunogenicity and healing abilities. The first human study for a third-generation therapeutic vaccine for visceral leishmaniasis and PKDL was carried out on healthy volunteers in the United Kingdom using CHAd63-KH vaccine. The CHAd63-KH vaccine is a replication-defective simian adenovirus expressing a novel synthetic gene (KH) encoding two *Leishmania* proteins KMP-11 and HASPB. The vaccine was shown to be safe and immunogenic [51–53].

6. Adjuvants for *Leishmania* vaccines

A plethora of adjuvants, live organisms (BCG), cytokines, oligonucleotide (CpG), minerals and particulate lipids, and polymer-based adjuvants are under investigations for *Leishmania* vaccine. Although there is an urgent need for studies on adjuvants in disease-endemic areas, access to potent adjuvants is the main hurdle for investigators and researchers in leishmaniasis-endemic countries [54–61].

7. Conclusion

Failure of second- and third-generation vaccines to reach phase III, rising drug resistance, and continued devastating VL pandemics make it a necessity to study further first-generation vaccines.

Author details

Eltahir Awad Gasim Khalil

Address all correspondence to: eltahirk@iend.org

Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan

References

[1] Pearson RD, Sousa AQ. Clinical spectrum of Leishmaniasis. Clinical Infectious Diseases. 1996;**22**:1-13

[2] Zijlstra EE, El-Hassan AM. Leishmaniasis in Sudan. Visceral leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2001;**95**(Suppl 1):S27-S58

[3] Sacks D, Kamhawi S. Molecular aspects of parasite-vector and vector–host interactions in leishmaniasis. Annual Review of Microbiology. 2001;**55**:453-483

[4] Alvar J, Canavate C, Molina R, Moreno J, Nieto J. Canine leishmaniasis. Advances in Parasitology. 2004;**57**:1-88

[5] Desjeux P. Leishmaniasis: Current situation and new perspectives. Comparative Immunology, Microbiology and Infectious Diseases. 2004;**27**:305-318

[6] Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, et al. Visceral leishmaniasis: What are the needs for diagnosis, treatment and control? Nature Reviews. Microbiology. 2007;**5**:873-882

[7] Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. The Lancet Infectious Diseases. 2007;**7**:581-596

[8] Postigo JA. Leishmaniasis in the World Health Organization Eastern Mediterranean Region. International Journal of Antimicrobial Agents. 2010;**36**(Suppl 1):S62-S65

[9] Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;**7**:e35671

[10] Khalil EAG, Musa AM, Elgawi SH, Meshasha A, Gamar Eldawla I, Elhassan MO, Eljaleel KA, Younis BM, Elfaki MEE, El-Hassan AM. Revival of a leishmaniasis focus in White Nile state, Sudan. Annals of Tropical Medicine & Parasitology 2008;**102**(1):79-80

[11] Krolewiecki AJ, Almazan MC, Quipildor M, Juarez M, Gil JF, Espinosa M, Canabire M, Cajal SP. Reappraisal of Leishmanin Skin Test (LST) in the management of American Cutaneous Leishmaniasis: A retrospective analysis from a reference center in Argentina. PLoS Neglected Tropical Diseases. 2017;**11**(10):e0005980. DOI: 10.1371/journal.pntd.0005980

[12] Musa AM, Noazin S, Khalil EAG, Modabber F. Immunological stimulation for the treatment of leishmaniasis: A modality worthy of serious consideration. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2010;**104**(1):1-2

[13] Sundar S, Singh A. What steps can be taken to counter the increasing failure of miltefosine to treat visceral leishmaniasis? Expert Review of Anti-Infective Therapy. 2013;**11**:117-119

[14] Lindoso JAL, Cunha MA, Queiroz IT. Moreira. Leishmaniasis–HIV coinfection: Current challenges. HIV/AIDS (Auckland, N.Z.). 2016;**8**:147-156. Published online 2016 Oct 7. DOI: 10.2147/HIV.S93789

[15] Kimutai R, Musa AM, Njoroge S, Omollo R, Alves F, Hailu A, et al. Safety and effectiveness of sodium stibogluconate and paromomycin combination for the treatment of visceral Leishmaniasis in eastern Africa: Results from a Pharmacovigilance Programme. Clinical Drug Investigation. 2017;**37**:259-272

[16] Carvalho EM, Bacellar O, Barral A, Badaro R, Johnson WD. Antigen-specific immunosuppression in visceral leishmaniasis is cell mediated. The Journal of Clinical Investigation. 1989;**83**(3):860-864. DOI: 10.1172/JCI113969

[17] Rodrigues V, da Silva JS, Campos-Neto A. Transforming growth factor β and immunosuppression in experimental visceral Leishmaniasis. Infection and Immunity. 1998;**66**(3):1233-1236

[18] Mohamed SN, Khalil EAG, Musa AM, Younis BM, Omer SA, Sharief AH, EL-Hassan AM. Anti-Leishmania donovani antibodies enhance promastigotes internalization into host macrophage. Journal of Microbiology and Antimicrobials. 2012;**4**(7):110-114. Available online: http://www.academicjournals.org/JMA. DOI: 10.5897/JMA11.047

[19] Khalil EAG, Khidir SA, Musa AM, Musa BY, Elfaki MEE, Elkadaru AMY, Zijlstra EE, Mohamed El-Hassan AM. Post kala-azar dermal leishmaniasis: A paradigm of paradoxical immune reconstitution syndrome in non-HIV/AIDS patients. Journal of Tropical Medicine. 2013. 7 p. Article ID 275253. http://dx.doi.org/10.1155/2013/275253

[20] Hailu A, Musa A, Wasunna M, Balasegaram M, Yifru S, Mengistu G, et al. Geographical variation in the response of visceral leishmaniasis to paromomycin in East Africa: A multicentre, open-label, randomized trial. PLoS Neglected Tropical Diseases. 2010;**4**(10):e709

[21] Hassabelgawi SH, Musa AM, Khalil EAG, Abebe T, Younis BM, Mona EEE, AM EL-H, Hailu A, Bart A. Probable genetic hybrids between Leishmania species in Sudanese isolates. Journal of Microbiology and Antimicrobials. 2011;**3**(6):142-145

[22] Khalil EAG, El Hassan AM, Zijlstra EE, Mukhtar MM, Ghalib HW, Musa B, Ibrahim ME, Kamil AA, Elsheikh M, Babiker A, Modabber F. Autoclaved Leishmania major vaccine for prevention of visceral leishmaniasis: A randomised, double-blind, BCG-controlled trial in Sudan. Lancet. 2000;**356**(9241):1565-1569

[23] Kamil AA, Khalil EAG, Musa AM, Modabber F, Mukhtar MM, Ibrahim ME, Zijlstra EE, Sacks D, Smith PG, Zicker F, Elhassan AM. Alum-precipitated autoclaved L. major plus BCG, a candidate vaccine for visceral leishmaniasis: Safety, skin delayed hypersensitivity response and dose finding in healthy volunteers. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2003;**97**(3):365-368

[24] EAG K, Ayed NB, Musa AM, Ibrahim ME, Mukhtar MM, Zijlstra EE, Elhassan IM, Smith PG, Kieny PM, Ghalib HW, Zicker F, Modabber F, Elhassan AM. Dichotomy of protective

cellular immune responses to human visceral leishmaniasis. Clinical and Experimental Immunology. 2005;**140**:349-353

[25] Khalil EAG, Musa AM, Modabber F, El-Hassan AM. Safety & immunogenicity of a candidate vaccine for visceral leishmaniasis (alum-precipitated autoclaved *L. major* +BCG) in children: An extended phase II study. Annals of Tropical Paediatrics. 2006;**26**(4):357-361

[26] Manzur A, Ul Bari A. Sensitivity of leishmanin skin test in patients of acute cutaneous leishmaniasis. Dermatol Online Journal. 2006;**12**(4):2

[27] Melby PC. Vaccination against cutaneous leishmaniasis: Current status. American Journal of Clinical Dermatology. 2002;**3**(8):557-570

[28] Khalil EAG, Elhassan AM, Zisltra EE, Mukhtar MM, Ibrahim ME, et al. Safety & immunogenicity of an autoclaved L. major vaccine. East African Medical Journal. 2000;**77**(9):468-470

[29] Bern C, Amann J, Haque R, Chowdhury R, Ali M, Kurkjian KM, Vaz L, Wagatsuma Y, Breiman RF, Secor WE, Maguire JH. Loss of leishmanin skin test antigen sensitivity and potency in a longitudinal study of visceral leishmaniasis in Bangladesh. The American Journal of Tropical Medicine and Hygiene. 2006;**75**(4):744-748

[30] Weigle KA, Valderrama L, Arias AL, Santrich C, Saravia NG. Leishmanin skin test standardization and evaluation of safety, dose, storage, longevity of reaction and sensitization. The American Journal of Tropical Medicine and Hygiene. 1991;**44**(3):260-271

[31] Hailu A, Berhe N, Ali A, Gemetchu T. Use of Leishmania major derived leishmanin for skin test surveys of visceral leishmaniasis in Ethiopia. East African Medical Journal. 1997;**74**(1):41-45

[32] Satti I, El-Hassan AM, Khalil EAG, Akuffo H. The effect of repeated leishmanin skin testing on the immune responses to Leishmania antigen in healthy volunteers. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2002;**96**:565-567

[33] Gidwani K, Rai M, Chakravarty J, Boelaert M, Sundar S. Evaluation of leishmanin skin test in Indian visceral leishmaniasis. The American Journal of Tropical Medicine and Hygiene. 2009;**80**(4):566-567

[34] Khamesipour A, Dowlati Y, Asilian A, Hashemi-Fesharki R, Javadi A, Noazin S, Modabber F. Leishmanization: Use of an old method for evaluation of candidate vaccines against leishmaniasis. Vaccine. 2005;**25**(28):3642-3648

[35] Leishmanization. In: Mehlhorn H, editor. Encyclopedia of Parasitology. Berlin, Heidelberg: Springer; 2008

[36] Musa AM, Khalil EAG, Ismail A, Elhassan IM, Fesharki H, Khamesipour A, Modabber F, Zijlstra EE, El-Hassan AM. Safety, immunogenicity and possible efficacy of immunochemotherapy of persistent post kala-azar dermal leishmaniasis (PKDL). Sudanese Journal of Dermatology. 2005;**3**:62-72

[37] Musa AM, Khalil EAG, Mahgoub FA, Hassab Elgawi SH, Modabber F, Elkadaru AMY, Aboud MH, Noazin S, Ghalib HW, El-Hassan AM. Immunochemotherapy of persistent

post kala-azar dermal leishmaniasis: A novel approach of treatment. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008;**102**(1):58-63 Epub 2007 Oct 25

[38] Musa AM, Younis B, Fadlalla A, Royce C, Balasegaram M, Wasunna M, et al. Paromomycin for the treatment of visceral leishmaniasis in Sudan: A randomized, open-label, dose-finding study. PLoS Neglected Tropical Diseases. 2010b;**4**(10):e855

[39] Satti IN, Osman HY, Daifalla NS, Younis SA, Khalil EAG, Zijlstra EE, El Hassan AM, Ghalib HW. Immunogenicity and safety of autoclaved Leishmania major plus BCG vaccine in healthy Sudanese volunteers. Vaccine. 2001;**19**(15-16):2100-2106

[40] Noazin S, Khamesipour A, Moulton LH, Tanner M, Nasseri K, Modabber F, Sharifi I, Khalil EAG, Velez Bernal ID, Antunes CMF, Smith PG. Efficacy of killed whole-parasite vaccines in the prevention of leishmaniasis: Meta-analysis. Vaccine. 2009;**27**:4747-4753. DOI: 10.1016/j. Vaccine. 2009.05.084

[41] Noazin S, Modabber F, Khamesipour A, Smith PG, Moulton LH, Nasseri K, Sharifi I, Khalil EA, Bernal ID, Antunes CM, Kieny MP, Tanner M. First generation leishmaniasis vaccines: A review of field efficacy trials. Vaccine. 2008;**26**:6759-6767 Epub 2008 Oct 23

[42] Chakravarty J, Kumar S, Trivedi S, Rai V, Singh A, Ashman J, et al. Vaccine. 2011;**29**: 3531-3537

[43] Nagill R, Kaur S. Vaccine candidates for leishmaniasis: A review. International Immuno pharmacology. 2011;**11**(10):1464-1488. DOI: 10.1016/j.intimp.2011.05.008

[44] Sundar S, Piazza F. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+MPL-SE vaccine for use in the prevention of visceral leishmaniasis. Vaccine. 2011;**29**(19):3531-3537. DOI: 10.1016/j.vaccine.2011.02.096

[45] Elfaki ME, Khalil EAG, De Groot AS, Musa AM, Gutiérrez Núñez A, Younis BM, Salih KA, El-Hassan AM. Immunogenicity and immune modulatory effects of *in silico* predicted *L. donovani* candidate peptide vaccines. Human Vaccines & Immunotherapeutics. 2012;**8**(12):1769-1774. [Epub ahead of print]

[46] Elfaki MEE, De Groot AS, Gutierrez AH, Younis BM, Tassone R, Terry F, Musa AM, Elhassan AM, Khalil EAG. *In silico* prediction of immunogenic T cell epitopes of Leishmania donovani GP63 protein: An alternative approach for anti-parasite vaccine development. Jacobs Journal of Vaccines and Vaccination. 2015;**1**(2):008

[47] Coler RN, Duthie MS, Hofmeyer KA, Guderian J, Jayashankar L, Vergara J, et al. From mouse to man: Safety, immunogenicity and efficacy of a candidate leishmaniasis vaccine LEISH-F3+GLA-SE. Clinical & Translational Immunology. 2015;**4**(4):e35. DOI: 10.1038/cti.2015.6. eCollection 2015 Apr

[48] Saeed WSE, Khalil EAG. Immune response modifying effects of bee venom protein [melittin]/autoclaved *L. donovani* complex in CD1 mice: The search for new vaccine adjuvants. Journal of Vaccines and Vaccination. 2017;**8**:372. ISSN: 2157-7560. DOI: 10.4172/2157-7560.1000372

[49] Saeed WSE, Khalil EAG. Toxic effects and safety of bee venom protein [Melittin] in mice: Search for natural vaccine adjuvants. Journal of Natural Products and Resources. 2017b;**3**(1):111-114 ISSN: 2455-0299

[50] Dunning N. Leishmania vaccines: From leishmanization to the era of DNA technology. Bioscience Horizons. 2009;**2**(1):73-82. DOI: 10.1093/biohorizons/hzp004

[51] Ghaffarifar F, Jorjani O, Sharifi Z, Dalimi A, Hassan ZM, Tabatabaie F, et al. Enhancement of immune response induced by DNA vaccine cocktail expressing complete LACK and TSA genes against Leishmania major. APMIS. 2013;**121**(4):290-298. DOI: 10.1111/j.1600-0463.2012.02968.x Epub 2012 Sep 18

[52] Soto M, Corvo L, Garde E, Ramírez L, Iniesta V, Bonay P. Coadministration of the three antigenic leishmania infantum poly (A) binding proteins as a DNA vaccine induces protection against leishmania major infection in BALB/c mice. PLOS Neglected Tropical Diseases. 2015;**9**(5):e0003751. DOI: 10.1371/journal.pntd.0003751. eCollection 2015 May

[53] Osman M, Mistry A, Keding A, Gabe R, Cook E, Forrester S, et al. PLOS Neglected Tropical Diseases. A third generation vaccine for human visceral leishmaniasis and post kala azar dermal leishmaniasis: First-in-human trial of ChAd63-KH. 2017;**11**(5):e0005527. DOI: 10.1371/journal.pntd.0005527. eCollection 2017 May

[54] Gurunathan S, Prussin C, Sacks DL, et al. Vaccine requirements for sustained cellular immunity to an intracellular parasitic infection. Nature Medicine. 1998;**4**:1409-1415

[55] Campos-Neto A. Anti-leishmania vaccine. In: Farrell JP, editor. Leishmania: World Class Parasites. Vol. 4. Boston, MA: Springer; 2002. pp. 169-190

[56] Vajdy M, Srivastava I, Polo J, Donnelly J, O'Hagan D, Singh M. Mucosal adjuvants and delivery systems for protein-, DNA- and RNA-based vaccines. Immunology & Cell Biology. 2004;**82**:617-627

[57] Garçon N, Chomez P, Van Mechelen M. GlaxoSmithKline adjuvant systems in vaccines: Concepts, achievements and perspectives. Expert Review of Vaccines. 2007;**6**:723-739

[58] Palatnik-de-Sousa CB. Vaccines for leishmaniasis in the fore coming 25 years. Vaccine. 2008;**26**:1709-1724

[59] Reed SG, Bertholet S, Coler RN, Fierde M. New horizons in adjuvants for vaccine development. Trends in Immunology. 2009;**30**:23-32

[60] Badiee A, Shargh VH, Khamesipour A, Jaafari MR. Micro/nanoparticle adjuvants for antileishmanial vaccines: Present and future trends. Vaccine. 2013;**31**:735-749

[61] Higgins SC, Mills KH. TLR, NLR agonists, and other immune modulators as infectious disease vaccine adjuvants. Current Infectious Disease Reports. 2010;**12**:4-12

Section 5

Therapeutic Targets and Inhibitors

Chapter 7

The Polyamine Pathway as a Potential Target for Leishmaniases Chemotherapy

Juliana Ide Aoki, Sandra Marcia Muxel,
Juliane Cristina Ribeiro Fernandes and
Lucile Maria Floeter-Winter

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75867

Abstract

Considering the limitations of the current leishmaniases chemotherapy and the lack of effective vaccines, the identification of novel drugs and/or vaccine approaches for the leishmaniases treatment and control is urgently required. In fact, a rational strategy for the parasite control can be based on the identification of essential metabolic pathways of the parasite. One of the most important pathways is the polyamine biosynthesis. *Leishmania* is auxotrophic for many amino acids, such as L-arginine, a precursor of ornithine, putrescine, and spermidine. These metabolites are essential for parasite replication and establishment of infection in the mammalian host. In addition, *Leishmania* has a specific and complex machinery to uptake and metabolize exogenous sources of those molecules. In this chapter, we will focus on the main aspects of the polyamine pathway as a potential target for infection control aiming for new targets for *Leishmania* chemotherapy.

Keywords: amastigote, L-arginine metabolism, putrescine, ornithine, spermidine, spermine, amino acid permease 3, amino acid transport, nitric oxide, nitric oxide synthase, glycosome

1. Introduction

Leishmaniases are diseases characterized by cutaneous, mucocutaneous, diffuse, or visceral clinical manifestations [1]. They are currently endemic in 98 countries and territories worldwide, with estimated 700,000 to 1 million new cases and 20,000–30,000 deaths occurring annually [2]. The incidence of this disease is mainly in underdeveloped countries within South East

Asia, East Africa, and Latin America; however, it is also endemic in several Mediterranean countries leading to highlight the importance of transmission in travelers [3]. *Leishmania*-HIV co-infection has emerged as an opportunistic infection and has been described as important clinical, diagnostic, and epidemiological implications [4]. The virus and the parasite compromise the immune response, leading to the replication of both and consequent progression of *Leishmania* infection [5].

Leishmaniases are caused by the protozoan parasites of *Leishmania* genus. The parasite presents two main morphological forms during its life cycle. The promastigote, an extracellular long and flagellated form, proliferates in the digestive tract of the invertebrate host, and the amastigote, an obligate intracellular form with a nonapparent flagellum, proliferates in the phagolysosome of the mammalian host macrophage [1, 6]. These two distinct host environments submit the parasite to a rapid adaptation in gene/protein expression, cellular signaling, metabolism, and morphology to survival during promastigote-to-amastigote differentiation [7–11]. In fact, the parasite can sense temperature, pH, and nutrient availability, controlling the amino acid and purine transport and osmoregulation to establish the infection [12].

The immune response in *Leishmania* infection is mediated by phagocytic cells such as neutrophils, macrophages, and dendritic cells. The monocyte recruitment and macrophage differentiation result in the recognition of the parasite, its phagocytosis, and consequent induction of inflammatory response with nitric oxide (NO) production through nitric oxide synthase 2 (NOS2) and reactive oxygen species production [13–16]. These actions coordinate the innate immune response and can promote the parasite killing, as showed for *L. amazonensis*, *L. major*, and *L. donovani* [17]. On the other hand, *Leishmania* is able to escape from these defense mechanisms leading to amastigote differentiation and proliferation in the macrophage phagolysosome [18]. Therefore, the antibodies have little or negligible effect in the infection. These coordinate mechanisms of evasion can be mediated by *Leishmania* polyamine pathway through induction of parasite-arginase (*L*-ARG) activity to produce polyamines [12, 19–22]. It is interesting to note that L-arginine is the common substrate for NOS2 and arginase 1 (ARG1). Both enzymes are competitively regulated by cytokines from T helper 1 (Th1), such as interferon gamma (IFN-γ), or T helper 2 (Th2), as interleukins IL-4, IL-13, and TGF-β, inducing the macrophages M1 or M2 polarization, respectively [23–27]. M2 macrophages contribute to susceptibility in cutaneous leishmaniasis [28].

The preconized treatment for leishmaniases is the use of antimonials, the same treatment used since its description by Gaspar Vianna in 1912 [6]. First, the recommendation was based on the use of trivalent antimonial; however, it was replaced with pentavalent antimonial, more efficient, and less toxic [29]. The pentavalent antimonial formulations are represented by meglumine antimoniate and sodium stibogluconate and still considered as the main line for leishmaniases treatment today [30]. These compounds have side effects, the treatment is long, and they are administered through intramuscular injections or intravenous infusions, requiring the patient hospitalization [30]. The high toxicity of these compounds can be due to the high concentration used in the current treatment (about four times higher than 20 years ago) and to the acquired resistance by the parasite as a result of long exposure of the drug and inadequate dosages [30]. An alternative line of treatment can be based on the use of amphotericin and its liposomal derivatives; however, they also present side effects, which restrict its use

http://dx.doi.org/10.5772/intechopen.75867

besides the high cost of the treatment [30]. Pentamidine has the mechanism of action based on the inhibition of polyamine synthesis [31, 32] and can also be used for unresponsive antimonial treatment [33]. Miltefosine, which is administrated as an oral drug, is a promisor alternative for leishmaniases treatment [34] with high effective and tolerated rate in visceral leishmaniasis in India [35] and later also effective for cutaneous leishmaniasis [36–38]. However, miltefosine-unresponsive cases have been reported in regions outside India [36]. Paromomycin is another promising treatment for visceral leishmaniasis control in India, and it has been described as effective in monotherapy as well as in combination with other drugs [39]. Azole antifungal agents, like ketoconazole, have been also used in the leishmaniases treatment for decades [40], and its mechanism of action is based on the inhibition of ergosterol biosynthesis [41].

The current leishmaniases chemotherapy, as represented in **Figure 1**, is based on these seven compounds, which present different origins of discovery, unique structures, and distinct modes of action [42].

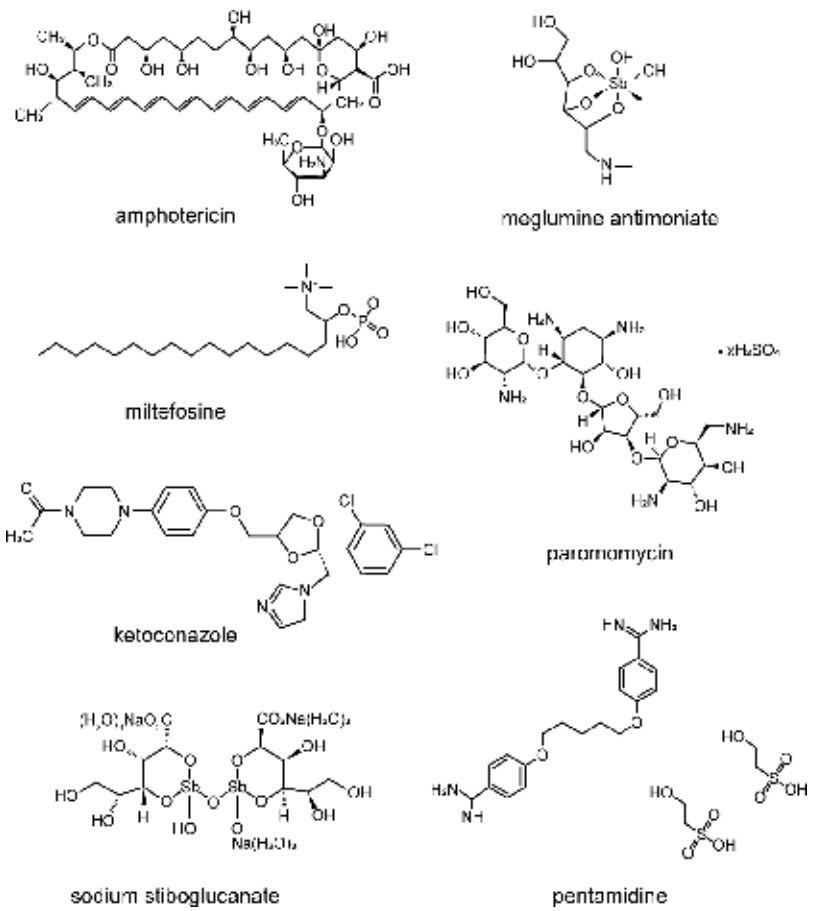


Figure 1. Chemical structure of the current antileishmanial drugs: amphotericin, meglumine antimoniate, miltefosine, paromomycin, ketoconazole, sodium stibogluconate, and pentamidine.

To date, there is no single effective treatment for leishmaniases. In fact, the leishmaniases treatment is complicated due to the complexity of the different species of *Leishmania* and their interaction with the host cells. The available therapies show high toxicity, low efficacy, long duration of treatment, high costs, and increased rate of resistant parasites. Then, there is an emergency and challenge in the development of new drugs for leishmaniases chemotherapy. The emergency is due to the increased incidence of case reports, and the challenge is based on the leishmaniasis classification as a neglected tropical disease. The high incidence in underdeveloped regions of the world implicates a lack of interest in research development and minimum financial funding from pharmaceutical industries. There are many studies describing potential targets for vaccine approaches; however, no licensed vaccine is available for human treatment.

A rational strategy for the parasite control can be developed based on the identification of fundamental metabolic pathways of both the parasite and the host, such as polyamine biosynthesis. Polyamines are involved in chromatin structure and DNA replication. They interact with both DNA and RNA, promoting gene expression regulation and transport mechanisms [43].

In this review, we will point aspects of the current treatment, relevant targets and highlight the potential for polyamine pathway for leishmaniases chemotherapy because it is essential for parasite replication and survival.

2. The current chemotherapy

The leishmaniases chemotherapy was discovered in an empiric way, which means all drugs used are re-purposed from other therapeutic prescriptions. In fact, this scenario is far to be optimal for a disease whose incidence is increasing in the endemic areas.

Then, we will review the current leishmaniases chemotherapy with description of the drugs and its mechanism of action.

2.1. Pentavalent antimonials

Pentavalent antimonials are represented by the formulations meglumine antimoniate and sodium stibogluconate (**Figure 1**) and consist in the most frequently used drug for leishmaniases treatment because they are effective for both visceral and cutaneous leishmaniases [30]. Both formulations present poor oral absorption, leading to an intramuscular or intravenous administration [44]. Besides its accumulation in the tissues, antimonials can cause severe cardiotoxicity, pancreatitis, and nephrotoxicity effects, requiring hospitalization and close monitoring of patients [45, 46]. In addition to these side effects, the long period of treatment leads to noncompliance and abandonment, favoring the emergence of resistant parasites and the drug efficacy varying from region to region compromising its use [33].

The antimonial mechanism of action involves the depletion of intracellular ATP due to interference in glycolysis and fatty acids β oxidation [47]. There are some studies evidencing that antimony kills the parasite by a process of apoptosis involving DNA fragmentation and externalization of phosphatidylserine on the membrane surface [48].

http://dx.doi.org/10.5772/intechopen.75867

2.2. Pentamidine

Pentamidine is an aromatic diamidine (**Figure 1**) used mainly in the treatment of cutaneous leishmaniasis unresponsive to pentavalent antimonial treatment [33]. Most regimens are based on intramuscular injections or intravenous infusions per day for about 30 days [49]. However, due to its toxicity and rapidly emerging resistance, pentamidine was abandoned in India in 1990 and replaced by amphotericin B, as the recommended treatment [50]. In contrast, pentamidine is the first line of choice for treatment in French Guiana, where it is the only available drug [51].

The pentamidine mechanism of action is related to inhibition of the polyamine synthesis [31, 32], activity of *S*-adenosyl-L-methionine decarboxylase [52], the alteration of the membrane fluidity, lipid metabolism, mitochondrial activity [53], the calcium transport [54], disintegration of the kinetoplast and mitochondria, and collapse of the mitochondrial membrane [55]. Pentamidine binds to DNA essentially in AT-rich regions, such as the kDNA, affecting the transcription and replication process [53]. Additionally, *L. amazonensis* and *L. donovani* parasites treated with pentamidine showed decrease in arginine, ornithine, and putrescine pools, while the levels of spermidine remain intact [31]. In *L. donovani*, pentamidine is described as a competitive inhibitor in the arginine uptake [56] and a noncompetitive inhibitor of putrescine and spermidine transport in *L. infantum* [57], *L. donovani*, and *L. mexicana* [58]. In fact, pentamidine uses polyamine transporters to enter in the parasite leading to an altered polyamine uptake in pentamidine-resistant *Leishmania* [31, 59].

2.3. Amphotericin B

Amphotericin B (**Figure 1**) and its lipid formulation have been considered as the most striking advances for visceral leishmaniasis treatment [60, 61]. This antifungal antibiotic has also been considered as the first-line drug for treatment due to its high efficacy against antimonial-unresponsive cases [62]. Amphotericin B is administrated through intravenous infusion and can present side effects, such as nephrotoxicity and myocarditis, leading to close monitoring and hospitalization for 4–5 weeks [63]. The advent of liposome technology allows minimization of dose-limiting toxicity, providing highly effective and safe therapy. The ambisome formulation is probably the most efficient of all currently available drugs for leishmaniases treatment, and it has been used as the first-line drug for treatment worldwide [64, 65].

The mechanism of action of amphotericin B is based on the sterols metabolism. It interferes in the ergosterol biosynthesis of the cell membrane of *Leishmania*, causing changes in the membrane permeability and leakage of intracellular components that damage the cell, triggering parasite killing [66].

2.4. Miltefosine

Miltefosine (**Figure 1**) was the first effective oral agent for visceral leishmaniasis treatment. This drug was originally used for cancer treatment, but it showed high efficacy for leishmaniases unresponsive to antimonial treatment [67]. Since it has been described effective for leishmaniases treatment, it has been used worldwide, however, with a variable rate of efficacy [37, 38, 68]. Drug-resistant cases have been reported, and increasing relapse rates can be due to the reflection of its long half-life in case of inadequate use [69].

Miltefosine interferes with cell membrane composition by inhibiting phospholipid metabolism with reduction of phosphatidylcholine content and enhancement of phosphatidylethanolamine content in the membrane of *L. donovani* [70]. Resistance to miltefosine is easily selected *in vitro* [71, 72]. The resistance mechanisms can be due to drug pressure inducing the mechanisms of regulation in *Leishmania* lipid metabolism by a defect in drug internalization mediated by miltefosine transporter machinery [69, 70, 72].

2.5. Paromomycin

Paromomycin is an aminoglycoside antibiotic (**Figure 1**) used to treat bacterial infections and requires metabolic energy from electron transport chain across plasmatic membrane [73, 74]. Paromomycin has shown high cure rate in leishmaniases treatment in India [75]. When orally administered, paromomycin is poorly absorbed, limiting its use to intramuscular injections.

The mechanism of paromomycin action in *Leishmania* is not precisely known, but protein synthesis has been proposed as target, based on studies with bacteria. Other possible mechanisms had been proposed, including alteration of membrane fluidity and effects on the mitochondria membrane potential. The ribosomal complex, responsible for translating the genetic information from mRNA to protein, is the usual site of action for aminoglycoside antibiotics. Based on paromomycin-resistant *L. donovani*, the upregulation of ribosomal proteins was observed in the resistant parasite, suggesting that protein synthesis machinery is the site of action in *Leishmania* [76, 77]. Transcriptomic profile of paromomycin-resistant *L. donovani* shows decreased protein synthesis and degradation and the role in oxidative phosphorylation, glycosomal succinate fermentation, DNA synthesis and repair, and also alteration in the NO production during macrophage infection [78].

2.6. Ketoconazole

Ketoconazole is an oral antifungal drug (**Figure 1**) that inhibits ergosterol biosynthesis. Ergosterol is the major sterol in *Leishmania*, and it is a potential target of some drugs because it is absent in mammalian cells, in which cholesterol is the main sterol. The mechanism of action of ketoconazole causes the accumulation of methyl sterols due to changes in membrane permeability [41]. Ketoconazole treatment of murine macrophage infected with *L. mexicana* altered the levels of free sterols in amastigotes [41]. Oral ketoconazole treatment resulted in failure in the control of *L. braziliensis* cutaneous lesions or ulcers [79, 80]. On the other hand, some studies have shown efficacy in controlling *L. braziliensis* cutaneous lesions [40] and *L. amazonensis* murine infection *in vitro* and *in vivo* [81]. Ketoconazole associated with antimony presented toxicity to amastigote forms of *L. amazonensis* [82].

3. Biological targets for therapy

In the course of the *Leishmania* life cycle, environmental changes inside the invertebrate and mammalian host represent important external signals for gene expression regulation. These signals start with the starvation of nutrients, such as amino acids, signaling for metacyclogenesis during

the transformation from promastigote procyclic forms into promastigote metacyclic forms. In this step, procyclic forms are adhered to epithelia of insect midgut, and the starvation promotes their release and starts the differentiation to infective metacyclic forms accompanied with their migration to the insect proboscis. From this site, the metacyclic forms are regurgitated during a new blood meal [83]. The following signal is the temperature shift from the invertebrate host (25°C) to the mammalian host (37°C), representing a challenge for parasite survival and differentiation into amastigote forms. The heat-shock proteins are examples of gene activation that allows parasite survival in rapid temperature changes [10, 84, 85]. The pH change, from 7.0 to 5.5, is the last signal, due to the phagosome and lysosome fusion to form the phagolysosome inside the phagocytic cells, where the amastigotes replicate [9–11].

Despite these environmental changes, promastigotes and amastigotes have common metabolic features that distinguish them from their hosts and can be used as new antiparasitic targets. New potential drug targets have been described along the years, and we will describe here some of them, such as protein kinases, glycolate enzyme, purine pathway, and polyamine pathway. Additionally, besides considering the future of leishmaniases treatment, encompassing new methodological approaches available today, the study of metabolic pathways allows the understanding of the biology and physiology of the parasite. Recent approaches, such as "omics", have been provided new insights into fundamental pathways of the parasite and/or in the *Leishmania*-host interactions that can be explored as potential new targets.

3.1. Protein kinases

The protein kinases are involved in several essential biological processes, including metabolism, gene expression, cell proliferation, motility, differentiation, and death [86]. Protein kinases act on serine, threonine, tyrosine, or histidine residues of proteins leading to phosphorylation. Phosphorylation can modify the function of a protein in a variety of ways, such as protein activity, stabilization, or degradation. The localization within a particular compartment of a cell can initiate or disrupt its interaction with other proteins [87]. These kinases along with phosphatases play a major role in protein and enzyme regulation. *Leishmania*-activated kinases play an important role in parasite thermo-tolerance and virulence [88].

These enzymes have been explored as therapeutic target by pharmaceutical industry, with a focus on the discovery of non-ATP-competitive kinase inhibitors, directing these modulators to target sites that can regulate specific protein kinases [89, 90].

3.2. Glycolytic enzymes

The glycolytic enzymes of trypanosomes are attractive drug targets because the glycolysis is essential for energy requirements. The ATP synthesis through glycolytic enzymes starts with glucose to produce glycerol and pyruvate, maintaining the ATP and NAD balance of glycosome compartment. The glycolytic enzymes, localized in glycosome and cytosol, have emerged as drug targets in parasitic diseases [76, 91]. Targeting the glycosomal enzymes could alter the production of energy and NADH by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and its oxidization through mitochondrial glycerol-3-phosphate oxidase to shift the electrons [91].

The inhibition of energy production in *Leishmania* can be based on the use of adenosine analogs, blocking the binding of NAD+ to GAPDH [92–95]. Some analogs have been described as inhibitor of *Leishmania* growth due the block of energy production.

3.3. Purine metabolism

Another interesting pathway to explore as new targets for leishmaniases chemotherapy is the purine metabolism. *Leishmania* and other protozoa are unable to synthesize purine nucleotides *de novo* and must uptake them from the host [96]. The parasite uptake of preformed purine host is mediated by nucleoside transporters [97].

Purine nucleotides and their derivatives are precursors of vast cellular and metabolic processes, including energy production, cell signaling, synthesis of nucleic acids, modulation of enzymatic activities, and synthesis of co-enzymes [96, 97]. This unique characteristic may be the basis for susceptibility of *Leishmania* to purine analogs [97, 98]. Purine analogs have been described for use in the parasite control, such as tubercidin (TUB) [98–100]. TUB is effective against promastigotes from *L. amazonensis*, *L. braziliensis*, *L. infantum chagasi*, and *L. major* [98, 101]. The same antiparasitic efficacy is described for amastigotes from *L. amazonensis* when associated with a specific and selective inhibitor of nucleoside transport for mammalian cells, the nitrobenzylthioinosine (NMBPR) [98]. The selectivity of TUB-NBMPR combined treatment for *Leishmania* produces highly selective toxicity against the parasite, inhibition of mammalian nucleoside transporter, supporting the hypothesis that these transporters are different between the parasite and its host [98].

In addition, based on tubercidin-resistant *L. major* parasites, the upregulation of the tubercidin-resistant protein, an endoplasmic reticulum protein is observed in these resistant line, suggesting that protein synthesis machinery is the site of action of TUB in *Leishmania* [99].

3.4. Polyamine pathway

The polyamine pathway is important for parasite replication and to the establishment of infection in the host [102]. Fundamental differences in this pathway are described between the parasite and its host, pointing to antileishmanial chemotherapy targets (**Figure 2**). Polyamines (putrescine, spermidine, and spermine) are essential substrates in all cells, including parasitic protozoa. Their intracellular concentration may be regulated at the level of their biosynthesis, interconversion, degradation, and transport [103]. In *Leishmania*, the hydrolysis of L-arginine by *L*-ARG to produce ornithine and urea is a crucial initial step for polyamines production [12, 102, 104–106] and for parasite growth and survival in promastigote and amastigote forms [107–110]. Thus, an inhibition of polyamine synthesis represents a promisor target for leishmaniases chemotherapy (**Figure 2**).

The polyamines biosynthetic pathway is characterized by the decarboxylation of the amino acid ornithine to putrescine and catalyzed by ornithine decarboxylase (ODC), a key enzyme on this pathway. Putrescine is then converted into spermidine by the action of a spermidine synthase (SpdS). Finally, spermidine is used to form both spermine by spermine synthase (SpmS) and trypanothione through trypanothione synthase (TryS). Trypanothione is an important regulator of intracellular thiol redox balance [111, 112]. *Leishmania* also uses L-arginine to

http://dx.doi.org/10.5772/intechopen.75867

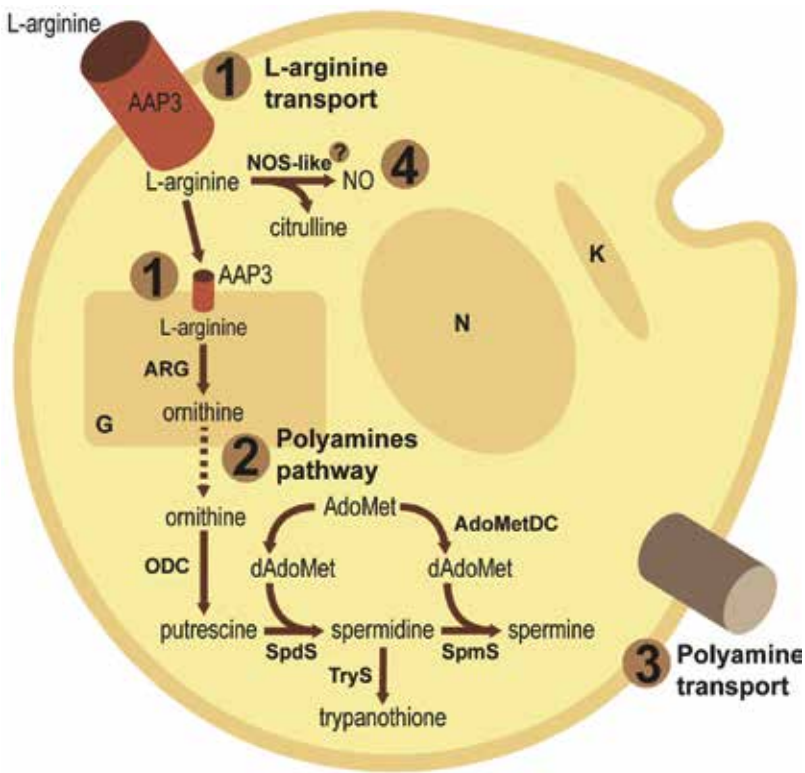


Figure 2. Schematic representation of polyamine-related chemotherapeutic approaches for *Leishmania*, such as the (1) inhibition of amino acid permease 3 (AAP3) in both plasmatic membrane and glycosome, preventing the L-arginine uptake; (2) inhibition of polyamines synthesis by arginase (ARG), ornithine decarboxylase (ODC), *S*-adenosylmethionine decarboxylase (AdoMetDC), spermidine synthase (SpdS), spermine synthase (SpmS), and trypanothione synthase (TryS); (3) inhibition of polyamines, preventing the parasite replication; and (4) inhibition of the parasite nitric oxide synthase (NOS-like), preventing the nitric oxide (NO) production and amastigote differentiation and replication. (?) predicted but not confirmed cytosolic localization, (N) nucleus, (K) kinetoplast, and (G) glycosome.

produce NO and citrulline by enzyme NOS-like activity [113–115]. The controversial NO production by the parasite is still not completely understood because NO is the same molecule produced by host macrophages to promote the parasite's killing [105, 109, 116]. However, NO production has been related to metacyclogenesis signaling in promastigote forms and to amastigote replication of *L. amazonensis* in a dependent way to the L-arginine availability and *L*-ARG activity [7]. The participation of a NOS-like enzyme in this pathway was recently described based on *in silico* analysis of oxidoreductase family domains [7]. Besides this, previous metabolome evidences of NO and citrulline production also indicated the activity of the enzyme in *L. amazonensis* [115]. Although this enzyme is not completely characterized, NOS-like enzyme could also be a potential drug target (**Figure 2**), as an inhibitor of NO production decreasing amastigote differentiation and replication.

Once L-arginine is not synthesized *de novo*, the parasite developed molecular mechanisms to sense the amino acid availability and activity of enzymes involved in polyamines production [115–120]. *L*-ARG is an enzyme with regulatory roles, as the modulation of L-arginine

availability with consequently regulation of polyamine synthesis [121]. *L*-ARG presents a glycosomal import signal, the three amino acids SKL [122, 123]. Glycosomes are peroxisome-like organelles, essential to parasite survival, likely due to compartmentalization of key metabolic enzymes [124]. Glycosomes are not only involved in glycolysis but are also predicted to carry out gluconeogenesis, reaction of the hexose-monophosphate pathway, purine salvage and pyrimidine biosynthesis, fatty acids β-oxidation, fatty acids elongation, and biosynthesis of other lipids. In addition, they seem to have involved in oxidant stress protection [123]. Another role for the existence of glycosome in kinetoplastid organisms can be related with the importance of sequestering metabolic pathways into this compartmentalized organelle and then facilitated the parasite development because the turnover of pathways (or part of them) is more rapidly and efficiently upon induction when they are compartmentalized than when present in the cytosol as individual enzymes [124].

Besides the glycosomal signal, *L*-ARG was demonstrated in fact localized in the glycosome compartment of *L. mexicana* and *L. amazonensis* promastigotes [102, 104, 122]. In addition, this glycosomal localization is maintained in intracellular amastigotes in macrophage infections [102]. Interestingly, the glycosomal localization is crucial for its activity because the mislocalization of the enzyme reduced *in vitro* and *in vivo* infectivity [102]. RNA-seq data revealed that *L*-ARG expression is downregulated in *L. amazonensis* axenic amastigotes when compared to promastigotes [8]. In contrast, an upregulation is observed in *Leishmania* intracellular amastigotes from BALB/c macrophages infection [12]. Altogether, these data reinforce the existence of a differential modulation of the enzyme activity under different environment conditions [8].

Leishmania amino acid uptake can also be considered for new antileishmanial target (**Figure 2**). L-Arginine uptake in macrophages is mediated by cationic amino acids (CAT1, CAT2A, CAT2B, and CAT3) [125]. A reduction in CAT2B expression and L-arginine uptake by treatment with melatonin, hormone of dark signal to biological rhythm, impairs *in vitro L. amazonensis* infectivity in murine model [126]. In contrast, *Leishmania* has a complex and specific machinery to uptake this amino acid. L-Arginine uptake is mediated by amino acid permease 3 (AAP3) in *L. donovani* and *L. amazonensis* [116, 119, 120]. Furthermore, the AAP3 dual localization in the plasma membrane and in the glycosome from promastigotes and axenic amastigotes of *L. amazonensis* and *L. donovani* [102, 118, 119] can be an indicative that the inhibition of L-arginine trafficking through the plasma membrane and/or through the glycosome suggests a promisor target for leishmaniases chemotherapy (**Figure 2**) [116, 119].

The drugs targeting enzymes involved in the polyamines production could reduce the parasite growth and survival. The 3-aminooxy-1-aminopropane (APA) and L-α-difluoromethylornithine (DFMO) are ODC inhibitors. APA is an isosteric analog of putrescine and inhibits the growth of *L. donovani* promastigotes and amastigotes [127]. However, DFMO presents a controversial data as a leishmanicidal compound. DFMO is described successful against African sleeping sickness [128], and it is also efficient in inhibition of *L. donovani*, *L. infantum*, and *L. guyanensis* infections but not for *L. major* and *L. mexicana* [129, 130]. Furthermore, other studies described DFMO inefficacy against *Leishmania* [131]. The basis for the selectivity toxicity of DFMO in the parasite is complex. The ODC from the parasite is no less susceptible to inhibition by DFMO than ODC from the host. However, many metabolic differences between parasites and

mammals have been identified, such as the inability of the parasite to obtain exogenous polyamines, the synthesis of trypanothione that could interfere in the susceptibility to DFMO [132] and its metabolic stability. ODC has a long half-life (more than 6 h), unlike the host protein (less than 30 min), which can be the basis for susceptibility of DFMO in the parasite [133].

A 5′-((Z)-4-amino-2-butenyl)methylamino)-5′-deoxyadenosine (MDL73811) can also be used as an inhibitor of adenosyl methionine decarboxylase (AdometDC), enzyme that forms decarboxylate *S*-adenosyl methionine. This molecule can be used with putrescine by SpdS to produce spermidine and with spermidine to produce spermine [58, 102, 121, 134–137], as described for *L. donovani* [137].

In addition, the importance of polyamine pathway has been described with the generation of *L. donovani*, *L. major*, *L. mexicana*, and *L. amazonensis* null mutants of essential enzymes involved in this pathway, such as ARG, ODC, AdoMetDC, or SpdS. Knockout parasites of these enzymes have been providing data of how these parasites synthesize polyamines and depend on supplementation of products of these enzymes for survival [7, 8, 102, 105, 106, 138]. The role of polyamine pathway in intracellular amastigotes is controversial; *in vitro* and *in vivo* infections with *L. amazonensis* arginase knockout parasites present lower infection index [102, 119]. In contrast, *in vivo* infection with *L. donovani* arginase knockout parasites reduces the parasite burden in the liver of mice but does not impact the parasite burden in the spleen [106]. Supplementation with putrescine recovered the levels of infectivity of *L. amazonensis* in murine macrophages with reduced levels of L-arginine transport [126]. A comparison of the polyamine pathway in these two different species reveals a variation in the polyamine pool within the phagolysosome, and consequently difference in the polyamine uptake by the parasite may contribute to differences in virulence [138].

3.5. "Omics"

The application of "omics" approaches in *Leishmania* research has been providing new insights into the biological processes that drive the replication and differentiation steps of the parasite. They can also provide insights into drug transport and metabolism. Furthermore, these approaches have been revealing as a fundamental tool for the biology, the discovery of new targets for chemotherapy, and the determination of drug-resistance mechanisms [139]. The progress of *Leishmania* spp. genome annotations has been providing a lot of information, including a review of previous genome annotations data and its misassembling, in an attempt to improve the current genome and gene annotations [8, 140, 141]. Since genomes are characterized by a high degree of synteny among the species, the genomic annotations can explain the specificity for tissues tropisms, differential immune responses, variations in drug susceptibility, gene content, and gene expression regulation, among the species.

Leishmania exhibit many unique features in their biology, and the elucidation of the molecular basis of them may lead to the development of new strategies for the control of the disease. *Leishmania* parasite genome is organized as large cluster of genes in the 5′–3′ direction on the DNA strand. The polycistronic transcription occurs in an initiation site, forming a primary RNA transcript across the chromosome. The maturation of mRNA, as well as its abundance, occurs by post-transcriptional mechanisms [142, 143].

Based on that, the following approaches can be helpful for the future chemotherapy targets discovery. The genomic studies allow the identification of single-nucleotide polymorphisms (SNPs), copy number variations (CNVs), genomic rearrangements, and genomic annotations, using whole genome sequencing and *exome* techniques. The knowledge of biological targets can point to the selectivity of the drug and the use of known validated targets allowing a better understanding of their biological mechanisms. Gazanion et al. demonstrated by next-generation sequencing an unprecedented number of drug-resistance/target genes against all drugs currently used in leishmaniases chemotherapy [139]. This screening method can be useful to discover the drug targets and to understand the resistance mechanisms [139].

The transcriptomic studies have been extensively used allowing the discovery of mRNA stability, mRNA processing, and gene expression regulation, through microarrays or RNA-seq techniques [7, 8, 119]. Previous studies revealed sequence elements that control the abundance of mRNAs by influencing their maturation and stability. These changes in transcripts abundance during the life cycle of the parasite may lead to the identification of essential genes and thus pointing them as potential candidates for vaccine or drug targets [144]. A previous study demonstrated that the differentiation of promastigotes to amastigotes from *L. amazonensis* leads to a modulation of genes involved in the polyamine biosynthesis [8]. Furthermore, the absence of arginase activity in promastigotes of *L. amazonensis* leads to a differential level of metabolites from this pathway: citrulline, L-arginine, and L-glutamate increase levels, whereas aspartate, proline, ornithine, and putrescine decrease levels [8]. These findings reveal the importance of *L*-ARG in parasite survival and differentiation and indicate the existence of a coordinate response in the absence of *L*-ARG activity in the polyamine pathway.

The proteomic studies allow the discovery of cellular components, protein expression, post-translation modification, and protein interaction, using quantitative proteomics by two-dimensional gel electrophoresis (2DE), liquid chromatography mass spectrometry (LC-MS), or stable isotope labeling by amino acids in cell culture (SILAC). Post-translation modifications are of particular interest in *Leishmania* because the parasite regulates gene expression at post-transcriptional and post-translational levels [143]. The metabolomic studies allow the discovery of metabolite signatures through MS or nuclear magnetic resonance spectroscopy (NMR) methodologies. This approach can be used to evaluate how specific metabolites respond under different environmental or physiological conditions, providing interesting data about the mode of action and resistance mechanisms of drugs in parasitic protozoa [145]. The metabolome fingerprints obtained with *L. amazonensis* in the absence of *L*-ARG activity and/or under amino acids starvation demonstrated how *Leishmania* is able to use an alternative route to provide substrates for the polyamine pathway [115]. In addition, metabolome fingerprints of *L. infantum* resistant to antimonials showed metabolite profile modification in *Leishmania* pathways, corresponding mainly to amino acids or their alternative metabolites in the polyamine pathways with the thiol-dependent redox metabolism [146].

4. Concluding remarks

In the absence of effective vaccine and vector control, the eradication of leishmaniases is mostly dependent on chemotherapy. Besides other vials, such as the protein kinases, glycolytic

enzymes, and purine metabolism, studies involving the polyamine pathway have been increasing over the years due to its consideration as a promisor target for leishmaniases chemotherapy. The importance of this pathway for *Leishmania* replication is unquestionable, and the polyamine pathway exhibits significant differences compared to its host pathway. *Leishmania* amastigotes reside in the phagolysosome of host macrophage, but the ability to uptake polyamines may vary depending on the *Leishmania* species and/or the type of host macrophage. Then, the focus on the polyamine pathway as chemotherapeutic approaches, such as inhibition of polyamine transport, inhibition of L-arginine transport, inhibition of polyamine synthesis, inhibition of polyamine interconversion, or inhibition of NOS-like enzyme, may be considered in the future of leishmaniases chemotherapy.

Acknowledgements

Funding: Sao Paulo Research Foundation (FAPESP) (grants #2014/50717-1, #2016/03273-6 and #2016/19815-2).

Author details

Juliana Ide Aoki*, Sandra Marcia Muxel*, Juliane Cristina Ribeiro Fernandes and Lucile Maria Floeter-Winter

*Address all correspondence to: juaoki@usp.br and sandrammuxel@usp.br

Department of Physiology, Institute of Bioscience, University of São Paulo, São Paulo, Brazil

References

[1] Ashford RW. The leishmaniases as emerging and reemerging zoonoses. International Journal for Parasitology. 2000;**30**(12-13):1269-1281. PubMed PMID: 11113254

[2] WHO. Leishmaniasis. 2017. [updated April 2017; cited 2017]. Available from: http://www.who.int/mediacentre/factsheets/fs375/en/

[3] Ehehalt U, Schunk M, Jensenius M, van Genderen PJ, Gkrania-Klotsas E, Chappuis F, et al. Leishmaniasis acquired by travellers to endemic regions in Europe: A EuroTravNet multi-centre study. Travel Medicine and Infectious Disease. 2014;**12**(2):167-172. DOI: 10.1016/j.tmaid.2013.12.003. PubMed PMID: 24388687

[4] Alvar J, Aparicio P, Aseffa A, Den Boer M, Cañavate C, Dedet JP, et al. The relationship between leishmaniasis and AIDS: The second 10 years. Clinical Microbiology Reviews. 2008;**21**(2):334-59, table of contents. DOI: 10.1128/CMR.00061-07. PubMed PMID: 18400800; PubMed Central PMCID: PMCPMC2292576

[5] Bernier R, Turco SJ, Olivier M, Tremblay M. Activation of human immunodeficiency virus type 1 in monocytoid cells by the protozoan parasite Leishmania donovani. Journal

of Virology. 1995;**69**(11):7282-5. PubMed PMID: 7474154; PubMed Central PMCID: PMCPMC189654

[6] Rey L. Parasitologia. Guanabara Koogan: Brazil; 2008. 888 p

[7] Acuna SM, Aoki JI, Laranjeira da Silva MF, Zampieri RA, Fernandes JC, Muxel SM, et al. Arginase expression modulates nitric oxide production in Leishmania (Leishmania) amazonensis. PLoS One. 2017;**12**(11):1-21. e018786. DOI: 10.1371/journal.pone.0187186

[8] Aoki JI, Muxel SM, Zampieri RA, Laranjeira-Silva MF, Muller KE, Nerland AH, et al. RNA-seq transcriptional profiling of Leishmania amazonensis reveals an arginase-dependent gene expression regulation. PLoS Neglected Tropical Diseases. 2017;**11**(10):e0006026). DOI: 10.1371/journal.pntd.0006026. PubMed PMID: 29077741

[9] Saar Y, Ransford A, Waldman E, Mazareb S, Amin-Spector S, Plumblee J, et al. Characterization of developmentally-regulated activities in axenic amastigotes of Leishmania donovani. Molecular and Biochemical Parasitology. 1998;**95**(1):9-20. PubMed PMID: 9763285

[10] Bates PA, Robertson CD, Tetley L, Coombs GH. Axenic cultivation and characterization of Leishmania mexicana amastigote-like forms. Parasitology. 1992;**105**(Pt 2):193-202. PubMed PMID: 1454417

[11] Zilberstein D, Shapira M. The role of pH and temperature in the development of Leishmania parasites. Annual Review of Microbiology. 1994;**48**:449-470. DOI: 10.1146/annurev.mi.48.100194.002313. PubMed PMID: 7826014

[12] Muxel SM, Laranjeira-Silva MF, Zampieri RA, Floeter-Winter LM. Leishmania (Leishmania) amazonensis induces macrophage miR-294 and miR-721 expression and modulates infection by targeting NOS2 and L-arginine metabolism. Scientific Reports. 2017;**7**:44141. DOI: 10.1038/srep44141. PubMed PMID: 28276497; PubMed Central PMCID: PMC5343489

[13] Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(16):8841-8848. PubMed PMID: 10922044; PubMed Central PMCID: PMC34021

[14] Janeway CA Jr, Medzhitov R. Innate immune recognition. Annual Review of Immunology. 2002;**20**:197-216. DOI: 10.1146/annurev.immunol.20.083001.084359. PubMed PMID: 11861602

[15] Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007;**449**(7164):819-826. DOI: 10.1038/nature06246. PubMed PMID: 17943118

[16] Naderer T, McConville MJ. The Leishmania-macrophage interaction: A metabolic perspective. Cellular Microbiology. 2008;**10**(2):301-308. DOI: 10.1111/j.1462-5822.2007.01096.x. PubMed PMID: 18070117

[17] Matte C, Maion G, Mourad W, Olivier M. Leishmania donovani-induced macrophages cyclooxygenase-2 and prostaglandin E2 synthesis. Parasite Immunology. 2001;**23**(4):177-184. PubMed PMID: 11298294

[18] Gregory DJ, Olivier M. Subversion of host cell signalling by the protozoan parasite Leishmania. Parasitology. 2005;**130**(Suppl):S27-S35. DOI: 10.1017/S0031182005008139. PubMed PMID: 16281989

[19] Nasseri M, Modabber FZ. Generalized infection and lack of delayed hypersensitivity in BALB/c mice infected with Leishmania tropica major. Infection and Immunity. 1979;**26**(2):611-614. PubMed PMID: 546790; PubMed Central PMCID: PMC414661

[20] Bacellar O, D'Oliveira A Jr, Jeronimo S, Carvalho EM. IL-10 and IL-12 are the main regulatory cytokines in visceral leishmaniasis. Cytokine. 2000;**12**(8):1228-1231. DOI: 10.1006/cyto.2000.0694. PubMed PMID: 10930301

[21] Kane MM, Mosser DM. The role of IL-10 in promoting disease progression in leishmaniasis. Journal of Immunology. 2001;**166**(2):1141-1147. PubMed PMID: 11145695

[22] Yang Z, Mosser DM, Zhang X. Activation of the MAPK, ERK, following Leishmania amazonensis infection of macrophages. Journal of Immunology. 2007;**178**(2):1077-1085. PubMed PMID: 17202371; PubMed Central PMCID: PMC2643020

[23] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends in immunology. 2004;**25**(12):677-686. DOI: 10.1016/j.it.2004.09.015. PubMed PMID: 15530839

[24] Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage m1-m2 polarization balance. Frontiers in Immunology. 2014;**5**:614. DOI: 10.3389/fimmu.2014.00614. PubMed PMID: 25506346; PubMed Central PMCID: PMC4246889

[25] Hrabak A, Bajor T, Temesi A, Meszaros G. The inhibitory effect of nitrite, a stable product of nitric oxide (NO) formation, on arginase. FEBS Letters. 1996;**390**(2):203-206. PubMed PMID: 8706860

[26] Boucher JL, Moali C, Tenu JP. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. Cellular and Molecular Life Sciences. 1999;**55**(8-9):1015-1028. PubMed PMID: 10484661

[27] Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: An immunologic functional perspective. Annual Review of Immunology. 2009;**27**:451-483. DOI: 10.1146/annurev.immunol.021908.132532. PubMed PMID: 19105661

[28] Holscher C, Arendse B, Schwegmann A, Myburgh E, Brombacher F. Impairment of alternative macrophage activation delays cutaneous leishmaniasis in nonhealing BALB/c mice. Journal of Immunology. 2006;**176**(2):1115-1121. PubMed PMID: 16394000

[29] Steck EA. The leishmaniases. Progress in Drug Research. 1974;**18**:289-351. PubMed PMID: 4453608

[30] Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. Lancet. 2005;**366**(9496):1561-1577. DOI: 10.1016/S0140-6736(05)67629-5. PubMed PMID: 16257344

[31] Basselin M, Badet-Denisot MA, Lawrence F, Robert-Gero M. Effects of pentamidine on polyamine level and biosynthesis in wild-type, pentamidine-treated, and pentamidine-resistant Leishmania. Experimental Parasitology. 1997;**85**(3):274-282. DOI: 10.1006/expr.1996.4131. PubMed PMID: 9085924

[32] Bachrach U, Brem S, Wertman SB, Schnur LF, Greenblatt CL. Leishmania spp.: Effect of inhibitors on growth and on polyamine and macromolecular syntheses. Experimental Parasitology. 1979;**48**(3):464-470. PubMed PMID: 510448

[33] Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clinical Microbiology Reviews. 2006;**19**(1):111-126. DOI: 10.1128/CMR.19.1.111-126.2006. PubMed PMID: 16418526; PubMed Central PMCID: PMCPMC1360270

[34] Croft SL, Coombs GH. Leishmaniasis—Current chemotherapy and recent advances in the search for novel drugs. Trends in Parasitology. 2003;**19**(11):502-508. PubMed PMID: 14580961

[35] Jha TK, Sundar S, Thakur CP, Bachmann P, Karbwang J, Fischer C, et al. Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. The New England Journal of Medicine. 1999;**341**(24):1795-1800. DOI: 10.1056/NEJM199912093412403. PubMed PMID: 10588964

[36] Soto J, Arana BA, Toledo J, Rizzo N, Vega JC, Diaz A, et al. Miltefosine for new world cutaneous leishmaniasis. Clinical Infectious Diseases. 2004;**38**(9):1266-1272. DOI: 10.1086/383321. PubMed PMID: 15127339

[37] Soto J, Berman J. Treatment of new world cutaneous leishmaniasis with miltefosine. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;**100**(Suppl 1):S34-S40. DOI: 10.1016/j.trstmh.2006.02.022. PubMed PMID: 16930649

[38] Soto J, Toledo JT. Oral miltefosine to treat new world cutaneous leishmaniasis. The Lancet Infectious Diseases. 2007;**7**(1):7. DOI: 10.1016/S1473-3099(06)70665-X. PubMed PMID: 17182338

[39] Sinha PK, Jha TK, Thakur CP, Nath D, Mukherjee S, Aditya AK, et al. Phase 4 pharmacovigilance trial of paromomycin injection for the treatment of visceral leishmaniasis in India. Journal of Tropical Medicine. 2011;**2011**:645203. DOI: 10.1155/2011/645203. PubMed PMID: 22174722; PubMed Central PMCID: PMCPMC3235903

[40] Saenz RE, Paz H, Berman JD. Efficacy of ketoconazole against Leishmania braziliensis panamensis cutaneous leishmaniasis. The American Journal of Medicine. 1990;**89**(2):147-155. PubMed PMID: 2166429

[41] Berman JD, Holz GG Jr, Beach DH. Effects of ketoconazole on growth and sterol biosynthesis of Leishmania mexicana promastigotes in culture. Molecular and Biochemical Parasitology. 1984;**12**(1):1-13. PubMed PMID: 6087138

[42] Nagle AS, Khare S, Kumar AB, Supek F, Buchynskyy A, Mathison CJ, et al. Recent developments in drug discovery for leishmaniasis and human African trypanosomiasis. Chemical Reviews. 2014;**114**(22):11305-11347. DOI: 10.1021/cr500365f. PubMed PMID: 25365529; PubMed Central PMCID: PMCPMC4633805

[43] Müller S, Coombs GH, Walter RD. Targeting polyamines of parasitic protozoa in chemotherapy. Trends in Parasitology. 2001;**17**(5):242-249. PubMed PMID: 11323309

[44] Chulay JD, Fleckenstein L, Smith DH. Pharmacokinetics of antimony during treatment of visceral leishmaniasis with sodium stibogluconate or meglumine antimoniate. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1988;**82**(1):69-72. PubMed PMID: 2845611

[45] Olliaro PL, Bryceson AD. Practical progress and new drugs for changing patterns of leishmaniasis. Parasitology Today. 1993;**9**(9):323-328. PubMed PMID: 15463794

[46] Berman JD. Human leishmaniasis: Clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clinical Infectious Diseases. 1997;**24**(4):684-703. PubMed PMID: 9145744

[47] Berman JD, Waddell D, Hanson BD. Biochemical mechanisms of the antileishmanial activity of sodium stibogluconate. Antimicrobial Agents and Chemotherapy. 1985;**27**(6):916-920. PubMed PMID: 2411217

[48] Sereno D, Holzmuller P, Mangot I, Cuny G, Ouaissi A, Lemesre JL. Antimonial-mediated DNA fragmentation in Leishmania infantum amastigotes. Antimicrobial Agents and Chemotherapy. 2001;**45**(7):2064-2069. DOI: 10.1128/AAC.45.7.2064-2069.2001. PubMed PMID: 11408224; PubMed Central PMCID: PMCPMC90601

[49] Thakur CP, Kumar M, Pandey AK. Evaluation of efficacy of longer durations of therapy of fresh cases of kala-azar with sodium stibogluconate. The Indian Journal of Medical Research. 1991;**93**:103-110. PubMed PMID: 1649790

[50] Sundar S. Drug resistance in Indian visceral leishmaniasis. Tropical Medicine & International Health. 2001;**6**(11):849-854. PubMed PMID: 11703838

[51] Roussel M, Nacher M, Frémont G, Rotureau B, Clyti E, Sainte-Marie D, et al. Comparison between one and two injections of pentamidine isethionate, at 7 mg/kg in each injection, in the treatment of cutaneous leishmaniasis in French Guiana. Annals of Tropical Medicine and Parasitology. 2006;**100**(4):307-314. DOI: 10.1179/136485906X105561. PubMed PMID: 16762111

[52] Bitonti AJ, Dumont JA, McCann PP. Characterization of Trypanosoma brucei brucei S-adenosyl-L-methionine decarboxylase and its inhibition by Berenil, pentamidine and methylglyoxal bis(guanylhydrazone). The Biochemical Journal. 1986;**237**(3):685-689. PubMed PMID: 3800910; PubMed Central PMCID: PMCPMC1147045

[53] Basselin M, Robert-Gero M. Alterations in membrane fluidity, lipid metabolism, mitochondrial activity, and lipophosphoglycan expression in pentamidine-resistant Leishmania. Parasitology Research. 1998;**84**(1):78-83. PubMed PMID: 9491432

[54] Vercesi AE, Docampo R. Ca^{2+} transport by digitonin-permeabilized Leishmania donovani. Effects of Ca^{2+}, pentamidine and WR-6026 on mitochondrial membrane potential in situ. Biochemical Journal. 1992;**284**(Pt 2):463-467. PubMed PMID: 1376113; PubMed Central PMCID: PMCPMC1132661

[55] Croft SL, Brazil RP. Effect of pentamidine isethionate on the ultrastructure and morphology of Leishmania mexicana amazonensis in vitro. Annals of Tropical Medicine and Parasitology. 1982;**76**(1):37-43. PubMed PMID: 7082077

[56] Kandpal M, Fouce RB, Pal A, Guru PY, Tekwani BL. Kinetics and molecular characteristics of arginine transport by Leishmania donovani promastigotes. Molecular and Biochemical Parasitology. 1995;**71**(2):193-201. PubMed PMID: 7477101

[57] Reguera R, Balaña Fouce R, Cubria JC, Alvarez Bujidos ML, Ordoñez D. Putrescine uptake inhibition by aromatic diamidines in Leishmania infantum promastigotes. Biochemical Pharmacology. 1994;**47**(10):1859-1866. PubMed PMID: 8204103

[58] Basselin M, Coombs GH, Barrett MP. Putrescine and spermidine transport in Leishmania. Molecular and Biochemical Parasitology. 2000;**109**(1):37-46. PubMed PMID: 10924755

[59] Basselin M, Denise H, Coombs GH, Barrett MP. Resistance to pentamidine in Leishmania mexicana involves exclusion of the drug from the mitochondrion. Antimicrobial Agents and Chemotherapy. 2002;**46**(12):3731-3738. PubMed PMID: 12435669; PubMed Central PMCID: PMCPMC132791

[60] Olliaro PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen JA, Sundar S. Treatment options for visceral leishmaniasis: A systematic review of clinical studies done in India, 1980-2004. The Lancet Infectious Diseases. 2005;**5**(12):763-774. DOI: 10.1016/S1473-3099(05)70296-6. PubMed PMID: 16310148

[61] Thakur CP, Sinha GP, Pandey AK, Barat D, Sinha PK. Amphotericin B in resistant kala-azar in Bihar. National Medical Journal of India. 1993;**6**(2):57-60. PubMed PMID: 8477209

[62] Sundar S, Goyal AK, More DK, Singh MK, Murray HW. Treatment of antimony-unresponsive Indian visceral leishmaniasis with ultra-short courses of amphotericin-B-lipid complex. Annals of Tropical Medicine and Parasitology. 1998;**92**(7):755-764. PubMed PMID: 9924533

[63] Thakur CP, Kumar P, Kumar N, Singh GN, Singh AK, Narain S. A randomized comparison of classical mode of administration of amphotericin B with its newer modes of administration in kala-azar. The Journal of the Association of Physicians of India. 1998;**46**(9):779-783. PubMed PMID: 11229246

[64] Berman JD, Badaro R, Thakur CP, Wasunna KM, Behbehani K, Davidson R, et al. Efficacy and safety of liposomal amphotericin B (AmBisome) for visceral leishmaniasis in endemic developing countries. Bulletin of the World Health Organization. 1998;**76**(1): 25-32. PubMed PMID: 9615494; PubMed Central PMCID: PMCPMC2305623

[65] Thakur CP. A single high dose treatment of kala-azar with Ambisome (amphotericin B lipid complex): A pilot study. International Journal of Antimicrobial Agents. 2001;**17**(1):67-70. PubMed PMID: 11137652

[66] Ramos H, Valdivieso E, Gamargo M, Dagger F, Cohen BE. Amphotericin B kills unicellular leishmanias by forming aqueous pores permeable to small cations and anions. The Journal of Membrane Biology. 1996;**152**(1):65-75. PubMed PMID: 8660406

[67] Sundar S, Olliaro PL. Miltefosine in the treatment of leishmaniasis: Clinical evidence for informed clinical risk management. Therapeutics and Clinical Risk Management. 2007;**3**(5):733-40. PubMed PMID: 18472998; PubMed Central PMCID: PMCPMC2376078

[68] Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, et al. Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. Clinical Infectious Diseases. 2012;**55**(4):543-550. DOI: 10.1093/cid/cis474. PubMed PMID: 22573856

[69] Pérez-Victoria FJ, Sánchez-Cañete MP, Seifert K, Croft SL, Sundar S, Castanys S, et al. Mechanisms of experimental resistance of Leishmania to miltefosine: Implications for clinical use. Drug Resistance Updates. 2006;**9**(1-2):26-39. DOI: 10.1016/j.drup.2006.04.001. PubMed PMID: 16814199

[70] Rakotomanga M, Blanc S, Gaudin K, Chaminade P, Loiseau PM. Miltefosine affects lipid metabolism in Leishmania donovani promastigotes. Antimicrobial Agents and Chemotherapy. 2007;**51**(4):1425-1430. DOI: 10.1128/AAC.01123-06. PubMed PMID: 17242145; PubMed Central PMCID: PMCPMC1855451

[71] Coelho AC, Boisvert S, Mukherjee A, Leprohon P, Corbeil J, Ouellette M. Multiple mutations in heterogeneous miltefosine-resistant Leishmania major population as determined by whole genome sequencing. PLoS Neglected Tropical Diseases. 2012;**6**(2):e1512. DOI: 10.1371/journal.pntd.0001512. PubMed PMID: 22348164; PubMed Central PMCID: PMCPMC3279362

[72] Coelho AC, Trinconi CT, Costa CH, Uliana SR. In vitro and in vivo miltefosine susceptibility of a Leishmania amazonensis isolate from a patient with diffuse cutaneous leishmaniasis. PLoS Neglected Tropical Diseases. 2014;**8**(7):e2999. DOI: 10.1371/journal.pntd.0002999. PubMed PMID: 25033218; PubMed Central PMCID: PMCPMC4102453

[73] Bryan LE, Kwan S. Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. Antimicrobial Agents and Chemotherapy. 1983;**23**(6):835-845. PubMed PMID: 6351731; PubMed Central PMCID: PMC184978

[74] Jhingran A, Chawla B, Saxena S, Barrett MP, Madhubala R. Paromomycin: Uptake and resistance in Leishmania donovani. Molecular and Biochemical Parasitology. 2009;**164**(2):111-117. DOI: 10.1016/j.molbiopara.2008.12.007. PubMed PMID: 19146886; PubMed Central PMCID: PMC3039421

[75] Sundar S, Jha TK, Thakur CP, Sinha PK, Bhattacharya SK. Injectable paromomycin for visceral leishmaniasis in India. The New England Journal of Medicine. 2007;**356**(25):2571-2581. DOI: 10.1056/NEJMoa066536. PubMed PMID: 17582067

[76] Chawla B, Madhubala R. Drug targets in Leishmania. Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology. 2010;**34**(1):1-13. DOI: 10.1007/s12639-010-0006-3. PubMed PMID: 21526026; PubMed Central PMCID: PMC3081701

[77] Chawla B, Jhingran A, Panigrahi A, Stuart KD, Madhubala R. Paromomycin affects translation and vesicle-mediated trafficking as revealed by proteomics of paromomycin-susceptible-resistant Leishmania donovani. PLoS One. 2011;**6**(10):e26660. DOI: 10.1371/journal.pone.0026660. PubMed PMID: 22046323; PubMed Central PMCID: PMCPMC3203147

[78] Verma A, Bhandari V, Deep DK, Sundar S, Dujardin JC, Singh R, et al. Transcriptome profiling identifies genes/pathways associated with experimental resistance to paromomycin in Leishmania donovani. International Journal for Parasitology Drugs and Drug Resistance. 2017;**7**(3):370-377. DOI: 10.1016/j.ijpddr.2017.10.004. PubMed PMID: 29035735; PubMed Central PMCID: PMC5645162

[79] Dedet JP, Jamet P, Esterre P, Ghipponi PM, Genin C, Lalande G. Failure to cure Leishmania braziliensis guyanensis cutaneous leishmaniasis with oral ketoconazole. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1986;**80**(1):176. PubMed PMID: 3726992

[80] Dan M, Verner E, El-On J, Zuckerman F, Michaeli D. Failure of oral ketoconazole to cure cutaneous ulcers caused by Leishmania braziliensis. Cutis. 1986;**38**(3):198-199. PubMed PMID: 3769557

[81] Pirson P, Leclef B, Trouet A. Activity of ketoconazole derivatives against Leishmania mexicana amazonensis within mouse peritoneal macrophages. Annals of Tropical Medicine and Parasitology. 1990;**84**(2):133-139. PubMed PMID: 2383093

[82] Nunes DCO, Bispo-da-Silva LB, Napolitano DR, Costa MS, Figueira M, Rodrigues RS, et al. In vitro additive interaction between ketoconazole and antimony against intramacrophage Leishmania (Leishmania) amazonensis amastigotes. PLoS One. 2017;**12**(6):e0180530. DOI: 10.1371/journal.pone.0180530. PubMed PMID: 28662149; PubMed Central PMCID: PMC5491259

[83] Bates PA, Tetley L. Leishmania mexicana: Induction of metacyclogenesis by cultivation of promastigotes at acidic pH. Experimental Parasitology. 1993;**76**(4):412-423. DOI: 10.1006/expr.1993.1050. PubMed PMID: 8513879

[84] Krobitsch S, Brandau S, Hoyer C, Schmetz C, Hubel A, Clos J. Leishmania donovani heat shock protein 100. Characterization and function in amastigote stage differentiation. Journal of Biological Chemistry. 1998;**273**(11):6488-6494. PubMed PMID: 9497383

[85] Hombach A, Ommen G, MacDonald A, Clos J. A small heat shock protein is essential for thermotolerance and intracellular survival of Leishmania donovani. Journal of Cell Science. 2014;**127**(Pt 21):4762-4773. DOI: 10.1242/jcs.157297. PubMed PMID: 25179594; PubMed Central PMCID: PMC4215717

[86] Naula C, Parsons M, Mottram JC. Protein kinases as drug targets in trypanosomes and Leishmania. Biochimica et Biophysica Acta. 2005;**1754**(1-2):151-159. DOI: 10.1016/j.bbapap.2005.08.018. PubMed PMID: 16198642; PubMed Central PMCID: PMCPMC1452262

[87] Hanks SK, Quinn AM. Protein kinase catalytic domain sequence database: Identification of conserved features of primary structure and classification of family members. Methods in Enzymology. 1991;**200**:38-62. PubMed PMID: 1956325

[88] Cardenas D, Carter PM, Nation CS, Pizarro JC, Guidry J, Aiyar A, et al. LACK, a RACK1 ortholog, facilitates cytochrome c oxidase subunit expression to promote Leishmania major fitness. Molecular Microbiology. 2015;**96**(1):95-109. DOI: 10.1111/mmi.12924. PubMed PMID: 25582232

[89] Sanderson L, Yardley V, Croft SL. Activity of anti-cancer protein kinase inhibitors against Leishmania spp. The Journal of Antimicrobial Chemotherapy. 2014;**69**(7):1888-1891. DOI: 10.1093/jac/dku069. PubMed PMID: 24668412

[90] Tirado-Duarte D, Marín-Villa M, Ochoa R, Blandón-Fuentes G, Soares MJ, Robledo SM, et al. The Akt-like kinase of Leishmania panamensis: As a new molecular target for drug discovery. Acta Tropica. 2018;**177**:171-178. DOI: 10.1016/j.actatropica.2017.10.008. PubMed PMID: 29037519

[91] Verlinde CL, Hannaert V, Blonski C, Willson M, Perie JJ, Fothergill-Gilmore LA, et al. Glycolysis as a target for the design of new anti-trypanosome drugs. Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy. 2001;**4**(1):50-65. DOI: 10.1054/drup.2000.0177. PubMed PMID: 11512153

[92] Kim H, Feil IK, Verlinde CL, Petra PH, Hol WG. Crystal structure of glycosomal glyceraldehyde-3-phosphate dehydrogenase from Leishmania mexicana: Implications for structure-based drug design and a new position for the inorganic phosphate binding site. Biochemistry. 1995;**34**(46):14975-14986. PubMed PMID: 7578111

[93] Aronov AM, Suresh S, Buckner FS, Van Voorhis WC, Verlinde CL, Opperdoes FR, et al. Structure-based design of submicromolar, biologically active inhibitors of trypanosomatid glyceraldehyde-3-phosphate dehydrogenase. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**(8):4273-4278. PubMed PMID: 10200252; PubMed Central PMCID: PMC16322

[94] Bressi JC, Verlinde CL, Aronov AM, Shaw ML, Shin SS, Nguyen LN, et al. Adenosine analogues as selective inhibitors of glyceraldehyde-3-phosphate dehydrogenase of Trypanosomatidae via structure-based drug design. Journal of Medicinal Chemistry. 2001;**44**(13):2080-2093. PubMed PMID: 11405646; PubMed Central PMCID: PMC2957370

[95] Suresh S, Bressi JC, Kennedy KJ, Verlinde CL, Gelb MH, Hol WG. Conformational changes in Leishmania mexicana glyceraldehyde-3-phosphate dehydrogenase induced by designed inhibitors. Journal of Molecular Biology. 2001;**309**(2):423-435. DOI: 10.1006/jmbi.2001.4588. PubMed PMID: 11371162

[96] Hammond DJ, Gutteridge WE. Purine and pyrimidine metabolism in the Trypanoso matidae. Molecular and Biochemical Parasitology. 1984;**13**(3):243-261. PubMed PMID: 6396514

[97] Landfear SM, Ullman B, Carter NS, Sanchez MA. Nucleoside and nucleobase transporters in parasitic protozoa. Eukaryotic Cell. 2004;**3**(2):245-254. PubMed PMID: 15075255; PubMed Central PMCID: PMCPMC387651

[98] Aoki JI, Yamashiro-Kanashiro EH, Ramos DCC, Cotrim PC. Efficacy of the tubercidin antileishmania action associated with an inhibitor of the nucleoside transport. Parasitology Research. 2009;**104**(2):223-228. DOI: 10.1007/s00436-008-1177-z. PubMed PMID: WOS: 000262652600004

[99] Aoki JI, Coelho AC, Muxel SM, Zampieri RA, Sanchez EM, Nerland AH, et al. Characterization of a novel endoplasmic reticulum protein involved in Tubercidin resistance in Leishmania major. PLoS Neglected Tropical Diseases. 2016;**10**(9):e0004972. DOI: 10.1371/journal.pntd.0004972. PubMed PMID: 27606425; PubMed Central PMCID: PMCPMC5015992

[100] el Kouni MH. Potential chemotherapeutic targets in the purine metabolism of parasites. Pharmacology & Therapeutics. 2003;**99**(3):283-309. PubMed PMID: 12951162

[101] Baer HP, Serignese V, Ogbunude PO, Dzimiri M. Nucleoside transporters in Leishmania major: Diversity in adenosine transporter expression or function in different strains. The American Journal of Tropical Medicine and Hygiene. 1992;**47**(1):87-91. PubMed PMID: 1636887

[102] da Silva MF, Zampieri RA, Muxel SM, Beverley SM, Floeter-Winter LM. Leishmania amazonensis arginase compartmentalization in the glycosome is important for parasite infectivity. PLoS One. 2012;**7**(3):e34022. DOI: 10.1371/journal.pone.0034022. PubMed PMID: 22479507; PubMed Central PMCID: PMC3316525

[103] Vieira LL, Cabantchik ZI. Amino acid uptake and intracellular accumulation in Leishmania major promastigotes are largely determined by an H(+)-pump generated membrane potential. Molecular and Biochemical Parasitology. 1995;**75**(1):15-23. PubMed PMID: 8720171

[104] Roberts SC, Tancer MJ, Polinsky MR, Gibson KM, Heby O, Ullman B. Arginase plays a pivotal role in polyamine precursor metabolism in Leishmania. Characterization of gene deletion mutants. Journal of Biological Chemistry. 2004;**279**(22):23668-23678. DOI: 10.1074/jbc.M402042200. PubMed PMID: 15023992

[105] Laranjeira-Silva MF, Floeter-Winter LM. Arginase in *Leishmania*. In: Andre LS Santos MHB, d'Avila-Levy CM, Kneipp LF, Sodre CL, editors. Proteins and Proteomics of Leishmania and Trypanossoma. Subcellular Biochemistry. Vol. 67. Springer: Springer; 2014. pp. 103-118

[106] Boitz JM, Gilroy CA, Olenyik TD, Paradis D, Perdeh J, Dearman K, et al. Arginase is essential for survival of Leishmania donovani promastigotes but not intracellular amastigotes. Infection and Immunity. 2017;**85**(1). DOI: 10.1128/IAI.00554-16. PubMed PMID: 27795357

[107] Camargo EP, Coelho JA, Moraes G, Figueiredo EN. Trypanosoma spp., Leishmania spp. and Leptomonas spp.: Enzymes of ornithine-arginine metabolism. Experimental Parasitology. 1978;**46**(2):141-144. PubMed PMID: 569593

[108] Mukhopadhyay R, Madhubala R. Effects of bis(benzyl)polyamine analogs on Leishmania donovani promastigotes. Experimental Parasitology. 1995;**81**(1):39-46. DOI: 10.1006/expr.1995.1090. PubMed PMID: 7628565

[109] Wanasen N, Soong L. L-arginine metabolism and its impact on host immunity against Leishmania infection. Immunologic Research. 2008;**41**(1):15-25. DOI: 10.1007/s12026-007-8012-y. PubMed PMID: 18040886; PubMed Central PMCID: PMC2639710

[110] Wanasen N, MacLeod CL, Ellies LG, Soong L. L-arginine and cationic amino acid transporter 2B regulate growth and survival of Leishmania amazonensis amastigotes in macrophages. Infection and Immunity. 2007;**75**(6):2802-2810. DOI: 10.1128/IAI.00026-07. PubMed PMID: 17387163; PubMed Central PMCID: PMC1932894

[111] Oza SL, Shaw MP, Wyllie S, Fairlamb AH. Trypanothione biosynthesis in Leishmania major. Molecular and Biochemical Parasitology. 2005;**139**(1):107-116. DOI: 10.1016/j.molbiopara.2004.10.004. PubMed PMID: 15610825

[112] Krauth-Siegel RL, Meiering SK, Schmidt H. The parasite-specific trypanothione metabolism of trypanosoma and leishmania. Biological Chemistry. 2003;**384**(4):539-549. DOI: 10.1515/BC.2003.062. PubMed PMID: 12751784

[113] Genestra M, Souza WJ, Guedes-Silva D, Machado GM, Cysne-Finkelstein L, Bezerra RJ, et al. Nitric oxide biosynthesis by Leishmania amazonensis promastigotes containing a high percentage of metacyclic forms. Archives of Microbiology. 2006;**185**(5):348-354. DOI: 10.1007/s00203-006-0105-9. PubMed PMID: 16575586

[114] Genestra M, Guedes-Silva D, Souza WJ, Cysne-Finkelstein L, Soares-Bezerra RJ, Monteiro FP, et al. Nitric oxide synthase (NOS) characterization in Leishmania amazonensis axenic amastigotes. Archives of Medical Research. 2006;**37**(3):328-333. DOI: 10.1016/j.arcmed.2005.07.011. PubMed PMID: 16513480

[115] Castilho-Martins EA, Canuto GA, Muxel SM, da Silva MF, Floeter-Winter LM, Del Aguila C, et al. Capillary electrophoresis reveals polyamine metabolism modulation in Leishmania (Leishmania) amazonensis wild type and arginase knockout mutants under arginine starvation. Electrophoresis. 2015. DOI: 10.1002/elps.201500114. PubMed PMID: 26202519

[116] Darlyuk I, Goldman A, Roberts SC, Ullman B, Rentsch D, Zilberstein D. Arginine homeostasis and transport in the human pathogen Leishmania donovani. The Journal of Biological Chemistry. 2009;**284**(30):19800-19807. DOI: 10.1074/jbc.M901066200. PubMed PMID: 19439418; PubMed Central PMCID: PMC2740405

[117] Landfear SM. Nutrient transport and pathogenesis in selected parasitic protozoa. Eukaryotic Cell. 2011;**10**(4):483-493. DOI: 10.1128/EC.00287-10. PubMed PMID: 21216940; PubMed Central PMCID: PMC3127635

[118] Goldman-Pinkovich A, Balno C, Strasser R, Zeituni-Molad M, Bendelak K, Rentsch D, et al. An arginine deprivation response pathway is induced in Leishmania during macrophage invasion. PLoS Pathogens. 2016;**12**(4):e1005494. DOI: 10.1371/journal.ppat.1005494. PubMed PMID: 27043018; PubMed Central PMCID: PMC4846328

[119] Aoki JI, Muxel SM, Zampieri RA, Acuna SM, Fernandes JCR, Vanderlinde RH, et al. L-arginine availability and arginase activity: Characterization of amino acid permease 3 in Leishmania amazonensis. PLoS Neglected Tropical Diseases. 2017;**11**(10):e0006025. DOI: 10.1371/journal.pntd.0006025. PubMed PMID: 29073150

[120] Castilho-Martins EA, Laranjeira da Silva MF, dos Santos MG, Muxel SM, Floeter-Winter LM. Axenic Leishmania amazonensis promastigotes sense both the external and internal arginine pool distinctly regulating the two transporter-coding genes. PLoS One. 2011;**6**(11):e27818. DOI: 10.1371/journal.pone.0027818. PubMed PMID: 22114701; PubMed Central PMCID: PMC3218042

[121] Wu G, Morris SM Jr. Arginine metabolism: Nitric oxide and beyond. The Biochemical Journal. 1998;**336**(Pt1):1-17. PubMed PMID: 9806879; PubMed Central PMCID: PMC 1219836

[122] da Silva ER, da Silva MF, Fischer H, Mortara RA, Mayer MG, Framesqui K, et al. Biochemical and biophysical properties of a highly active recombinant arginase from Leishmania (Leishmania) amazonensis and subcellular localization of native enzyme. Molecular and Biochemical Parasitology. 2008;**159**(2):104-111. DOI: 10.1016/j.molbiopara.2008.02.011. PubMed PMID: 18400316

[123] Opperdoes FR, Szikora JP. In silico prediction of the glycosomal enzymes of Leishmania major and trypanosomes. Molecular and Biochemical Parasitology. 2006;**147**(2):193-206. DOI: 10.1016/j.molbiopara.2006.02.010. PubMed PMID: 16546274

[124] Gualdrón-López M, Brennand A, Hannaert V, Quiñones W, Cáceres AJ, Bringaud F, et al. When, how and why glycolysis became compartmentalised in the Kinetoplastea. A new look at an ancient organelle. International Journal for Parasitology. 2012;**42**(1): 1-20. DOI: 10.1016/j.ijpara.2011.10.007. PubMed PMID: 22142562

[125] Closs EI, Simon A, Vekony N, Rotmann A. Plasma membrane transporters for arginine. The Journal of Nutrition. 2004;**134**(10 Suppl):2752S-2759S; discussion 65S-67S. PubMed PMID: 15465780

[126] Laranjeira-Silva MF, Zampieri RA, Muxel SM, Floeter-Winter LM, Markus RP. Melatonin attenuates Leishmania (L.) amazonensis infection by modulating arginine metabolism. Journal of Pineal Research. 2015. DOI: 10.1111/jpi.12279. PubMed PMID: 26383232

[127] Singh S, Mukherjee A, Khomutov AR, Persson L, Heby O, Chatterjee M, et al. Antileishmanial effect of 3-aminooxy-1-aminopropane is due to polyamine depletion. Antimicrobial Agents and Chemotherapy. 2007;**51**(2):528-534. DOI: 10.1128/AAC.01055-06. PubMed PMID: 17101681; PubMed Central PMCID: PMC1797741

[128] Van Nieuwenhove S, Schechter PJ, Declercq J, Boné G, Burke J, Sjoerdsma A. Treatment of gambiense sleeping sickness in the Sudan with oral DFMO (DL-alpha-difluoromethylornithine), an inhibitor of ornithine decarboxylase; first field trial. Transactions of the Royal Society of Tropical Medicine and Hygiene 1985;**79**(5):692-698. PubMed PMID: 3938090

[129] Gradoni L, Iorio MA, Gramiccia M, Orsini S. In vivo effect of eflornithine (DFMO) and some related compounds on Leishmania infantum preliminary communication. Farmaco. 1989;**44**(12):1157-1166. PubMed PMID: 2517472

[130] Keithly JS. FAH. Inhibition of Leishmania Species by α-Difluoromethylornithine. In: Hart DT, editor. Leishmaniasis. NATO ASI Series (Series A: Life Sciences). Boston, MA: Springer; 1989

[131] Kaur K, Emmett K, McCann PP, Sjoerdsma A, Ullman B. Effects of DL-alpha-difluoromethylornithine on Leishmania donovani promastigotes. The Journal of Protozoology. 1986;**33**(4):518-521. PubMed PMID: 3098971

[132] Fairlamb AH, Blackburn P, Ulrich P, Chait BT, Cerami A. Trypanothione: A novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids. Science. 1985;**227**(4693):1485-1487. PubMed PMID: 3883489

[133] Carrillo C, Cejas S, Cortés M, Ceriani C, Huber A, González NS, et al. Sensitivity of trypanosomatid protozoa to DFMO and metabolic turnover of ornithine decarboxylase. Biochemical and Biophysical Research Communications. 2000;**279**(2):663-668. DOI: 10.1006/bbrc.2000.3996. PubMed PMID: 11118342

[134] da Silva ER, Castilho TM, Pioker FC, Tomich de Paula Silva CH, Floeter-Winter LM. Genomic organisation and transcription characterisation of the gene encoding Leishmania (Leishmania) amazonensis arginase and its protein structure prediction. International Journal for Parasitology 2002;**32**(6):727-737. PubMed PMID: 12062491

[135] Gilroy C, Olenyik T, Roberts SC, Ullman B. Spermidine synthase is required for virulence of Leishmania donovani. Infection and Immunity. 2011;**79**(7):2764-2769. DOI: 10.1128/IAI.00073-11. PubMed PMID: 21536795; PubMed Central PMCID: PMC3191959

[136] Roberts SC, Jiang Y, Jardim A, Carter NS, Heby O, Ullman B. Genetic analysis of spermidine synthase from Leishmania donovani. Molecular and Biochemical Parasitology. 2001;**115**(2):217-226. PubMed PMID: 11420108

[137] Roberts SC, Scott J, Gasteier JE, Jiang Y, Brooks B, Jardim A, et al. S-adenosylmethionine decarboxylase from Leishmania donovani. Molecular, genetic, and biochemical characterization of null mutants and overproducers. Journal of Biological Chemistry. 2002;**277**(8):5902-5909. DOI: 10.1074/jbc.M110118200. PubMed PMID: 11734561

[138] Roberts S, Ullman B. Parasite polyamines as pharmaceutical targets. Current Pharmaceutical Design. 2017;**23**(23):3325-3341. DOI: 10.2174/1381612823666170601101644. PubMed PMID: 28571553

[139] Gazanion É, Fernández-Prada C, Papadopoulou B, Leprohon P, Ouellette M. Cos-Seq for high-throughput identification of drug target and resistance mechanisms in the protozoan parasite Leishmania. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**(21):E3012-E3021. DOI: 10.1073/pnas.1520693113. PubMed PMID: 27162331; PubMed Central PMCID: PMCPMC4889358

[140] Fiebig M, Kelly S, Gluenz E. Comparative life cycle transcriptomics revises Leishmania mexicana genome annotation and links a chromosome duplication with parasitism of vertebrates. PLoS Pathogens. 2015;**11**(10):e1005186. DOI: 10.1371/journal.ppat.1005186. PubMed PMID: 26452044; PubMed Central PMCID: PMC4599935

[141] Alonso G, Rastrojo A, Lopez-Perez S, Requena JM, Aguado B. Resequencing and assembly of seven complex loci to improve the Leishmania major (Friedlin strain) reference genome. Parasites & Vectors. 2016;**9**:74. DOI: 10.1186/s13071-016-1329-4. PubMed PMID: 26857920; PubMed Central PMCID: PMC4746890

[142] Stiles JK, Hicock PI, Shah PH, Meade JC. Genomic organization, transcription, splicing and gene regulation in Leishmania. Annals of Tropical Medicine and Parasitology. 1999;**93**(8):781-807. PubMed PMID: 10715672

[143] Clayton CE. Life without transcriptional control? From fly to man and back again. EMBO Journal. 2002;**21**(8):1881-8. DOI: 10.1093/emboj/21.8.1881. PubMed PMID: 11953307; PubMed Central PMCID: PMCPMC125970

[144] Holzer TR, McMaster WR, Forney JD. Expression profiling by whole-genome interspecies microarray hybridization reveals differential gene expression in procyclic promastigotes, lesion-derived amastigotes, and axenic amastigotes in Leishmania mexicana. Molecular and Biochemical Parasitology. 2006;**146**(2):198-218. DOI: 10.1016/j.molbiopara.2005.12.009. PubMed PMID: 16430978

[145] Vincent IM, Barrett MP. Metabolomic-based strategies for anti-parasite drug discovery. Journal of Biomolecular Screening. 2015;**20**(1):44-55. DOI: 10.1177/1087057114551519. PubMed PMID: 25281738

[146] Canuto GA, Castilho-Martins EA, Tavares M, López-Gonzálvez A, Rivas L, Barbas C. CE-ESI-MS metabolic fingerprinting of Leishmania resistance to antimony treatment. Electrophoresis. 2012;**33**(12):1901-1910. DOI: 10.1002/elps.201200007. PubMed PMID: 22740478

Chapter 8

3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGR) Enzyme of the Sterol Biosynthetic Pathway: A Potential Target against Visceral Leishmaniasis

Sushma Singh and N. Kishore Babu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75480

Abstract

Sterol biosynthetic pathway is explored for its therapeutic potential for Visceral Leishmaniasis. In *Leishmania*, this pathway produces ergosterol which is absent in host and therefore is a promising strategy to combat proliferation of both extracellular and intracellular forms of the parasite with minimal host toxicity. The present chapter focuses on 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) enzyme which is the rate-limiting enzyme of the ergosterol biosynthesis. HMGR gene of *L. donovani* was biochemically and biophysically characterized for the first time. HMGR over expressing transgenic parasites were generated to evaluate its role in parasite growth and infection ability. A series of statins like atorvastatin, simvastatin and mevastatin were evaluated for its therapeutic efficacy and mode of action elucidated. Atorvastatin and mevastatin were found to be killing both the promastigote and amastigote forms of the parasite without exhibiting host cytotoxicity. Besides, non-statin class of molecules like resveratrol and glycyrrhizic acid were also analyzed for antileishmanial potential. Two antidepressants, ketanserin and mianserin were found to kill both *L. donovani* promastigotes and intracellular amastigotes with no apparent toxicity to the host cells. Since targeting of the sterol biosynthetic pathway enzymes may be useful therapeutically, the present work may have implications in treatment of Leishmaniasis.

Keywords: Visceral Leishmaniasis, HMGR, ergosterol, statins, antidepressants

1. Introduction

A variety of *Leishmania* species are reported to cause disease, which afflicts about 12 million people in 98 countries of which Indian subcontinent, Sudan and Brazil are the major

regions with higher incidence of Leishmaniasis. The World Health Organization (WHO) has considered Leishmaniasis to be one of the six priority diseases of its special programme for Research and Training in tropical diseases. Visceral Leishmaniasis (VL) being a neglected tropical disease has been of concern for several years. Antimonial compounds remain the first line drug for VL treatment with amphotericin B and pentamidine being the second line drugs. However, both the classes have high toxicity and serious side effects. Drug resistance, toxicity and long-term treatment profile are some of the issues which plague the treatment regimen. In the wake of this problem, there are increasing efforts to identify vaccine candidates and drug target candidates with equal focus on drug repositioning. Till date, several enzymes of various crucial metabolic pathways such as the pentose phosphate pathway, trypanothione biosynthesis pathway and sterol biosynthetic pathway have been explored in parasites [1]. With the whole genome sequence of *Leishmania donovani* now available, it has become feasible to identify new genes and explore its essentiality in parasite survival and host infectivity. Structural analysis of identified enzymes would throw light on potential active site for designing pharmacophore. Based on this, *in silico* ligand screening is performed to identify potential compounds from already existing library. This would further lead to design and synthesis of new chemical entities whose potency can be evaluated in cell-based and target-based screening assays.

Sterol biosynthetic pathway is an important metabolic pathway in fungi and trypanosomatids. In recent years, attention has been focused on the sterol metabolism of *Leishmania* as a potential drug target for therapy. In sterol biosynthetic pathway, condensation of two acetyl-CoA units leads to formation of acetoacetyl-CoA, followed by the addition of a third unit to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), which is further reduced by NADPH to produce mevalonic acid. The mevalonate pathway comprises of three steps and is catalyzed by acetoacetyl-CoA thiolase, and two mitochondrial enzymes HMG-CoA synthase and HMG-CoA reductase, in yeast [2] and in trypanosomatids [3]. Sterols are important components of the cell membrane that are important for cellular function and maintenance of cell structure. Unlike mammalian cells which have cholesterol as the major membrane sterol, trypanosomatids synthesize ergosterol and other 24-methyl sterols that are required for their growth and viability. *Leishmania* parasite contains predominantly ergostane-based sterols such as ergosterol, which differ from cholesterol by the presence of a 24-methyl group at $\Delta 7$ and $\Delta 22$ bonds [4]. Therefore, the sterol biosynthetic pathway from *Leishmania* is considered to be an important drug target. Squalene synthase (SQS) catalyzes the first committed step of sterol synthesis by coupling two farnesyl molecules to form squalene. Two quinuclidine derivatives, ER-119884 and E5700, have been shown to be potent antileishmanial and anti-trypanosomal agents. The inhibition of SQS by these compounds decreased endogenous sterol levels of the parasite and caused an anti-proliferative effect on the parasite [5]. Sterol 24-C-methyltranferase (SMT) is unique to the parasite and validated as a potential drug target against trypanosomatid parasites. Azosterols like ketoconazole are known to inhibit SMT in fungi. They were also found to be anti-proliferative in *Leishmania amazonensis* [6].

One of the enzymes of the sterol biosynthetic pathway which is focused in this chapter is 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR, EC:1.1.1.34). HMGR catalyzes the NADPH

http://dx.doi.org/10.5772/intechopen.75480

dependent synthesis of mevalonate from HMG-CoA and is a rate limiting step [7]. There are two classes of HMG-CoA reductase: class I (eukaryotic HMGRs) and class II (prokaryotic HMGRs). The class I HMGR has an N-terminal membrane domain and is present in eukaryotes and several archaea. Class II HMGR lacks this domain and occurs in *Pseudomonas mevalonii*, *Archaeoglobus fulgidus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptomycetes* [8–11]. *L. major* HMGR enzyme lacks the N-terminal domain and is the only eukaryote with soluble HMGR protein. Among kinetoplastids, HMGR has been earlier characterized in *L. major* and *Trypanosoma cruzi* [12, 13]. Given that ergosterol is an important component of *Leishmania* membrane, we focused our research on identification and validation of HMGR from *L. donovani* as a potential drug target candidate.

L. donovani HMGR gene was identified via a BLAST search of the genome using *L. major* HMGR sequence (www.ebi.ac.uk/parasites/LGN) as the template. *Ld*HMGR gene was amplified, cloned in pET30a (+) vector and sequenced (GenBank accession no. JX036280.1). *Ld*HMGR exhibited only 25.2% identity (35% similarity) with human HMGR. This signifies that host HMGR is significantly different from parasite HMGR. HMGR enzyme is constitutively expressed in *Leishmania* promastigotes as shown by western blot analysis [14, 27].

1.1. Functional analysis of *Ld*HMGR overexpressors

Next, we were interested to see whether HMGR has any role in parasite growth and infectivity. For this, HMGR was cloned in a *Leishmania* specific overexpression vector. HMGR overexpression in *L. donovani* promastigotes was confirmed by measuring of HMGR activity, estimation of ergosterol levels and western blot analysis confirmed the overexpression of HMGR gene [15].

1.2. Growth curve analysis of HMGR transfectants

The growth profile of transfected and wild-type parasites *in vitro* was studied by measuring OD at 600 nm of the plated cells for every 24 h. We monitored the growth of parasites and *Ld*HMGR transfectants exhibited ~ 1.5 fold increase in growth than compared to wild-type and psp vector transfected parasites (**Figure 1A**).

1.3. Role of HMGR in parasite infection ability

The transfectants were tested for their ability to infect THP-1 differentiated macrophages. The stationary phase of wild-type and HMGR overexpressing promastigotes were used to infect THP-1 differentiated macrophages. The percentage of infection with wild type was considered as 100% and relatively percentage of infection was calculated for psp and HMGR overexpressors. The HMGR transfectants exhibited ~2 fold change in the infectivity compared to wild-type parasites (**Figure 1B**).

In other organisms like yeast, it was reported that combined overexpression of genes (*ERG1* and *ERG11*) leads to significant increase in the amount of total sterols by threefold in comparison with a wild-type strain in yeast. The HMG-CoA reductase controls the entering

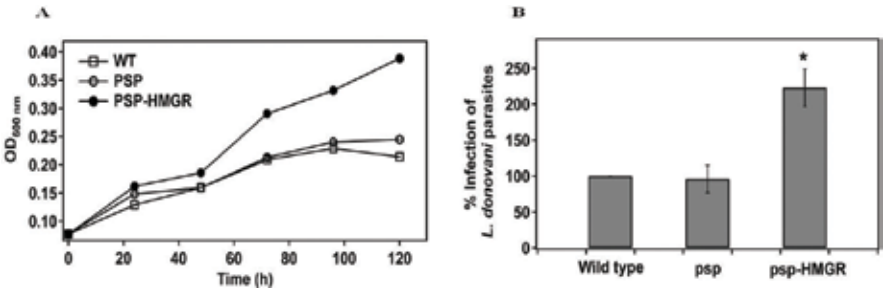


Figure 1. Functional analysis of *Ld*HMGR overexpressors. (A) Growth analysis of wild-type (WT), psp and *Ld*HMGR overexpressing parasites; (B) evaluation of infection efficiency of wild-type (WT), psp and *Ld*HMGR overexpressors. Data were expressed as mean ± standard deviations from three independent experiments. *$p \leq 0.05$.

of intermediates on the pre-squalene part of the pathway and Erg1p and Erg11p seem to control the transfer of intermediates into the post-squalene part of sterol biosynthetic pathway [16]. Other studies reported that overexpression of HMGR in yeast leads to increased linalool production which is a plant monoterpene which display antiparasitic, antimicrobial and antiviral properties as well as a plethora of promising health benefits [17].

2. HMGR inhibition profiles

2.1. Evaluation of antileishmanial effect of class I statin (simvastatin), class II statin (atorvastatin) and mevastatin

The inhibitors used in the present study were atorvastatin, simvastatin and mevastatin. The concentrations of atorvastatin and mevastatin at which 50% growth of *L. donovani* promastigotes was inhibited (IC_{50}) were 19.4 ± 3.07 μM and 23.8 ± 4.2 μM respectively [14, 18, 25]. The IC_{50} value of simvastatin was 73.2 ± 3.7 μM and at 100 μM it caused only 63% inhibition. The cytotoxicity of atorvastatin, simvastatin and mevastatin against THP-1 differentiated macrophages was determined by using MTT assay. The IC_{50} value of three drugs were found to be above 100 μM, that is, noncytotoxic to host macrophage cell line. Miltefosine inhibited promastigote growth with an IC_{50} value of 14.6 μM. Atorvastatin was found to inhibit *L. donovani* promastigotes at low micromolar concentrations compared to mevastatin and simvastatin. The concentrations of atorvastatin, simvastatin and mevastatin at which 50% growth of L. donovani amastigotes was inhibited (IC_{50}) were 6.75 ± 0.353 μM, 21.5 ± 4.94 μM and 7.5 ± 1.1 μM respectively. The amastigotes were approximately threefold more sensitive to atorvastatin and resveratrol than promastigotes. Miltefosine was taken as the reference drug, and its IC_{50} value for amastigotes was 3.9 ± 1.27 [14, 25]. The IC_{50} values are depicted in **Table 1**.

The inhibitors were screened for their ability to inhibit the catalytic efficiency of recombinant *Ld*HMGR. The IC_{50} value of atorvastatin, simvastatin and mevastatin was found to be half maximal at around 315 ± 2.12 nM, 43.66 ± 31.5 μM and 42.2 ± 3.0 μM respectively. Atorvastatin (1 μM) resulted in 93.5 ± 7.2% inhibition of the recombinant HMGR. **Table 1** shows the IC_{50} values of the statins on recombinant HMGR.

http://dx.doi.org/10.5772/intechopen.75480

Inhibitors	IC_{50} values (μM)				
	L. donovani promastigotes	*L. donovani* amastigotes	THP-1 differentiated macrophages	SI values	rHMGR
Atorvastatin[a]	19.4 ± 3.07	6.75 ± 0.353	>100	>14.8	0.315 ± 2.12
Simvastatin[a]	73.2 ± 3.7	21.5 ± 4.94	>100	>4.65	43.66 ± 31.5
Mevastatin[a]	23.8 ± 4.2	7.5 ± 1.1	>100	>13.3	42.2 ± 3.0
Miltefosine[b]	14.6 ± 1.7	3.9 ± 1.27	43.6 ± 5.5	11.17	—

[a]Dinesh et al. [14, 25].
[b]Dinesh et al. [18].

Table 1. Antileishmanial effect of statins.

2.2. Evaluation of antidepressants as HMGR inhibitors

Tricyclic drugs, antidepressants and antipsychotics are reported to be toxic to both the promastigote and amastigote forms of *Leishmania* [19]. Imipramine, a tricyclic antidepressant belonging to the same class of cationic amphiphilic drugs, when administered orally was found to be active against both antimony-sensitive and antimony resistant clinical isolates of *L. donovani* [20].

Ketanserin is a serotonin S2-receptor antagonist which is used as an antihypertensive agent. The IC_{50} value of ketanserin for *L. donovani* promastigotes was 37.8 μM and intracellular amastigotes was 28.5 μM. It was however found to be noncytotoxic up to a concentration of 100 μM, when tested on differentiated THP-1 cells. Miltefosine inhibited amastigote growth with an IC_{50} value of 3.4 μM which correlated with the previously reported data [21]. However, the standard drug killed the macrophage cells at an IC_{50} value of 43.6 μM. This was well correlated with the already published results on the effect of miltefosine on THP-1 and J774A.1 cell line [22, 23]. These results showed that ketanserin displayed antileishmanial activity at noncytotoxic concentrations. We evaluated the effect of ketanserin on recombinant *Ld*HMGR and found its IC_{50} value to be 43 ± 2.5 μM. This data showed that ketanserin binds to the *Ld*HMGR enzyme active site and inhibits its activity (**Table 2**) [15].

Mianserin hydrochloride is a noradrenergic and specific serotonergic antidepressant (NaSSA) with a tetracyclic structure and is used for the treatment of depressive illness and depression associated with anxiety [24]. Mianserin strongly blocks postsynaptic 5-HT2 receptors and only weakly blocks post synaptic 5-HT1 and 5-HT3 receptors and blocks moderately presynaptic α2 receptors [24]. The effect of mianserin was investigated on the proliferation rate of *L. donovani* promastigotes and amastigotes. The dose-dependent antileishmanial effect of mianserin against *L. donovani* promastigotes resulted in significant reduction in viable parasites compared to the untreated parasites. The concentration of mianserin at which 50% of the promastigote and amastigote growth was inhibited was 21 ± 3.7 μM and 46.4 ± 5.2 μM respectively. Mianserin up to 100 μM failed to cause any toxic effect on viability of THP-1 differentiated macrophages indicating that mianserin selectively inhibits *Leishmania* promastigotes. Mianserin inhibited recombinant *L. donovani* HMGR enzyme with an IC_{50} value of 19.8 ± 3.1 μM (**Table 2**) [14, 25].

Inhibitors	IC_{50} values (μM)				
	L. donovani promastigotes	*L. donovani* amastigotes	THP-1 differentiated macrophages	SI values	rHMGR
Mianserin[a]	21.0 ± 3.7	46.4 ± 5.2	>100	>2.15	19.8 ± 3.1
Ketanserin[b]	37.8 ± 3.3	28.5 ± 1.9	>100	>3.5	43.0 ± 2.5
Miltefosine[a]	14.6 ± 1.7	3.4 ± 0.9	43.6 ± 5.5	12.8	—

[a]Dinesh et al. [14, 25].
[b]Singh et al. [15].

Table 2. Antileishmanial effect of antidepressants.

3. Natural products as inhibitors of HMGR

The inhibitors used in the present study were resveratrol and glycyrrhizic acid. The concentrations of resveratrol at which 50% growth of *L. donovani* promastigotes was inhibited (IC_{50}) was 36.1 ± 3.6 μM. The cytotoxicity of resveratrol against THP-1 differentiated macrophages was determined by using MTT assay. The results showed the IC_{50} value of three drugs was found to be above 100 μM, that is, noncytotoxic to host macrophage cell line. The concentrations of resveratrol at which 50% growth of *L. donovani* amastigotes was inhibited IC_{50} value was 9.5 ± 2.12 μM [14, 25]. The data are depicted in **Table 3**.

Glycyrrhiza glabra, which is popularly known as liquorice is used for the treatment of pulmonary diseases and inflammatory processes [26]. Glycyrrhizic acid (GA), licochalone A and Glycyrrhetinic acid which have been reported to exert antileishmanial properties are the major bioactive components in liquorice root [27, 29, 30]. GA exhibits potent antileishmanial and immunomodulatory properties with enhanced parasite clearance [27]. A dose-dependent inhibition of the viability of *L. donovani* promastigotes was observed in the presence of GA. The IC_{50} determined from the graph was approximately 34 ± 2.9 μM. GA was found to inhibit intracellular amastigotes with an IC_{50} value of 20 ± 4.2 μM. GA did not cause macrophage killing up

Inhibitors	IC_{50} values (μM)				
	L. donovani promastigotes	*L. donovani* amastigotes	THP-1 differentiated macrophages	SI values	rHMGR
Glycyrrhizic acid[a]	34.0 ± 2.9	20.0 ± 4.24	>100	>5.0	24.0 ± 4.3
Resveratrol[b]	36.1 ± 3.6	9.5 ± 2.12	>100	>10.5	46.3 ± 16.4
Miltefosine[a]	15.3 ± 2.1	3.8 ± 1.2	44.2 ± 5.29	11.5	—

[a]Dinesh et al. [28].
[b]Dinesh et al. [14, 25].

Table 3. Natural products as inhibitors of HMGR.

to 100 μM concentration. GA was tested against recombinant *Ld*HMGR enzyme at the range of 10–100 μM concentration. The IC_{50} value was found to be 24 ± 4.3 μM (**Table 3**).

In *Leishmania* sterol, biosynthetic pathway produces ergosterol which is absent in host. This makes *Ld*HMGR enzyme a potential drug target for designing parasite specific molecules. The present review encompasses functional characterization of *L. donovani* HMGR enzyme and the evaluation of the effect of various HMGR inhibitors as potential candidates for treatment of Leishmaniasis. Inhibitors which showed inhibition of both the extracellular and intracellular forms of the parasites at low micromolar range with no cytotoxicity to host cells are promising antileishmanial candidates. They can be further explored in an experimental animal model of VL to evaluate its anti-VL efficacy.

Author details

Sushma Singh* and N. Kishore Babu

*Address all correspondence to: sushmasingh@niper.ac.in

Department of Biotechnology, National Institute of Pharmaceutical Education and Research, Mohali, Punjab, India

References

[1] Chawla B, Madhubala R. Drug targets in *Leishmania*. Journal of Parasitic Diseases. 2010; **34**(1):1-13

[2] Trocha PJ, Sprinson DB. Location and regulation of early enzymes of sterol biosynthesis in yeast. Archives of Biochemistry and Biophysics. 1997;**174**:45-51

[3] Pena-Diaz J, Montalvetti A, Flores CL, Constan A, Hurtado-Guerrero R, De Souza W, Gancedo C, Ruiz-Perez LM, Gonzalez-Pacanowska D. Mitochondrial localization of the mevalonate pathway enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase in the Trypanosomatidae. Molecular Biology of the Cell. 2004;**15**:1356-1363

[4] Jimenez-Jimenez C, Carrero-Lerida J, Sealey-Cardona M, Ruiz Perez LM, Urbina JA, Gonzalez Pacanowska D. Delta 24(25)-sterol methenyltransferase: Intracellular localization and azasterol sensitivity in *Leishmania major* promastigotes overexpressing the enzyme. Molecular and Biochemical Parasitology. 2008;**160**:52-59

[5] Fernandes Rodrigues JC, Concepcion JL, Rodrigues C, Caldera A, Urbina JA, De Souza W. *In vitro* activities of ER-119884 and E5700, two potent squalene synthase inhibitors, against *Leishmania amazonensis*: Antiproliferative, biochemical, and ultra structural effects. Antimicrobial Agents and Chemotherapy. 2008;**52**:4098-4114

[6] Pirson P, Leclef B, Trouet A. Activity of ketoconazole derivatives against *Leishmania mexicana amazonesis* within mouse peritoneal macrophages. Annals of Tropical Medicine and Parasitology. 1990;**84**(2):133-139

[7] Caelles C, Ferrer A, Balcells L, Hegardt G, Boronat A. Isolation and structural characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3 methylglutaryl coenzyme A reductase. Plant Molecular Biology. 1989;**13**:627-638

[8] Hedl M, Rodwell VW. Inhibition of the class II HMG-CoA reductase of *Pseudomonas mevalonii*. Protein Science. 2004;**13**:1693-1697

[9] Kim DY, Stauffacher CV, Rodwell VW. Dual coenzyme specificity of *Archaeoglobus fulgidus* HMG-CoA reductase. Protein Science. 2000;**9**:1226-1234

[10] Wilding EI, Kim DY, Bryant AP, Gwynn MN, Lunsford RD, McDevitt D, Myers JEJ, Rosenberg M, Sylvester D, Stauffacher CV, Rodwell VW. Essentiality, expression, and characterization of the class II 3-hydroxy-3-methylglutaryl coenzyme A reductase of *Staphylococcus aureus*. Journal of Bacteriology. 2000;**182**:5147-5152

[11] Hedl M, Sutherlin A, Wilding EI, Mazzulla M, McDevitt D, Lane P, Burgner JW 2nd, Lehnbeuter KR, Stauffacher CV, Gwynn MN, Rodwell VW. *Enterococcusfaecalis* acetoacetyl-coenzyme A thiolase/3-hydroxy-3-methylglutarylcoenzyme A reductase, a dual-function protein of isopentenyl diphosphate biosynthesis. Journal of Bacteriology. 2002; **184**:2116-2122

[12] Takahashi S, Kuzuyama T, Purification SH. Characterization, and cloning of a eubacterial 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key enzyme involved in biosynthesis of terpenoids. Journal of Bacteriology. 1999;**181**:1256-1263

[13] Pena-Diaz J, Montalvetti A, Camacho A, Gallego C, Ruiz-Perez LM, Gonzalez- Pacanowska D. A soluble 3-hydroxy-3-methylglutaryl-CoA reductase in the protozoan *Trypanosoma cruzi*. The Biochemical Journal. 1997;**324**:619-626

[14] Dinesh N, Dheeraj SRP, Kaur PK, Babu NK, Singh S. Exploring *Leishmania donovani* 3-hydroxy-3-methylglutaryl coenzyme A reductase (*Ld* HMGR) as a potential drug target by biochemical, biophysical and inhibition studies. Microbial Pathogenesis. 2014;**66**:14-23

[15] Singh S, Dinesh N, Kaur PK, Shamiulla B. Ketanserin, an antidepressant, exerts its antileishmanial action via inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) enzyme of *Leishmania donovani*. Parasitology Research. 2014;**113**:2161-2168

[16] Veen M, Stahl U, Lang C. Combined overexpression of genes of the ergosterol biosynthetic pathway leads to accumulation of sterols in *Saccharomyces cerevisiae*. FEMS Yeast Research. 2003;**4**:87-95

[17] Rico J, Pardo E, Orejas M. Enhanced production of a plant monoterpene by overexpression of the 3-hydroxy-3-methylglutaryl coenzyme A reductase catalytic domain in *Saccharomyces cerevisiae*. Applied and Environmental Microbiology. 2010;**76**:6449-6454

[18] Dinesh N, Soumya N, Singh S. Antileishmanial effect of mevastatin is due to interference with sterol metabolism. Parasitology Research. 2015;**114**(10):3873-3883

[19] Zilberstein D, Dwyer DM. Antidepressants cause lethal disruption of membrane function in the human protozoan parasite *Leishmania*. Science. 1984;**226**:977-979

[20] Mukherjee S, Mukherjee B, Mukhopadhyay R, Naskar K, Sundar S, Dujardin JC, Das AK, Roy S. Imipramine is an orally active drug against both antimony sensitive and resistant *Leishmania donovani* clinical isolates in experimental infection. PLoS Neglected Tropical Diseases. 2012;**6**:e1987

[21] Corral MJ, Gonzalez-Sanchez E, Cuquerella M, Alunda JM. *In vitro* synergistic effect of amphotericin B and allicin on *Leishmania donovani* and *L. infantum*. Antimicrol Agents Chemother. 2014;**58**:1596-1602

[22] Calogeropoulou T, Angelou P, Detsi A, Fragiadaki I, Scoulica E. Design and synthesis of potent antileishmanial cyclo alkylidene substituted ether phospholipid derivatives. Journal of Medicinal Chemistry. 2008;**51**:897-908

[23] Dube A, Singh N, Saxena A, Lakshmi V. Antileishmanial potentialof a marine sponge, *Haliclona exigua* (Kirkpatrick) against experimental visceral leishmaniasis. Parasitology Research. 2007;**101**:317-324

[24] Schreiber S, Backer MM, Kaufman JP, Pick CG. Interaction between the tetracyclic antidepressant mianserin HCl and opioid receptors. European Neuropsychopharmacology. 1998;**8**:297-302

[25] Dinesh N, Kaur PK, Swamy KK, Singh S. Mianserin, an antidepressant kills *Leishmania donovani* by depleting ergosterol levels. Experimental Parasitology. 2014;**144**:84-90

[26] Davis EA, Morris DJ. Medicinal uses of licorice through the millennia: The good and plenty of it. Molecular and Cellular Endocrinology. 1991;**78**:1-6

[27] Bhattacharjee S, Bhattacharjee A, Majumder S, Majumdar SB, Majumdar S. Glycyrrhizic acid suppresses Cox-2-mediated anti-inflammatory responses during *Leishmania donovani* infection. The Journal of Antimicrobial Chemotherapy. 2012;**67**:1905-1914

[28] Dinesh N, Soumya N, Kumar V, Singh S. Glycyrrhizic acid attenuates growth of *Leishmania donovani* by depleting ergosterol levels. Experimental Parasitology. 2017;**176**:21-29

[29] Fu Y, Hsieh TC, Guo J, Kunicki J, Lee MY, Darzynkiewicz Z, Wu JM. Licochalcone-A, a novel flavonoid isolated from licorice root (*Glycyrrhiza glabra*), causes G2 and late-G1 arrests in androgen-independent PC-3 prostate cancer cells. Biochemical and Biophysical Research Communications. 2004;**322**:263-270

[30] Ukil A, Biswas A, Das T, Das PK. 18 Beta-glycyrrhetinic acid triggers curative Th1 response and nitric oxide up-regulation in experimental visceral leishmaniasis associated with the activation of NF-kappa B. Journal of Immunology. 2005;**175**:1161-1169

Section 6

Medicinal Plants as Source of Anti-leishmanial Metabolites

Chapter 9

Alternative Treatment for Leishmaniasis

Renata Mondêgo de Oliveira,
Solange de Araújo Melo,
Tatiane Aranha da Penha-Silva,
Fernando Almeida-Souza and Ana Lucia Abreu-Silva

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75895

Abstract

Leishmaniasis remains as one of the most important neglected diseases in the world and, after all these years, its treatment is still a problem, mainly because of the side effects caused by the first- and second-line drugs and the indiscriminate treatment, which leads to increasing cases of parasite resistance. The search for alternative therapies for the treatment of leishmaniasis is extremely important. In this context, the use of natural products arises as a promising alternative, combining the empirical knowledge disseminated in the population with researches that aim to scientifically prove the therapeutic effects of plants. Based on this, the use of medicinal plants is considered a desirable and accessible tool in the treatment of these diseases and considered by pharmacognosy as a valuable source for the development of new drugs and as adjuvant for conventional therapies.

Keywords: herbal medicine, *Leishmania* spp., natural products, visceral leishmaniasis, traditional medicine

1. Introduction

Protozoa of the genus *Leishmania* cause a broad spectrum of diseases collectively called Leishmaniasis, which represent a serious public health problem worldwide. Its clinical forms vary from cutaneous leishmaniasis (CL), characterized by tegumentary lesions that can heal and regress spontaneously, to visceral leishmaniasis (VL), more severe and potentially fatal, if not treated [1].

VL is an important zoonosis caused by parasites of the *Leishmania donovani* complex (*L. donovani* in India and Central Africa and *L. infantum* in America, Middle East, Central Asia, China, and the Mediterranean Basin) [2, 3]. It is present in 98 countries but, although widely distributed, more than 90% of cases are restricted to India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil [4, 5]. In the Americas, dogs are considered the main reservoir of the parasites, as well as an important link for the maintenance of the infection in the urban environment [6] (**Figure 1**).

Leishmania species have a complex life cycle, alternating between a permissive insect vector and a susceptible vertebrate host [8]. The transmission of the parasite occurs through the bite of an infected female sandfly, belonging to the genus *Phlebotomus*, in the Old World, or *Lutzomyia*, in the New World [9]. Once inside the vertebrate host, the promastigote forms inoculated by the insect will be phagocytosed by macrophages, transforming into amastigotes. After extensive multiplication, the amastigotes increase in quantity until the cell ruptures, leading to infection of other phagocytic cells, continuing the cycle [10].

Other forms of transmission have already been reported, such as vertical and/or sexual transmission [11], non-vector hematogenous [12, 13], and through other vectors, such as *Rhipicephalus sanguineus* [14], but their role in the maintenance of the disease is not totally clear yet.

In epidemiological terms, the dynamics of disease transmission is very complex and depends on several factors, such as the socioeconomic status of the population (poor living conditions, malnutrition), climate and environmental changes (which leads to sandfly adaptation and spread), host–parasite relationship (immunocompromised individuals, evasion mechanisms employed by the parasite), and population mobility (international travels and/or migration from non-endemic areas to endemic areas), which means that there may be differences in the pattern of disease spread, depending on the place [8, 15–18].

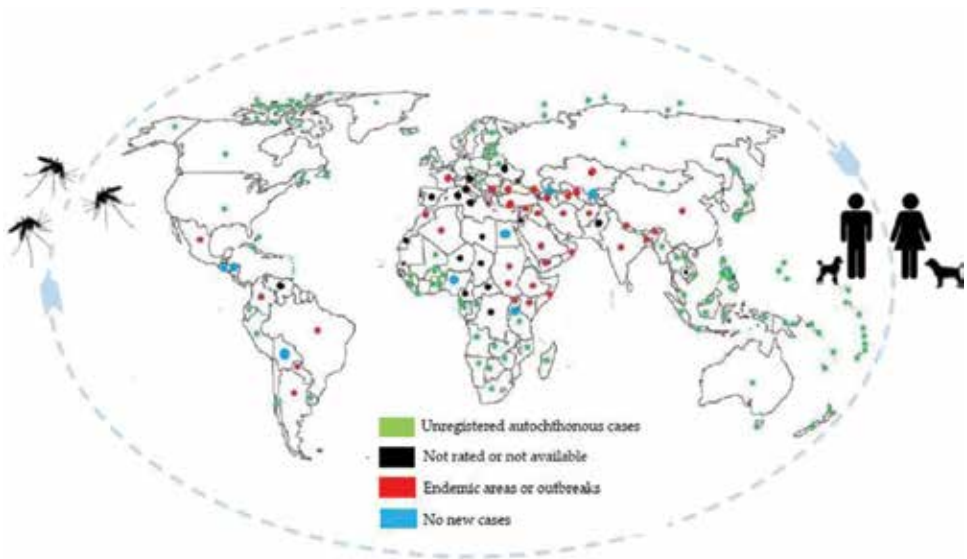


Figure 1. Status of endemicity of visceral leishmaniasis worldwide, 2015. Source: Adapted from WHO (2015) [7].

2. Therapeutic modalities for VL

2.1. Chemotherapy

Despite its importance for both human and animal health, there are few therapeutic options for VL treatment. The bases of therapeutic protocols in humans are the pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), but the need of hospitalization and the severe side effects caused by its administration leads to high dropout rates among the patients, which contributes for parasite resistance in case of disease relapse. Although widely used, the mechanism of action of antimonials is still poorly understood [19, 20].

As a second-line drug, amphotericin B was initially recommended for patients who did not respond to the treatment with pentavalent antimonials. It presents high cure rates, efficacy, and safety, but, once again, needs prolonged hospitalization, for close monitoring of renal functions, and has some adverse effects, such as fever and chills [21, 22].

Miltefosine was the first oral drug used for VL cases, which simplified the treatment in several aspects. It was originally designed for breast cancer and other solid tumors, but the gastrointestinal side effects limited its use [23, 24]. In vitro and in vivo evidences of the antileishmanial activity of miltefosine [25–27] conducted in clinical trials in humans and its release for the treatment of human VL in many countries [28–30]. However, it should not be used in pregnant women due to its teratogenic effect [23]. Besides that, its indiscriminate use, incomplete treatment, and the long half-life of the drug has increased the cases of parasitic resistance, which represents a serious concern [31].

Other drugs are commonly used as therapeutic alternatives for VL, such as paromomycin, pentamidine, and sitamaquine. However, all have variable side effects or cure rates lower than the reference drugs [22, 32–36].

In general, all available drugs have problems related to toxicity and high costs, which hinders the treatment of Leishmaniasis, especially in poor and developing countries, where the great majority of the cases are concentrated [8]. For this reason, it is of great importance the adoption of strategies for the search and development of new candidate drugs. In this context, we emphasize the emergence of phytotherapy as a promising therapeutic alternative, since the use of natural products is widely disseminated in the population [37]. Therefore, it is necessary to combine the empirical knowledge with researches, with the aim to scientifically prove the therapeutic effects of plants—crude extracts, fractions or isolated substances—against *Leishmania* species, especially the causative agents of VL.

2.2. Phytotherapy

Medicinal plants are defined as those administered to man or animals, by any routes, that exert some therapeutical activity [38]. Plants are used as sources of new compounds throughout the history of mankind and, even today, serve as basis of many products used in the medical routine [39].

The use of medicinal plants has become, especially in developing countries, an alternative to traditional health services, both in rural areas, deprived of public health resources, as in

urban areas, as an option or as a complement to allopathic medication [40]. This tradition has been passed on to the populations in every generation, and is configured as a new science, the phytotherapy [41].

In countries with a great diversity of flora, such as Brazil, there is a great potential for the rational exploitation of plant resources and for the diffusion of herbal practices. Such practices are able to generate benefits both in the cultural point of view such as contributions to the scientific validation of the use of plant species [42, 43], since many of these are consumed without their pharmacological properties are, in fact, known [44].

The scientific community reveals a growing interest in this field, recognizing the true health benefit that plants provide [45]. The World Health Organization itself recognizes that the solution to combating numerous diseases, especially the so-called "neglected diseases," lies in the traditional knowledge and in the development of new drugs derived from biodiversity products [46].

Historically, experiments with the use of plants in medicine with therapeutical and healing purposes have been reported, which demonstrates that man began to use plants not only as food but also as a therapeutic resource for many diseases. Currently, many plant drugs are pointed out and described as viable alternatives in the treatment of many diseases [47], progressively abandoning empirical use based on experiments, starting for rational use based on iatrochemistry [48], based on the evident undesirable effects of some synthetic drugs [49].

Tagboto and Townson [50] describe as challenging the path of validation of the use of natural drugs and this includes not only the discovery of new drugs, but also the certification of products already used, culminating in the preservation of biodiversity. These authors report that, due to the widespread use of natural drugs being used, especially in underdeveloped countries, there was a need for certification of these products, and that due to these reasons, in 2000, the World Health Organization created a demand in order to qualify and regulate with scientific bases some medicines whose principles are already known, as well as empirical ones, in order to identify new possibilities within pharmacognosy.

3. Alternate therapies—mechanism of action

3.1. Immunomodulation by antileishmanial plant products

The immunological condition of a patient infected with *Leishmania* represents a determinant point for a favorable treatment. In visceral leishmaniasis, the immune system is markedly shaken by secondary infections and other opportunistic infections associated with the clinical picture of the disease, which emphasizes the need for drugs that not only favor immune recovery but also present a leishmanicidal action [51]. The modern medicine has changed the focus regarding the treatment of several diseases, such as neoplasms and infectious diseases. Traditionally, the drugs were developed to act directly on the microorganisms or neoplastic cells, but now, the main goal is to strengthen the body's defenses. Plants have several secondary metabolites, for example, flavonoids, polysaccharides, lactones, alkaloids, diterpenoids, and glycosides that may activate

the immunological system [52]. Regarding leishmaniosis treatment, Chouhan et al. [53] describe the use of medicinal plants as an alternative for modulating the patient's immune response as an effective device in therapy. A combination of miltefosine and nanoparticles of curcumin displayed lymphocyte proliferation and increased the phagocytic capacity of peritoneal macrophages. This effect was attributed to curcumin [54]. A substance isolated of *Casearia arborea*, tricin, was able to modulate the respiratory burst, which favors the parasite elimination [55].

Awareness of the importance of modulating the immune system has been a crucial point in the prevention and treatment of various diseases, and for this reason, the immunomodulatory properties of plants have been extensively explored so that researchers seek not only to affect the permanence of the pathogen but also have sought to boost both the patient's natural and adaptive defenses [56, 57]. This fact was observed by Almeida-Souza et al. [58], demonstrating a hypothesis that determines compounds obtained by different extraction methods can favor the increase of mediators such as nitric oxide (NO), increasing the functions and abilities of macrophages in the elimination of amastigote forms.

3.2. Reactive oxygen species generation

Against obligate intracellular parasite, macrophages use various mechanisms of action to control infection, as the induction of reactive oxygen and nitrogen species. Hydrogen peroxide is a major source of hydroxyl radicals and other reactive oxygen species, which macrophages produce in greater quantities [59, 60]. Among reactive nitrogen species, nitric oxide (NO) has a potent microbicide effect against intracellular parasites, such as *Leishmania* [61]. NO is a freely diffusible gas produced by the activity of inducible NO synthase (iNOS) enzyme by the conversion of *L*-arginine to *L*-citrulline. iNOS is induced by various pro-inflammatory factors such as cytokines or endotoxins [62]. In its short life, NO acts directly on pathogens by inhibition of proliferation, DNA mutagenesis, disruption of [FeS] clusters, metabolic blockade, and inactivation of virulence factors or molecules associated with infectious pathogens [63]. The functions of NO also include immunostimulatory (pro-inflammatory) effects that together with antimicrobial activity contribute to the killing of intracellular *Leishmania* as previous reported [58].

3.3. Apoptosis-inducing potential

The mechanism of action of leishmanicidal drugs is not well elucidated. It has been reported that both conventional drugs and some plants extracts used in the treatment of visceral leishmaniasis may induce a phenomenon like apoptosis in the parasite. The ethanolic extract of seeds and leaves of *Azadiracta indica* [64] and essentials oils of *Artemisia campestris* and *Artemisia herba-alba* [65] act as an apoptosis inductor in promastigotes of *L. donovani* and *L. infantum,* respectively.

4. Plants with antileishmanial properties

The available drugs against leishmaniasis do not always present a satisfactory result and have been shown as an expressive challenge for current treatment protocols [66]. Many plants that

Plant	Part of plant	Preparation	Species	Reference
Withania somnifera	Leaves; whole plant	Alcoholic fractions F5 and F6; tablets; methanolic extract (fraction A6)	*L. donovani*	Chandrasekaran et al. [68] Kaur et al. [73] Sharma et al. [74]
Inula chritmoides	Not cited	Acetone and dichloromethane extracts	*L. infantum*	Oliveira et al. [75]
Casearia arborea	Leaves	Methanolic extract	*L. infantum*	Santos et al. [55]
Curcuma longa	Rhizome	Oral formulation based on nanoparticles	*L. donovani*	Tiwari et al. [54]
Spergularia rubra	Not cited	Acetone and dichloromethane extracts	*L. infantum*	Oliveira et al. [75]
Ocimum sanctum	Leaves	Ethanolic extract	*L. donovani*	Bhalla et al. [76]; Kaur et al. [73]
Cocos nucifera	Husk fiber	Aqueous extract	*L. donovani*	Bhalla et al. [76]
Sterculia villosa	Bark	Methanolic extract	*L. donovani*	Das et al. [77]
Coccinia grandis	Leaves	Extract	*L. donovai*	Pramanik et al. [78] Das et al. [79]
Morinda citrifolia	Fruits	Aqueous extract Fruit juice	*L. chagasi*	Almeida-Souza et al. [80]
Solanum tuberosum	Tuber	Sodium bisulphite extraction	*L. donovani*	Paik et al. [81] Paik et al. [82]
Moringa oleifera	Flower	Ethyl acetate fraction	*L. donovani*	Singh et al. [83]
Azadirachta indica	Leaves and seeds	Ethanolic fraction and ethyl acetate fraction	*L. donovani*	Chouhan et al. [84]; Dayakar et al. [85]
Croton caudatus	Leaves	Hexanic extract	*L. donovani*	Dey et al. [86]
Artemisia annua	Leaves and seeds	n-hexane fractions	*L. donovani*	Islamuddin et al. [87] Islamuddin et al. [88]
Asparagus racemosus	Whole plant	Tablets	*L. donovani*	Kaur et al. [89] Sachdeva et al. [90]
Syzygium aromaticum	Flower	Essential oil	*L. donovani*	Islamuddin et al. [91]
Croton cajucara	Leaves	Essential oil	*L. chagasi*	Rodrigues et al. [7]
Solanocia mannii	Leaves	Extract	*L. donovani*	Hubert et al. [92]
Solanum torvum	Leaves	Extract	*L. donovani*	Hubert et al. [92]
Coriandrum sativum	Seeds	Oleoresin	*L. chagasi*	Rondon et al. [93]
Lippia sidoides	Not cited	Essential oil	*L. chagasi*	Rondon et al. [93]
Copaifera reticulata	Seeds	Essential oil	*L. chagasi*	Rondon et al. [93]
Spondias mombin	Aerial parts	Ethanolic extract (Sm3 fraction)	*L. chagasi*	Accioly et al. [94]
Annona squamosa	Leaves	Alkaloid and acetogenic extract	*L. chagasi*	Vila-Nova et al. [95]

Plant	Part of plant	Preparation	Species	Reference
Annona muricata	Seeds	Alkaloid and acetogenic extract	*L. chagasi*	Vila-Nova et al. [95]
Aloe vera	Leaves	Extract	*L. infantum*	Rondon et al. [96]
Coriandrum sativum	Seeds	Extract	*L. infantum*	Rondon et al. [96]
Ricinus communis	Leaves	Extract	*L. infantum*	Rondon et al. [96]
Valeriana wallichii	Root	Methanol and chloroform extracts	*L. donovani*	Ghosh et al. [97]
Momordica charantia	Fruit	Crude extract	*L. donovani*	Gupta et al. [98]
Kalanchoe pinnata	Leaves	Aqueous extract	*L. chagasi*	Gomes et al. [99]
Allium sativum	Bulb	Methanolic extract (fraction G3)	*L. donovani*	Sharma et al. [74]
Piper betle	Leaves	Methanolic extract and essential oil	*L. donovani*	Misra et al. [100]
Nyctanthes arbor-tristis	Leaves	Methanolic extract (fraction calceolariosidea)	*L. donovani*	Poddar et al. [101]
Aloe vera	Leaves	Exudate	*L. donovani*	Dutta et al. [102]
Tinospora sinensis	Powdered stem	Ethanolic extract	*L. donovani*	Singh et al. [103]
Chenopodium ambrosioides	Aerial parts	Essential oil	*L. donovani*	Manzote et al. [104]
Annona crassiflora	Stem bark	Exanolic and ethanolic extract	*L. donovani*	Mesquita et al. [105]
Himatanthus obovatus	Root wood	Exanolic and ethanolic extract	*L. donovani*	Mesquita et al. [105]
Guarea kunthiana	Roots	Exanolic and ethanolic extract	*L. donovani*	Mesquita et al. [105]
Cupania vernalis	Leaves	Exanolic and ethanolic extract	*L. donovani*	Mesquita et al. [105]
Serjania lethalis	Root bark	Exanolic and ethanolic extract	*L. donovani*	Mesquita et al. [105]

Table 1. Antileishmanial activity of plants against visceral leishmaniasis.

present anti-infectious characteristics have been studied for the careful detection of new active compounds isolated [67] of antiparasitic action and also as immunomodulators, so that they are shown as a collection of bioactive compounds for the optimization of the treatment of leishmaniasis [68], as well as the presence of active compounds belonging to several chemical groups [69–71], such as flavonoids, isoflavonoids, saponins, alkaloids, sesquiterpenes, polysaccharides, tannins, indoles, and glucans [72].

Much information about plants and formulations employed in popular medicine is contained in the literature, and based on this information, new constituents have been successfully perfected and clinically tested, correlating traditional and modern medicine, combining science and empiricism (**Table 1**). Traditional medicine is based primarily on personal experience, with the use of compounds not yet fully validated, requiring complementary evidence to become safe and effective [106].

5. Conclusion

The drugs available for the treatment of visceral leishmaniasis have adverse effects, a high cost, and, in addition, parasitic resistance is frequent. These facts are a challenge for modern science, which uses traditional medicine as a research source to find a compound that is effective and has minimal side effects. Many studies have been carried out, but the results obtained are not very encouraging. Most of the plants studied did not present leishmanicidal effect but the immunomodulatory effect has often been emphasized. Summarizing, data in the literature show that the substances obtained from the study of plants may be excellent allies in the treatment of leishmaniasis because they have immunomodulatory effects, but none has a direct effect against the parasite.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa e Desenvolvimento Científico e Tecnológico do Maranhão – FAPEMA [grant numbers APP-00844/09, Pronex-241709/2014 to Abreu-Silva AL; DCR03438/16 to Almeida-Souza F]; Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq [grant numbers 309885/2017-5 to Abreu-Silva AL; 312765/2016-9 to Almeida-Souza F]; Secretaria da Ciência Tecnologia e Inovação do Estado do Maranhão [grant number DCR03438/16 to Almeida-Souza F]; and CAPES PNPD program [grant to Penha-Silva TA].

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this chapter.

Author details

Renata Mondêgo de Oliveira[1], Solange de Araújo Melo[1], Tatiane Aranha da Penha-Silva[1], Fernando Almeida-Souza[1,2]* and Ana Lucia Abreu-Silva[1]*

*Address all correspondence to: abreusilva.ana@gmail.com and fernandoalsouza@gmail.com

1 Universidade Estadual do Maranhão, São Luís, Brazil

2 Laboratório de Imunomodulação e Protozoologia, Instituto Oswaldo Cruz, Fioruz, Rio de Janeiro, Brazil

References

[1] Stanley AC, Engwerda CR. Balancing immunity and pathology in visceral leishmaniasis. Immunology Cell Biology. 2007;**85**:138-147. DOI: 10.1038/sj.icb7100011

[2] Baneth G, Koutinas AF, Solano-Gallego L, Bourdeau P, Ferrer L. Canine leishmaniosis – new concepts and insights on an expanding zoonosis: Part one. Trends in Parasitology. 2008;**24**:324-330. DOI: 10.1016/j.pt.2008.04.001

[3] CB1 P-d-S, Day MJ. One health: The global challenge of epidemic and endemic leishmaniasis. Parasites & Vectors. 2011;**4**:197. DOI: 10.1186/1756-3305-4-197

[4] Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;**7**(5):e35671. DOI: 10.1371/journal.pone.0035671

[5] Singh S. Changing trends in the epidemiology, clinical presentation, and diagnosis of *Leishmania*-HIV co-infection in India. International Journal of Infectious Diseases. 2014;**29**:103-112. DOI: 10.1016/j.ijid.2014.07.011

[6] Dantas-Torres F, Brandão-Filho SP. Visceral leishmaniasis in Brazil: Revisiting paradigms of epidemiology and control. Revista do Instituto de Medicina Tropical de São Paulo. 2006;**48**:151-156. DOI: /S0036-46652006000300007

[7] Rodrigues IA, Azevedo MM, Chaves FC, Bizzo HR, Corte-Real S, Alviano DS, Alviano CS, Rosa MS, Vermelho AB. In vitro cytocidal effects of the essential oil from Croton cajucara (red sacaca) and its major constituent 7-hydroxycalamenene against Leishmania chagasi. BMC Complementary and Alternative Medicine. 2013 Oct 2;**13**:249. DOI: 10.1186/1472-6882-13-249

[8] Savoia D. Recent updates and perspectives on leishmaniasis. Journal of Infection in Developing Countries. 2015;**9**:588-596. DOI: 10.3855/jidc.6833

[9] Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. The Lancet. 2005;**366**:1561-1577. DOI: 10.1016/S0140-6736(05)67629-5

[10] Bañuls AL, Hide M, Prugnolle F. *Leishmania* and the Leishmaniases: A parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. Advances in Parasitology. 2007;**64**:1-109. DOI: 10.1016/S0065-308X(06)64001-3

[11] Turchetti AP, Souza TD, Paixão TA, Santos RL. Sexual and vertical transmission of visceral leishmaniasis. Journal of Infection in Developing Countries. 2014;**8**:403-407. DOI: 10.3855/jidc.4108

[12] Owens SD, Oakley DA, Marryott K, Hatchett W, Walton R, Nolan TJ, Newton A, Steurer F, Schantz P, Giger U. Transmission of visceral leishmaniasis through blood transfusions from infected English foxhounds to anemic dogs. Journal of the American Veterinary Medical Association. 2001;**219**:1076-1083. DOI: 10.2460/javma.2001.219.1076

[13] Tabar MD, Roura X, Francino O, Altet L, Ruiz de Gopegui R. Detection of *Leishmania infantum* by real-time PCR in a canine blood bank. Journal of Small Animal Practice. 2008;**49**:325-328. DOI: 10.1111/j.1748-5827.2008.00542.x

[14] Viol MA, Guerrero FD, de Oliveira BC, de Aquino MC, Loiola SH, de Melo GD, de Souza Gomes AH, Kanamura CT, Garcia MV, Andreotti R, de Lima VM, Bresciani KD.

Identification of *Leishmania* spp. promastigotes in the intestines, ovaries, and salivary glands of *Rhipicephalus sanguineus* actively infesting dogs. Parasitology Research. 2016;**115**:3479-3484. DOI: 10.1007/s00436-016-5111-5

[15] Alvar J, Yactayo S, Bern C. Leishmaniasis and poverty. Trends in Parasitology. 2006;**22**: 552-557. DOI: 10.1016/j.pt.2006.09.004

[16] Cardenas R, Sandoval CM, Rodriguez-Morales AJ, Vivas P. Zoonoses and climate variability. Annals of the New York Academy of Sciences. 2008;**1149**:326-330. DOI: 10.1196/annals.1428.094

[17] Cecilio P, Perez-Cabezas B, Santarem N, Maciel J, Rodrigues V, Cordeiro da Silva A. Deception and manipulation: The arms of *Leishmania*, a successful parasite. Frontiers in Immunology. 2014;**5**:480. DOI: 10.3389/fimmu.2014.00480

[18] Leta S, Dao TH, Mesele F, Alemayehu G. Visceral leishmaniasis in Ethiopia: An evolving disease. PLoS Neglected Tropical Diseases. 2014;**8**:e3131. DOI: 10.1371/journal.pntd.0003131

[19] Frézard F, Demicheli C, Ribeiro RR. Pentavalent antimonials: New perspectives for old drugs. Molecules. 2009;**14**:2317-2336. DOI: 10.3390/molecules14072317

[20] Sundar S, Chakravarty J. An update on pharmacotherapy for leishmaniasis. Expert Opinion on Pharmacotherapy. 2015;**16**:237-252. DOI: 10.1517/14656566.2015.973850

[21] Chattopadhyay A, Jafurulla M. A novel mechanism for an old drug: Amphotericin B in the treatment of visceral leishmaniasis. Biochemical and Biophysical Research Communications. 2011;**416**:7-12. DOI: 10.1016/j.bbrc.2011.11.023

[22] Singh OP. Current challenges in treatment options for visceral leishmaniasis in India: A public health perspective. Infectious Diseases of Poverty. 2016;**5**:19. DOI: 10.1186/s40249-016-0112-2

[23] Sundar S, Olliaro PL. Miltefosine in the treatment of leishmaniasis: Clinical evidence for informed clinical risk management. Therapeutics and Clinical Risk Management. 2007;**3**:733-740

[24] Dummer R, Krasovec M, Röger J, Sindermann H, Burg G. Topical administration of hexadecylphosphocholine in patients with cutaneous lymphomas: Results of a phase I/II study. Journal of the American Academy of Dermatology. 1993;**29**:963-970. DOI: 10.1016/0190-9622(93)70275-X

[25] Croft SL, Neal RA, Pendergast W, Chan JH. The activity of alkyl phosphorylcholines and related derivatives against *Leishmania donovani*. Biochemical Pharmacology. 1987;**36**:2633-2636. DOI: 10.1016/0006-2952(87)90543-0

[26] Croft SL, Snowdon D, Yardley V. The activities of four anticancer alkyllysophospholipids against *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei*. Journal of Antimicrobial Chemotherapy. 1996;**38**:1041-1047

[27] Kuhlencord A, Maniera T, Eibl H, Unger C. Hexadecylphosphocholine: Oral treatment of visceral leishmaniasis in mice. Antimicrobial Agents and Chemotherapy. 1992;**36**:1630-1634. DOI: 0066-4804/92/081630-05$02.00/0

[28] Sundar S, Jha TK, Thakur CP, Engel J, Sindermann H, Fischer C, Junge K, Bryceson A, Berman J. Oral miltefosine for Indian visceral leishmaniasis. The New England Journal of Medicine. 2002;**347**:1739-1746. DOI: 10.1056/NEJMoa021556

[29] Berman J. Miltefosine to treat leishmaniasis. Expert Opinion on Pharmacotherapy. 2005;**6**:1381-1388. DOI: 10.1517/14656566.6.8.1381

[30] Sindermann H, Engel J. Development of miltefosine as an oral treatment for leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;**100**(Suppl 1): S17-S20. DOI: 10.1016/j.trstmh.2006.02.010

[31] Bhandari V, Kulshrestha A, Deep DK, Stark O, Prajapati VK, Ramesh V, Sundar S, Schonian G, Dujardin JC, Salotra P. Drug susceptibility in *Leishmania* isolates following miltefosine treatment in cases of visceral leishmaniasis and post kala-azar dermal leishmaniasis. PLoS Neglected Tropical Diseases. 2012;**6**:e1657. DOI: 10.1371/journal.pntd.0001657

[32] Neal RA, Allen S, McCoy N, Olliaro P, Croft SL. The sensitivity of *Leishmania* species to aminosidine. Journal of Antimicrobial Chemotherapy. 1995;**35**:577-584. DOI: 10.1093/jac/35.5.577

[33] Das VN, Ranjan A, Sinha AN, Verma N, Lal CS, Gupta AK, Siddiqui NA, Kar SK. A randomized clinical trial of low dosage combination of pentamidine and allopurinol in the treatment of antimony unresponsive cases of visceral leishmaniasis. The Journal of the Association of Physicians of India. 2001;**49**:609-613

[34] Dietze R, Carvalho SF, Valli LC, Berman J, Brewer T, Milhous W, Sanchez J, Schuster B, Grogl M. Phase 2 trial of WR6026, an orally administered 8-aminoquinoline, in the treatment of visceral leishmaniasis caused by *Leishmania chagasi*. American Journal of Tropical Medicine and Hygiene. 2001;**65**:685-689

[35] Jha TK, Sundar S, Thakur CP, Felton JM, Sabin AJ, Horton J. A phase II dose-ranging study of sitamaquine for the treatment of visceral leishmaniasis in India. American Journal of Tropical Medicine and Hygiene. 2005;**73**:1005-1011

[36] Wasunna MK, Rashid JR, Mbui J, Kirigi G, Kinoti D, Lodenyo H, Felton JM, Sabin AJ, Albert MJ, Horton J. A phase II dose-increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. American Journal of Tropical Medicine and Hygiene. 2005;**73**:871-876

[37] Harvey AL. Natural products in drug discovery. Drug Discovery Today. 2008;**13**:894-901. DOI: 10.1016/j.drudis.2008.07.004

[38] Almassy AA Jr et al. Folhas de chá: Plantas medicinais na terapêutica humana. Viçosa: UFV; 2005. p. 233

[39] Cragg GM, Grothaus PG, Newman DJ. Impact of natural products on developing new anti-cancer agents. Chemical Reviews. 2009;**109**:3012-3043. DOI: 10.1021/cr900019j

[40] Formagio AS, Kassuya CA, Neto FF, Volobuff CR, Iriguchi EK, Vieira Mdo C, Foglio MA. The flavonoid content and antiproliferative, hypoglycaemic, anti-inflammatory and free radical scavenging activities of Annona dioica St. Hill. BMC Complement Altern Med. 2013 Jan 11;**13**:14. DOI: 10.1186/1472-6882-13-14

[41] Lima JFJ, Dimesntein M. A fitoterapia na saúde pública: visão do odontólogo. Saúde em Revista. 2006;**8**:37-44

[42] Külkamp IC, Burin GD, de Souza MHM, da Silva P, Piovezan AP. Aceitação de práticas não-convencionais em saúde por estudantes de medicina da Universidade do Sul de Santa Catarina. Revista Brasileira de Educação Médica. 2007;**31**:229-235. DOI: 10.1590/S0100-55022007000300005

[43] Menezes VA, dos Anjos AGP, Pereira MRD, Leite AF, Granville-Garcia AF. Terapêutica com plantas medicinais: percepção de profissionais da estratégia de saúde da família de um município do agreste pernambucano. Odonto. 2012;**20**:111-122

[44] Santos OJ, Torres OJM. Phytotherapy evolution in the healing process in surgery. Arquivos Brasileiros de Cirurgia Digestiva. 2012;**25**:139. DOI: 10.1590/S0102-67202012000300001

[45] Miguel MD, Miguel OG. Desenvolvimento de fitoterápicos. Ribeirão Preto: Tecmed. 2004;115p

[46] WHO. World Health Organization. WHO police perspectives on medicines; 2010. Available from:http://apps.who.int/medicinedocs/documents/s16880e/s16880e.pdf?ua=1 [Accessed: February 06, 2018]

[47] Petrovska BB. Historical review of medicinal plants' usage. Pharmacognosy Reviews. 2012;**6**(11):1-5. DOI: 10.4103/0973-7847.95849

[48] Stojanoski N. Development of health culture in Veles and its region from the past to the end of the 20th century. Veles: Society of science and Art; 1999. pp. 13-34

[49] Kelly K. History of Medicine. New York: Facts on File; 2009. pp. 29-50

[50] Tagboto S, Townson S. Antiparasitic properties of medicinal plants and other naturally occurring products. Advances in Parasitology. 2001;**50**:199-295. DOI: 10.1016/S0065-308X(01)50032-9

[51] Berger BJ, Fairlamb AH. Interactions between immunity and chemotherapy in the treatment of the trypanosomiases and leishmaniases. Parasitology. 1992;**105**:S71-S78. DOI: 10.1017/S0031182000075375

[52] Jantan I, Ahmad W, Bukhari SN. Plant-derived immunomodulators: An insight on their preclinical evaluation and clinical trials Ibrahim. Frontiers in Plant Science. 2015;**6**. DOI: 10.3389/fpls.2015.00655

[53] Chouhan G, Islamuddin M, Sahal D, Afrin F. Exploring the role of medicinal plant-based immunomodulators for effective therapy of leishmaniasis. Frontiers in Immunology. 2014;**5**(193):29-35. DOI: 10.3389/fimmu.2014.00193

[54] Tiwari B, Pahuja R, Kumar P, Rath SK, Gupta KC, Goyala N. Nanotized curcumin and miltefosine, a potential combination for treatment of experimental visceral Leishmaniasis. Antimicrobial Agents and Chemotherapy. 2017;**61**:e01169-e01116. DOI: 10.1128/AAC.01169-16

[55] Santos AL, Yamamoto ES, Passero LFD, Laurenti MD, Martins LF, Lima ML, Uemi M, Soares MG, Lago JHG, Tempone AG, Sartorelli P. Antileishmanial activity and immunomodulatory effects of tricin isolated from leaves of Casearia *arborea* (Salicaceae). Chemistry and Biodiversity. 2017;**14**(5). DOI: 10.1002/cbdv.201600458

[56] Ali N, Nakhasi HL, Valenzuela JG, Reis AB. Targeted immunology for prevention and cure of VL. Frontiers in Immunology. 2014;**5**(193):29-35. DOI: 10.3389/fimmu.2014.00660

[57] Shukla S, Bajpai VK, Kim M. Plants as potential sources of natural immunomodulators. Reviews in Environmental Science and Bio/Technology. 2014;**13**(1):17-33. DOI: 10.1007/s11157-012-9303-x

[58] Almeida-Souza F, de Souza Cda S, Taniwaki NN, Silva JJ, de Oliveira RM, Abreu-Silva AL, Calabrese Kda S. Morinda citrifolia Linn. Fruit (noni) juice induces an increase in NO production and death of Leishmania amazonensis amastigotes in peritoneal macrophages from BALB/c. Nitric Oxide. 2016;**58**:51-58. DOI: 10.1016/j.niox.2016.06.004 a

[59] Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:8841-8848

[60] Horta MF, Mendes BP, Roma EH, Noronha FS, Macêdo JP, Oliveira LS, Duarte MM, Vieira LQ. Reactive oxygen species and nitric oxide in cutaneous leishmaniasis. J Parasitol Res. 2012;**2012**:203818. DOI: 10.1155/2012/203818. Epub 2012 Apr 12

[61] Bogdan C. Nitric oxide synthase in innate and adaptive immunity: An update. Trends in Immunology. 2015;**36**:161-178. DOI: 10.1016/j.it.2015.01.003

[62] Bredt DS. Endogenous nitric oxide synthesis: Biological functions and pathophysiology. Free Radical Research. 1999;**31**:577-596

[63] Fang FC. Antimicrobial reactive oxygen and nitrogen species: Concepts and controversies. Nature Reviews Microbiology. 2004;**2**:820-832

[64] Chouhan G, Islamuddin M, Want MY, Abdin MZ, Ozbak HA, Hemeg HA, Sahal D, Afrin F. Apoptosis mediated leishmanicidal activity of *Azadirachta indica* bioactive fractions is accompanied by Th1 immunostimulatory potential and therapeutic cure in vivo. Parasites & Vectors. 2015;**8**(183):1-24

[65] Aloui Z, Messaoud C, Haoues M, Neffati N, Bassoumi Jamoussi I, Essafi-Benkhadir K, Boussaid M, Guizani I, Karoui H. Asteraceae Artemisia campestris and Artemisia herba-alba essential oils trigger apoptosis and cell cycle arrest in *Leishmania infantum* Promastigotes. Evidence-Based Complementary and Alternative Medicine. 2016; **9147096**:1-15

[66] Tiuman TS, Santos AO, Ueda-Nakamura T, Filho BPD, Nakamura CV. Recent advances in leishmaniasis treatment. International Journal of Infectious Diseases. 2001;**15**(8):525-532. DOI: 10.1016/j.ijid.2011.03.021

[67] Rates SM. Plants as source of drugs. Toxicon. 2001;**39**:603-613. DOI: 10.1016/S0041-0101(00)00154-9

[68] Chandrasekaran S, Veronica J, Sundar S, Maurya R. Alcoholic fractions F5 and F6 from Withania somnifera leaves show a potent antileishmanial and immunomodulatory activities to control experimental visceral leishmaniasis. Frontiers in Medicine (Lausanne). 2017;**12**(4):55. DOI: 10.3389/fmed.2017.00055

[69] Adebayo OL, Suleman D, Samson AA. Natural products in antileishmanial drug discovery: A review. Journal of Asian Scientific Research. 2013;**3**(2):157-173

[70] Rocha LG, Almeida JR, Macêdo RO, Barbosa-Filho JM. A review of natural products with antileishmanial activity. Phytomedicine. 2005;**12**:514-535. DOI: 10.1016/j.phymed.2003.10.006

[71] Wang J, Peng Q, Li G. New compounds of natural resources in 2008. African Journal of Biotechnology. 2009;**8**:4299-4307 http://www.academicjournals.org/AJB

[72] Patwardhan B, Gautam M. Botanical immunodrugs: Scope and opportunities. Drug Discovery Today. 2005;**10**:495-502. DOI: 10.1016/S1359-6446(04)03357-4

[73] Kaur S, Bhardwaj K, Sachdeva H. Antileishmanial efficacy of Boerhaavia diffusa L. and Ocimum sanctum L. against experimental visceral leishmaniasis. Indian Journal of Experimental Biology. 2015 Aug;**53**(8):522-529

[74] Sharma U, Velpandian T, Sharma P, Singh S. Evaluation of anti-leishmanial activity of selected Indian plants known to have antimicrobial properties. Parasitology Research. 2009 Oct;**105**(5):1287-1293. DOI: 10.1007/s00436-009-1554-2

[75] Oliveira M, João Rodrigues M, Pereira C, Neto RLDM, Junior PAS, Neng NDR, Nogueira JMF, Varela J, Barreira L, Custódio L. First report of the in vitro antileishmanial properties of extremophile plants from the Algarve coast. Natural Product Research. 2017 May;**11**:1-5. DOI: 10.1080/14786419.2017.1326489

[76] Bhalla G, Kaur S, Kaur J, Kaur R, Raina P. Antileishmanial and immunomodulatory potential of Ocimum sanctum Linn. and Cocos nucifera Linn. In murine visceral leishmaniasis. Journal of Parasitic Disease. 2017 Mar;**41**(1):76-85. DOI: 10.1007/s12639-016-0753-x

[77] Das A, Das MC, Das N, Bhattacharjee S. Evaluation of the antileishmanial potency, toxicity and phytochemical constituents of methanol bark extract of Sterculia villosa. Pharmaceutical Biology. 2017 Dec;**55**(1):998-1009. DOI: 10.1080/13880209.2017.1285946

[78] Pramanik A, Paik D, Naskar K, Chakraborti T. Coccinia grandis (L.) Voigt leaf extract exhibits antileishmanial effect through pro-inflammatory response: An in vitro study. Current Microbiology. 2017 Jan;**74**(1):59-67. DOI: 10.1007/s00284-016-1151-4

[79] Das P, Paik D, Pramanik A, De T, Chakraborti T. Antiproteolytic and leishmanicidal activity of Coccinia grandis (L.) Voigt leaf extract against Leishmania donovani promastigotes. Indian Journal Experimental Biology. 2015;**53**(11):740-746

[80] Almeida-Souza F, Taniwaki NN, Amaral AC, de Souza Cda S, Calabrese Kda S, Abreu-Silva A. Ultrastructural changes and death of *Leishmania infantum* promastigotes induced by *Morinda citrifolia* Linn. Fruit (noni) juice treatment. Evidence-Based Complementary and Alternative Medicine. 2016;**2016**:5063540. DOI: 10.1155/2016/5063540 b

[81] Paik D, Das P, Naskar K, Pramanik PK, Chakraborti T. Protective inflammatory response against visceral leishmaniasis with potato tuber extract: A new approach of successful therapy. Biomedicine & Pharmacotherapy. 2016 Oct;**83**:1295-1302. DOI: 10.1016/j.biopha.2016.08.046

[82] Paik D, Das P, De T, Chakraborti T. In vitro anti-leishmanial efficacy of potato tuber extract (PTEx): Leishmanial serine protease(s) as putative target. Experimental Parasitolology. 2014 Nov;**146**:11-19. DOI: 10.1016/j.exppara.2014.08.009

[83] Singh MK, Paul J, De T, Chakraborti T. Bioactivity guided fractionation of Moringa oleífera lam. Flower targeting Leishmania donovani. Indian Journal of Experimental Biology. 2015;**53**:747-752

[84] Chouhan G, Islamuddin M, Want MY, Abdin MZ, Ozbak HA, Hemeg HA, Sahal D, Afrin F. Apoptosis mediated leishmanicidal activity of Azadirachta indica bioactive fractions is accompanied by Th1 immunostimulatory potential and therapeutic cure in vivo. Parasites and Vectors. 2015 Mar 26;**8**:183. DOI: 10.1186/s13071-015-0788-3

[85] Dayakar A, Chandrasekaran S, Veronica J, Sundar S, Maurya R. In vitro and in vivo evaluation of anti-leishmanial and immunomodulatory activity of Neem leaf extract in Leishmania donovani infection. Experimental Parasitology. 2015 Jun;**153**:45-54. DOI: 10.1016/j.exppara.2015.02.011

[86] Dey S, Mukherjee D, Chakraborty S, Mallick S, Dutta A, Ghosh J, Swapana N, Maiti S, Ghorai N, Singh CB, Pal C. Protective effect of Croton caudatus Geisel leaf extract against experimental visceral leishmaniasis induces proinflammatory cytokines in vitro and in vivo. Experimental Parasitology. 2015 Apr-May;**151-152**:84-95. DOI: 10.1016/j.exppara.2015.01.012

[87] Islamuddin M, Chouhan G, Farooque A, Dwarakanath BS, Sahal D, Afrin F. Th1-biased immunomodulation and therapeutic potential of Artemisia annua in murine visceral leishmaniasis. PLoS Neglected Tropical Disease. 2015 Jan 8;**9**(1):e3321. DOI: 10.1371/journal.pntd.0003321

[88] Islamuddin M, Farooque A, Dwarakanath BS, Sahal D, Afrin F. Extracts of Artemisia annua leaves and seeds mediate programmed cell death in Leishmania donovani. Journal of Medical Microbiology. 2012 Dec;**61**(Pt 12):1709-1718. DOI: 10.1099/jmm.0.049387-0

[89] Kaur S, Chauhan K, Sachdeva H. Protection against experimental visceral leishmaniasis by immunostimulation with herbal drugs derived from Withania somnifera and *Asparagus racemosus*. Journal of Medical Microbiology. 2014 Oct;**63**(Pt 10):1328-1338. DOI: 10.1099/jmm.0.072694-0

[90] Sachdeva H, Sehgal R, Kaur S. Asparagus racemosus ameliorates cisplatin induced toxicities and augments its antileishmanial activity by immunomodulation in vivo. Parasitology International. 2014 Feb;**63**(1):21-30. DOI: 10.1016/j.parint.2013.09.016

[91] Islamuddin M, Sahal D, Afrin F. Apoptosis-like death in Leishmania donovani promastigotes induced by eugenol-rich oil of Syzygium aromaticum. Journal of Medical Microbiology. 2014 Jan;**63**(Pt 1):74-85. DOI: 10.1099/jmm.0.064709-0

[92] Hubert DJ, Céline N, Michel N, Gogulamudi VR, Florence NT, Johnson BN, Bonaventure NT, Singh IP, Sehgal R. In vitro leishmanicidal activity of some Cameroonian medicinal plants. Experimental Parasitology. 2013 Jul;**134**(3):304-308. DOI: 10.1016/j.exppara.2013.03.023

[93] Rondon FC, Bevilaqua CM, Accioly MP, de Morais SM, de Andrade-Júnior HF, de Carvalho CA, Lima JC, Magalhães HC. In vitro efficacy of Coriandrum sativum, Lippia sidoides and Copaifera reticulata against Leishmania chagasi. Revista Brasileira de Parasitologia Veterinária. 2012 July–Sep;**21**(3):185-191

[94] Accioly MP, Bevilaqua CM, Rondon FC, de Morais SM, Machado LK, Almeida CA, de Andrade HF Jr, Cardoso RP. Leishmanicidal activity in vitro of Musa paradisiaca L. and Spondias mombin L. fractions. Veterinary Parasitology. 2012 Jun 8;**187**(1-2):79-84. DOI: 10.1016/j.vetpar.2011.12.029

[95] Vila-Nova NS, Morais SM, Falcão MJ, Machado LK, Beviláqua CM, Costa IR, Brasil NV, Andrade Júnior HF. Leishmanicidal activity and cytotoxicity of compounds from two Annonacea species cultivated in northeastern Brazil. Revista da Sociedade Brasileira de Medicina Tropical. 2011 Oct;**44**(5):567-571

[96] Rondon FC, Bevilaqua CM, Accioly MP, Morais SM, Andrade-Junior HF, Machado LK, Cardoso RP, Almeida CA, Queiroz-Junior EM, Rodrigues AC. In vitro effect of Aloe vera, Coriandrum sativum and Ricinus communis fractions on Leishmania infantum and on murine monocytic cells. Veterinary Parasitolology. 2011 June 10;**178**(3-4):235-240. DOI: 10.1016/j.vetpar.2011.01.007

[97] Ghosh S, Debnath S, Hazra S, Hartung A, Thomale K, Schultheis M, Kapkova P, Schurigt U, Moll H, Holzgrabe U, Hazra B. Valeriana wallichii root extracts and fractions with activity against Leishmania spp. Parasitology Research. 2011 Apr;**108**(4):861-871. DOI: 10.1007/s00436-010-2127-0

[98] Gupta S, Raychaudhuri B, Banerjee S, Das B, Mukhopadhaya S, Datta SC. Momordicatin purified from fruits of *Momordica charantia* is effective to act as a potent antileishmania agent. Parasitology International. 2010 June;**59**(2):192-197. DOI: 10.1016/j.parint.2010.01.004

[99] Gomes DC, Muzitano MF, Costa SS, Rossi-Bergmann B. Effectiveness of the immunomodulatory extract of Kalanchoe pinnata against murine visceral leishmaniasis. Parasitology. 2010 Apr;**137**(4):613-618. DOI: 10.1017/S0031182009991405

[100] Misra P, Kumar A, Khare P, Gupta S, Kumar N, Dube A. Pro-apoptotic effect of the landrace Bangla Mahoba of Piper betle on Leishmania donovani may be due to the high content of eugenol. Journal of Medical Microbiology. 2009 Aug;**58**:1058-1066. DOI: 10.1099/jmm.0.009290-0

[101] Poddar A, Banerjee A, Ghanta S, Chattopadhyay S. In vivo efficacy of calceolarioside a against experimental visceral leishmaniasis. Planta Medica. 2008 Apr;**74**(5):503-508. DOI: 10.1055/s-2008-1034373

[102] Dutta A, Sarkar D, Gurib-Fakim A, Mandal C, Chatterjee M. In vitro and in vivo activity of Aloe vera leaf exudate in experimental visceral leishmaniasis. Parasitology Research. 2008;**102**:1235-1242. DOI: 10.1007/s00436-008-0899-2

[103] Singh N, Kumar A, Gupta P, Chand K, Samant M, Maurya R, Dube A. Evaluation of antileishmanial potential of Tinospora sinensis against experimental visceral leishmaniasis. Parasitology Research. 2008 Feb;**102**(3):561-565. DOI: 10.1007/s00436-007-0822-2

[104] Manzote L, García M, Montalvo AM, Scull R, Miranda M, Abreu J. In vitro activity of an essential oil against Leishmania donovani. Phytotherapy Research. 2007 Nov;**21**(11):1055-1058

[105] Mesquita ML, Desrivot J, Bories C, Fournet A, Paula JE, Grellier P, Espindola LS. Antileishmanial and trypanocidal activity of Brazilian Cerrado plants. Memórias do Instituto Oswaldo Cruz. 2005;(7). DOI: 10.1590/S0074-02762005000700019

[106] World Health Organization. A Report of the Consultation Meeting on Traditional and Modern Medicine: Harmonizing two Approaches; November 22-26, 1999. Beijing: West Pacific Region; 2000

Chapter 10

Natural Compounds and Extracts from Mexican Medicinal Plants with Anti-Leishmanial Activity: An Update

María Adelina Jiménez-Arellanes and
Rosalba León-Díaz

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77247

Abstract

Leishmaniasis is considered an emerging, uncontrolled disease, and is endemic in 98 countries. Annually, about 2 million cases of cutaneous and 500,000 cases of visceral type leishmaniasis are recorded, and 60,000 persons die from the disease. In Mexico, cutaneous leishmaniasis is known as chiclero's ulcer and is reported in 22 states; it is considered a health problem. For its treatment, pentavalent antimonial drugs are administered; these drugs cause severe side effects, are costly, and drug-resistant cases have been reported and have been developing for >70 years. One alternative to the drugs that are currently available is to find active molecules in medicinal plants; dihydrocorynantheine, corynantheine, and corynantheidine are active against *Leishmania major*, while harmane, pleiocarpin, buchtienin, luteolin, and quercetin are active against *L. donovani*. In Mexico, about 20 medicinal plants have been evaluated against *L. mexicana*, among which the most active are *Tridax procumbens, Tridax procumbens, Pentalinon andrieuxii, Lantana camara, Schinus molle,* and *Prosopis laevigata*. From some of these plants, active compounds with $IC_{50} \leq 30$ μg/mL or μM have been isolated, such as 3(S)-16,17-didehydrofalcarinol or Oxylipin, cholestra-4,20,24-trien-3-one or pentalinosterol, 24-methylcholest-4-24(28)-dien-3-one, cholest-4-en-3-one, 6,7-dihy-droneridie-none, neridienone, cholest-5,20,24-trien-3β-ol, and isocordoin. Today, the only pentalinonsterol has been synthesized and assayed in the visceral leishmaniasis experimental model using BALB/c mice infected with *L. donovani*. Liposome formulation of this compound administered by intravenous route at 2.5 mg/kg showed a significant reduction of parasite load in mouse liver and spleen.

Keywords: active extracts, leishmaniasis, leishmanicidal activity, natural compounds, Mexican medicinal plants

1. Introduction

Leishmaniasis is caused by about 20 species of the protozoan parasite of the genus *Leishmania*. It is classified by the World Health Organization (WHO) as an emergent category one, uncontrolled disease, and it comprises one of the six most important tropical diseases, with 0.9–1.6 million new cases annually and 20,000–30,000 deaths. The *Leishmania* infection exhibits three main clinical manifestations: cutaneous, mucocutaneous, and visceral. It is endemic in 98 developing countries (tropical and subtropical regions) and is more frequent in males. Today, it is estimated that there are 12 million infected persons (all forms), 350 million of people are at risk, and an incidence has been reported of 1.5–2 million new cutaneous cases annually [1–4]. Each year, 500,000 cases of the visceral type are reported and 50,000 individuals die from the latter [4]. In Mexico, cases of leishmaniasis has been reported in 22 states, and it is considered endemic in the states of Coahuila, Nuevo León, Tamaulipas, Veracruz, Tabasco, Campeche, Yucatán, Quintana Roo, Chiapas, Oaxaca, Guerrero, Michoacán, Jalisco, Nayarit, San Luis Potosí, Morelos, Puebla, and Hidalgo, where it is commonly known *as* chiclero's ulcer [5–8]. For example, in one municipality of the state of Campeche, over a 2-year period, 76% of persons had skin lesions, and were diagnosed with cutaneous leishmaniasis. In this study, about 89% of cutaneous leishmaniasis is caused principally by *Leishmania mexicana* [9]. The most serious clinical manifestation of leishmaniasis is the visceral form (VL), which it is endemic in Guerrero and Morelos; and 921,273 people are considered at risk to be infected [10]. Recently, cases of leishmaniasis co-infection with HIV/AIDS have been reported, which have a poor prognosis. This co-infection has worldwide distribution and has been recorded in 35 countries. Infection by this parasite depends in great measure on the state of the host's immune system. Other risk factors that favor its dissemination are socioeconomic condition, migration, deforestation, and urbanization [3, 8].

Currently, treatment of leishmaniasis employs first-line drugs such as sodium stibogluconate (commercially known as Pentostam) and meglumine antimoniate (commercially known as Glucantime), and other options (second-line drugs) are pentamidine isothionate (commercially known as Pentamidine), amphotericin B, (Fungizone or Ambisome), miltefosine, and paramomycin sulfate (Aminosidine), although this latter option is not widely utilized in Mexico and is not effective when administered orally [11]. Even when administered in combination, the effectiveness of the drugs is less than optimal [12, 13].

Antimonial pharmaceuticals (pentostam, glucantime, and pentamidine) were developed >70 years ago, and are continue to be used to treat leishmaniasis. Some of these have not been effective due to the drug resistance developed by the parasite [2, 8, 14, 15], in addition to the scarce development of this drug type. These substances have severe side effects, such as kidney failure, acute pancreatitis, myalgia, teratogenicity, peripheral neuropathy, hepatotoxicity, and cardiotoxicity (cardiac arrhythmia), in addition to the fact that treatment is prolonged (>30 days) depending on the patient's immunity. Drug administration is by the parenteral route, some of these drugs are expensive, and they are not always effective due to the parasite's resistance. Sometimes, the patient has no access to health systems, and these drugs cannot be utilized in patients with kidney, hepatic, or cardiac failure, or in those with tuberculosis [8]. An alternative for the treatment of leishmaniasis is to find molecules active in medicinal plants that serve as active principles for the development of new pharmaceutical preparations.

http://dx.doi.org/10.5772/intechopen.77247

In the present text, an exhaustive bibliographic research (from 2001 to 2018) was carried out on leishmanicidal activity from the extracts and/or compounds obtained from Mexican medicinal plants against several *Leishmania* spp. in *vivo* and *in vitro* assays. The main scientific sources consulted were the Scopus and PubMed databases. Regarding this subject, we found 56 references. The keywords employed included: medicinal plants, Mexican medicinal plants, anti-leishmanial activity, and natural compounds.

2. An overview of the leishmanicidal potential of medicinal plants and compounds isolated from these

The development of drugs to treat parasitic diseases such as leishmaniasis has been scarce, due to the fact that these diseases are more often present in developing countries, and because the pharmaceutical industry does not receive high profits since it must develop low-cost medication that will be accessible to a population with a low socioeconomic condition [7, 15]. In this regard, the WHO has emphasized the urgent need to develop new drugs for the treatment of leishmaniasis [4]. An alternative to synthetic drugs is the search for active molecules from natural sources, such as the medicinal plants used in the treatment of leishmaniasis in ancient times. In this regard, medicinal plants biosynthesize several secondary metabolites, which constitute an important source of leishmanicidal agents [7, 16].

Natural products have been an important role in current therapy; between the years 1981–2006, 1184 novel drugs with a natural origin were obtained, and 28% of these derived from plants. On the other hand, 24% of the new synthetic drugs have as a base molecule or are derived from, active molecules obtained from medicinal plants [8–17]. Another report states that between the years 2000–2005, 23 new natural-origin drugs were introduced into the market, all of which exhibited structural and biological diversity. Therefore, natural products constitute an immeasurable wealth of chemical structures that has been and continues to be an important source of new drugs and that constitutes prototype molecules for the development of new active substances [18–20]. Some examples of the active agent obtained from medicinal plants utilized in current therapy are paclitaxel (isolated from *Taxus brevifolia*), camptothecin (isolated from *Camptotheca acuminata*), and vinblastine and vincristine (isolated from *Catharanthus roseus*); artemisinin, isolated from *Artemisia annua*; this compound is employed in malaria treatment.

Regarding the development of active compounds against *Leishmania* spp., to date only four molecules are potential candidates for the development of anti-leishmanial drugs (these substances are in phase I/II research) and include the following: miltefosine (an alkylphospholipid) that has been used in India since 2002, that was authorized for use in Colombia in 2005, and is in clinical phase research to determine its possible global use [2]; paromomycin (an aminoglycoside); 8-aminoquinoline; sitamaquine, and berberine (the latter, an alkaloid of vegetable origin, isolated from *Beberis vulgaris*). This latter compound has been utilized against this disease for >50 years and has demonstrated its activity both *in vitro* and *in vivo* [8, 20–24].

Recently, some secondary metabolites, such as quinones, naphthoquinones, lignans, neolignans, alkaloids (quinolines, isoquinoline, steroidal, and indole analogs), phenolic derivatives (chalcones and flavonoids), and terpenes (iridoids, sesquiterpenes, diterpenes, triterpenoids, and saponins) have been reported to possess leishmanicidal activity [23, 25–28]. Among these,

some alkaloids isolated from plant species have exhibited significant *in vitro* leishmanicidal activity. Some examples of these are isoguattouregidine, an indole alkaloid isolated from *Guatteria foliosa*, with a mean inhibitory concentration (IC_{50}) = 100 μg/mL against *L. donovani* and *L. amazonensis*, and coronaridine (isolated from *Peschirea australis*), which an IC_{50} = 12 μg/mL against *L. amazonensis*. In addition, indole alkaloids (dihydrocorinanteine, corinanteine, and corinanteidine), which were isolated from *Corynanthe pachyceras*, were active against *Leishmania major* with an IC_{50}~30 μM. Other indole alkaloids, including harmane, pleiocarpin, and buchtienin, which are isolated from the bark and leaves of *Kopsia griffithii*, were active against promastigotes of *L. donovani*, demonstrating IC_{50} = 6.25, 2, and 1.56 μg/mL, respectively [26–29]. The main disadvantage is that these alkaloids have been evaluated against different strains of *Leishmania* and on different growth stages, and none of these compounds, to our knowledge, is currently under clinical investigation. Other active alkaloids, such as ramiflorines A and B (isolated from *Aspidosperma ramiflorum*) showed a median lethal dose (LD_{50}) = 16.3 and 4.9 μg/mL against *L. amazonensis* promastigotes, respectively [26]. The alkaloid 4-hydroxy-1-tetralone (isolated from *Ampelocera edentula* bark) was active against *L. braziliensis*, *L. amazonensis*, and *L. donovani* promastigotes, with an IC_{50} = 10 μg/mL [30].

In addition, the *in vitro* activity of other two medicinal plant extracts, such as *Ambrosia miratima* and *Acacua nilotica* with IC_{50} < 8 μg/mL [31] have been reported; however, no compounds responsible for activity have been isolated from these active plant species. The ethanol extract and the dichloromethane and chloroform fractions from the leaves of *Azadirachta indica* presented IC_{50} = 38, 3.9, and 1.2 μg/mL against promastigotes of *L. amazonensis,* respectively, and against amastigotes, IC_{50} was 9.8, 1.1, and 0.6 μg/mL [16].

The tormentic acid-rich fraction, 2α,3β-dihydroxyursan-12-in-28-oic acid,2α,3β-dihydroxyolean-12-in-oic acid, ursolic acid, and oleanolic acid from *Pourouma guianensis* were active against *L. amazonensis* promastigotes, showing an IC_{50} = 100 μg/mL; in addition, ursolic acid, and oleanolic acid were also very active against intracellular amastigotes (IC_{50} = 27 and 11 μg/mL, respectively). These compounds were more active than glucantime (IC_{50} = 83 μg/mL) [23]. In addition, review described that the flavones luteolin and quercetin (isolated from *Vitex negundo* and *Fagopyrum esculentum*, respectively) were active against *L. donovani* amastigotes, with IC_{50} = 12.5, and 45.5 μM; the chalcone identified as licochalcone A (isolated from *Glycyrrhiza* spp.) showed an IC_{50} = 0.9 μg/mL (2.7 μM) against *L. donovani* amastigotes and against *L. major* promastigotes, demonstrating an IC_{50} = 7.2 μg/mL (21 μM). Also, 2′,6′-dihydroxy-4′-methoxy chalcone (isolated from *Piper aduncum*) inhibited the growth of promastigotes and intracellular amastigotes of *L. amazonensis*; median effective doses were 0.5 μg/mL (1.9 μM) and 24 μg/mL (89 μM), respectively. The nanoparticle polymeric formulation of this compound (440 μg) was administered during 42 days to BALB/c mice infected with *L. amazonensis*; the results revealed that this formulation reduced their skin ulcers by 53%, while the pure compound reduced ulcers by only 23% [27, 32]. A glucosecoiridoid, identified as amarogentin (isolated from *Swertia chirata*), was tested in an *in vivo* model (hamster), together with two formulations (liposomal and niosomal) in mice infected with *L. donovani*; the niosomal-amarogentin formula reduced the parasitic load by 90% in the spleen of the treated animals and was more efficacious than the liposomal amarogentin. Both of these formulations can be good candidates for developing leishmanicidal drugs [27, 33].

Plumbagin, a naphthoquinone isolated from the bark of *Pera benensis* and from some species of the genus *Plumbago*, resulted active against *L. donovani* promastigotes and intracellular

amastigotes (IC_{50} = 0.21 μM); also, against intracellular amastigotes of *L. donovani* and *L. amazonensis*, with IC_{50} = 0.42 and 1.1 μg/mL, respectively. *In vivo* studies have demonstrated that plumbagin delayed the development of *L. amazonensis* and *L. venezuelensis* infection and exhibited good activity at 2.5–5 mg/kg/day, respectively. Local treatment of a simple lesion with 8,8′-biplumbagin resulted in a better treatment than that of glucantime (reference drug). In addition, plumbagin and 8,8′-biplumbagin were very active against *L. amazonensis* amastigotes and against *L. brazilensis* (2903), *L. amazonensis* (PH8, H-142), and *L. donovani* (2682 and HS70) promastigotes, demonstrating values of IC_{90} = 5 μg/mL [27, 34–37].

Saponins mesabalide III and mesabalide VI (obtained from *Maesa balansae*), were very active against intracellular amastigotes of *L. infantum* (IC_{50} = 7 and 14 ng/mL, respectively) but, despite exhibiting significant leishmanicidal activity, these compounds are highly cytotoxic; thus, they are not candidates for continued research. The steroidal saponin racemoside A (isolated from *Asparagus racemosus*) induced apoptosis in *L. donovani* promastigotes and amastigotes and showed values of IC_{50} = 1.31 and 0.61 μg/mL, respectively [27]. α- and β-Hederine and hederacholchiside A (obtained from *Hedera helix*) demonstrated leishmanicidal activity; hederacholchiside A was more active, with an IC_{50} = 1.2 and 0.053 μM against *Lleishmania infantum* promastigotes and intracellular amastigotes, respectively [26]. Diospyrin (isolated from *Euclea natalensis*) was active against *L. donovani* promastigotes at 0.1 μg/mL. This compound is a specific inhibitor of the parasitic topoisomerase [38, 39].

3. Extract and pure compounds obtained from Mexican medicinal plants active against *Leishmania* spp.

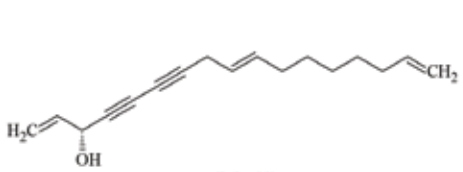
In Mexico, some unconventional treatments as cauterization with copper sulfate are routinely used to treat leishmaniasis. Employing hot automobile engine oil, red-hot coins, red metal utensils, hot animal bones, or a hot light bulb directly applied as thermotherapy are on the ulcer as thermotherapy; also, cryotherapy, which consists of placing ice on the wound is applied. Both therapies are used in Mexico and in some regions worldwide [22]. Antibacterials as penicillin or antifungal creams (such as miconazole, ketoconazole, or itraconazole) are applied on the lesion [40]. Mexican patients have also used acetic acid, boric acid, sulfuric acid (car battery acid), formalin, alcohol, hydrogen peroxide, wire and copper sulfate, among other remedies [22, 42]. While these methods only deform and accentuate the inflammation, patients continue to employ these approaches without knowing that they are dealing with a parasitosis, which requires professional medical care.

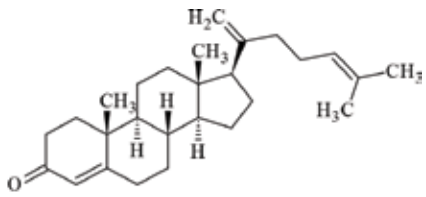
On the other hand, some plants are routinely used in Mexico to treat the skin lesions caused by *Leishmania* [22, 41, 42]. To date, there are scarce studies that explore theirs *in vitro* and/or *in vivo* leishmanicidal activity. Peraza-Sánchez et al. [42] described an *in vitro* evaluation of the methanolic extracts from 18 medicinal plants from the southeastern state of Yucatán, Mexico against *L. mexicana* promastigotes; these authors found that the extracts of *Aphelandra scabra* (leaves), *Byrsonima bucidaefolia* (bark), *Byrsonima crassifolia* (bark), *Clusia flava* (leaves), *Cupania dentata* (bark), *Diphysa carthagenensis* (leaves), *Dorstenia contrajerva* (complete plant), *Milleria quinqueflora* (root), *Tridax procumbens* (complete plant), and *Vitex gaumeri* (bark) were the most active, exhibiting IC_{50} values of <50 μg/mL. The same investigation group assayed 15 samples

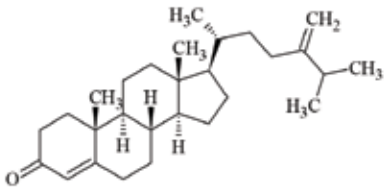
(extracts, fractions, and some pure compounds) obtained from *Urechites andrieuxii* (syn. *Pentalinon andrieuxii*), *Colubrina greggii*, *Dorstenia contrajerva*, and *Tridax procumbens*. One compound, identified as NCG-5C, and the fraction DCG-3A (with low polarity) obtained from *C. greggii* and the low-polarity fraction TPZ-24 obtained from *T. procumbens*, were the most active against *L. aethiopica* promastigotes; these samples demonstrated LD_{50} = 62.4, 7.2, and 18.5 μg/mL, respectively, while LD_{50} against amastigotes was 94.2, 27.1, and 95.2 μg/mL, respectively. In this study, it is also evaluated the same extracts and pure compounds against *L. major* and *L. tropica*, but these samples exhibited poor activity [1]. The methanol extract of *T. procumbens* and the compound identified as 3(S)-16,17-didehydrofalcarinol or oxylipin (1) inhibited the growth of *L. mexicana* promastigotes, showing IC_{50} = 3 and 0.478 μg/mL, respectively. In addition, pure oxylipin (1) was active against the intracellular amastigotes of *L. mexicana* [43, 44]. Gamboa-León et al. [45] described that the methanol extract of the *T. procumbens* (complete plant) mixed with the lyophilized aqueous extract of *Allium sativa* (bulbs) significantly reduced skin lesions caused by *L. mexicana* promastigotes (Hd18-MHET/MX/97/Hd18) in female CD-1 mice treated during 2 weeks with this mixture. Individually, these extracts also reduced the formation of lesions in a lower percentage than the mixture. These authors also described that the methanol extract from *U. andrieuxii* (syn. *P. andrieuxii*) leaves and roots, collected in Champotón, Mexico (Collection I), was the most active against promastigotes of *L. braziliensis*, of *L. amazonensis*, and of *L. donovani* [42] and was also active against *L. mexicana* promastigotes [46, 47]. The hexane fraction obtained from the methanol extract of *P. andrieuxii* roots was evaluated in an *in vivo* model for cutaneous leishmaniasis in male C57BL/6 mice infected with *L. mexicana* promastigotes. Topical application of 10 μg of the hexane fraction for 6 weeks significantly reduced the size of the lesions with respect to the vehicle. This fraction also inhibited the growth of *L. mexicana* in *vitro* condition, showing an IC_{50} = 43.04 μg/mL, while against macrophages infected with *L. mexicana* amastigotes, it exhibited an IC_{50} = 4.1 μg/mL, and in dendritic cells infected with *L. mexicana* amastigotes the IC_{50} value was 11.06 μg/mL [48].

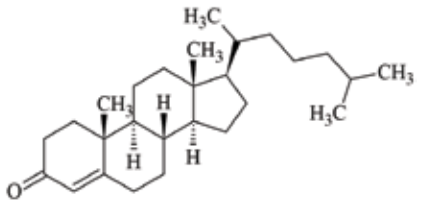
From the active hexane fraction (obtained by partition from active methanol extract) of the *U. andrieuxii* roots (syn. *P. andrieuxii*), the following compounds were isolated: cholestra-4,20,24-trien-3-one or pentalinosterol (2); 24-methylcholest-4-24(28)-dien-3-one (3), cholest-4-en-3-one (4),6,7-dihydroneridienone (5), and neridienone (6). All compounds (2–6) inhibited the growth of *L. mexicana* promastigotes, showing an IC_{50} of <30 μM; pentostam was used as positive control (IC_{50} = 346.1 μM). All of these compounds, together with cholest-5,20,24-trien-3β-ol (7), were active against *L. mexicana* amastigotes (IC_{50} < 14.5 μg/mL) in the *in vitro* assay [49]. The most active compound was cholest-4-en-3-one (4), which exhibited an IC_{50} value of 0.03 μM; all active compounds were non-cytotoxic in healthy bone marrow-derived macrophages (C57BL16 mice), demonstrating an IC_{50} of >100 μg/mL [48]. In addition, recently, pentalinonsterol (2) was synthesized and was tested in the visceral leishmaniasis experimental model using BALB/c mice infected with *L. donovani*. Pentalinonsterol (2.5 mg/kg) was administered by intravenous (IV) route in liposome formulation; this compound showed a significant reduction of parasite load in mouse liver and spleen, and it is a candidate for the development of a new leishmanicidal drug [50]. In addition, betulinic acid (8) has been isolated from the ethanol extract of *P. andrieuxii* leaves, but this was inactive against *L. amazonensis* and *L. brazilensis*, exhibiting an IC_{50} of >200 μM [1].

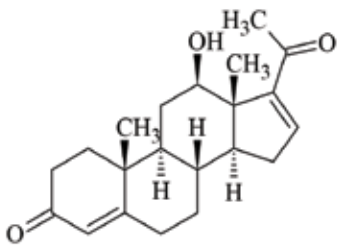
http://dx.doi.org/10.5772/intechopen.77247

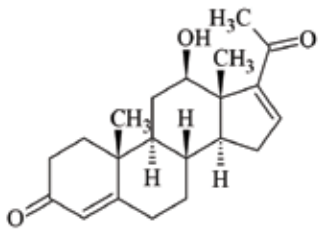

oxylipin (**1**)

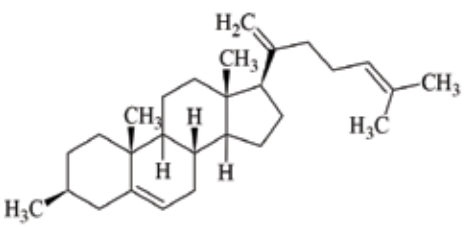

pentalinosterol (**2**)

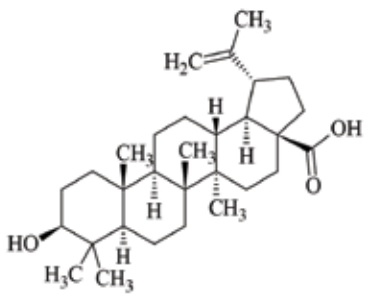

24-methylcholest-4-24(28)-dien-3-one (**3**)


cholest-4-en-3-one (**4**)


6,7-dihydroneridienone (**5**)


neridienone (**6**)

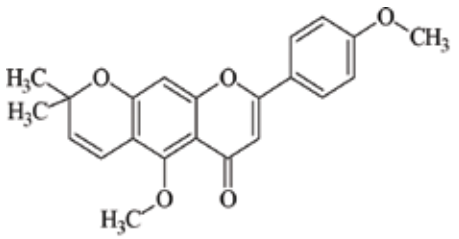

cholest-5,20,24-trien-3β-ol (**7**)

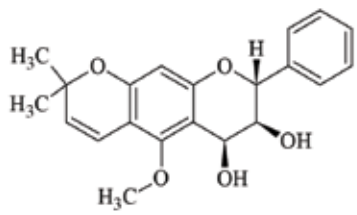

betulinic acid (**8**)

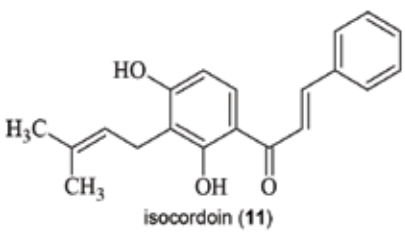
The hexane extract from *P. andrieuxii* roots at 10 μg/mL was very active against *L. mexicana* promastigotes (MHOM/MX/84/ISETGS). The effect observed was similar to that of glucantime (positive control); the parasites were completely destroyed after 100 h of exposure (LD_{50} = 6.10 vs. 173.9 μg/mL, respectively). In addition, the extracts of ethyl acetate and ethanol of this medicinal plant were also tested against *L. mexicana* but were inactive [51].

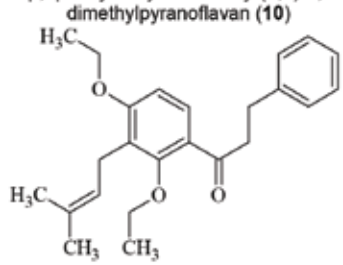
The flavone 5, 4-dimethoxy-(6, 7)-2′,2′-dimethyl-pyrano-favone (9), isolated from *Lonchocarpus xuul* and *Lonchocarpus yucatanensis* leaves) was active against promastigotes of *L. brazilensis* (MHOM/BR/75/M9203), *L. donovani* (MHOM/BR/74/PP75), and *L. amazonensis* (IFLA/BR/67/PH8), showing the similar value, an IC_{50} = 5.6 μg/mL. Also, 3β,4β-dihydroxy-5-methoxy-(7,6)-2,

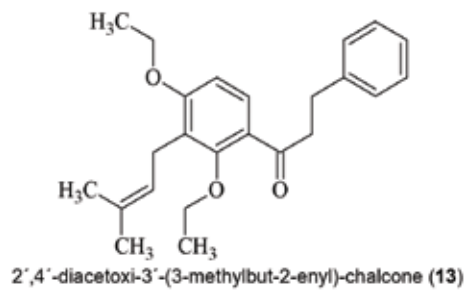
2-dimethylpyranoflavan (10) was isolated from both *Lonchocarpus* spp. and was tested against promastigotes of same *Leishmania* strain. Compound 10 was less active than compound 9, it showed an IC_{50} = 26.7–40 μg/mL against *L. brazilensis* and *L. amazonensis*, respectively, and was inactive against *L. donovani.* From *Lonchocarpus xuul* roots was isolated 2′,4′-dihydroxy-3′-(3-methyl-but-2-enyl) chalcone or isocordoin (11), this compound was active against promastigotes of the same *Leishmania* strains (*L. brazilensis*, *L. donovani*, and *L. amazonensis*), showing an IC_{50} values of 10, 40, and 26.7 μg/mL, respectively, also, this compound was active against the P-388 cell line with IC_{50} = 34–57 μM [52]. Isocordoin (11) and 2′,4′-dihydroxy-3′-(γ,γ-dimethylallyl)-dihydrochalcone or dihydroisocordoin (12), isolated from *Lonchocarpus xuul* roots were tested against *L. mexicana* promastigotes. These compounds showed an IC_{50} = 7.7–66.5 μM, respectively. In this study, some semisynthetic derivatives of these natural compounds were tested; the acetylated and methoxylated derivative [2′,4′-diacetoxi-3′-(3-methyl but-2-enyl)-chalcone (13) and 2′,4′-dimethoxy-3′-(3-methyl but-2-enyl)-chalcone (**14**)] were the most active, exhibiting an IC_{50} = 3.10–11.70 μM against *L. mexicana* promastigotes, these semisynthetic derivates were more active than natural compounds [53].

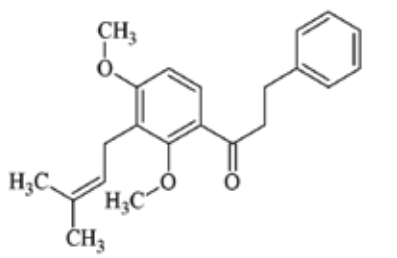












On the other hand, the chloroform and aqueous extracts (successive extracts) from *Laennecia confusa* aerial parts and the primary fraction of the chloroform extract demonstrated leishmanicidal properties. These extracts and fraction presented good activity on *L. donovani* promastigotes, with IC_{50} = 20, 20, and 200 μg/mL, respectively, after 72 h of exposure. However,

these samples (the aqueous and chloroform extracts and the primary fraction from chloroform extract) exhibited a cytotoxic effect on human-derived monocyte (THP-1) cells, with IC_{50} values of 24.8, 25, and 24.2 μg/mL, respectively [54]. The chloroform extract from *Lopezia racemosa* (aerial parts) and the hexane and methanol fractions demonstrated good activity against *L. donovani* promastigotes after 72 h incubation. The extract and hexane and methanol fractions reduced parasitic growth by approximately 88% (1×10^6 promastigotes/well). In addition, the chloroform extract was cytotoxic in macrophages (THP-1) cells, showing an IC_{50} = 28.58 μg/mL [55]. The author did not describe the active compounds.

The primary fractions (HE 5 and HE 14b) obtained from the hexane extract from the aerial parts of *gallium mexicanum* were active against *L. donovani* promastigotes (1×10^6 promastigotes/well). The HE 5 sample inhibited the growth of the parasites at 333 μg/mL after 72 h of exposure, and HE 14b was active at 999 μg/mL. The HE 5 fraction was not cytotoxic (IC_{50} = 1398 μg/mL), and the HE 14b fraction was cytotoxic (IC_{50} = 228.5 μg/mL) on the THP-1 cell line [56].

The chloroform and methanol extracts from *Echeveria leucotricha* reduced the growth of *L. donovani* promastigotes in 64–52%; however, these extracts were toxic in the human-derived monocyte-cell line THP-1. It is important to mention that the author did not describe the concentration of the extracts that they evaluated or their LD_{50} values [57].

Recently, 10 medicinal Mexican plants were evaluated against *L. amazonensis* (MHOM/77BR/LTB0016) promastigotes and amastigotes. Three of it showed a good activity (with IC_{50} < 30 μg/mL) against *L. amazonensis* promastigotes; being the most active *Lantana camara* (dichloromethane extract), *Schinus molle* (dichloromethane and dichloromethane:methanol 1:1 extracts) (SI = 5; SI = 6, respectively), and *Prosopis laevigata* (aqueous extract) (SI = 7). The SI = 5; SI = 6 extracts of *S. molle* showed IC_{50} = 15.4 and 29.4 μg/mL, respectively. The dichloromethane extract of *L. camara* exhibited IC_{50} = 11.7 μg/mL, and the aqueous extract of *P. laevigata* showed an IC_{50} = 22.8 μg/mL. The qualitative screening of the extracts revealed the presence of terpenoids in *S. molle*, the most active species. In addition, these extracts were cytotoxic against peritoneal macrophages Balb/c mice with CC_{50} > 186.8 μg/mL. In addition, both extract (dichloromethane and dichloromethane: methanol) of *S. molle* was active against *L. amazonensis* amastigotes with IC_{50} = 25.9 and 21.8 μg/mL, respectively. Also, the dichloromethane extract of *L. camara* and aqueous extract from *P. laevigata* exhibited IC_{50} = 21.8 and 35.2 μg/mL, respectively, against amastigotes of *L. amazonensis* [58].

Alamilla-Fonseca et al. [59] evaluated *Cleoserrata serrata* dichloromethane: methanol (1:1) extract. This Mexican medicinal plant is used in South-Central Mexico to treat skin infections and wounds. The extract showed activity against the *L. mexicana* amastigotes at the concentration of 10 μg/mL; and against *L. mexicana* promastigotes, the effect was dose-dependent; in this case, the author observed 60% of inhibition at 100 μg/mL and 85% of inhibition at 200 μg/mL. The LD_{50} doses were 23.5 μg/mL for promastigotes, and 6.11 μg/mL for the amastigotes [59]. This extract at 10 μg/mL showed leishmanicidal activity on amastigotes after 4 days of culture and at 100 μg/mL was leishmanicidal on promastigotes.

4. Conclusion

To date, there are few medicinal species in Mexico that have been evaluated to determine their leishmanicidal potential; from the studies performed, only seven medicinal species (*Tridax*

procumbens, *Lonchocarpus xuul*, *Pentalinon andrieuxii, L. camara, Schinus molle, Prosopis levigate,* and *Cleoserrata serrata*) have demonstrated significant activity *in vivo* against *L. mexicana* and can be considered potential candidates as leishmanicidal sources. From these species, eight active compounds have been isolated [Oxylipin, Isocordoin, 2′,4′-dihydroxy-3′-(γ,γ-dimethylallyl)-dihydrochalcone, cholestra-4,20,24-trien-3-one or pentalinosterol, 24-methyl-cholesta-4-24(28)-dien-3-one, cholest-4-en-3-one, 6,7-dihydroneridienone, neridienone, and cholest-5,20,24-trien-3β-ol], which have shown an IC_{50} of >30 μg/mL against *L. mexicana*; however, the real potential of these are not known, because only pentalinosterol has been synthesized and was tested in an *in vivo* experimental model using BALB/c mice infected with *L. donovani*. In addition, some organic extracts have demonstrated activity against other species of *Leishmania* (*L. brazilensis*, *L. donovani*, and *L. amazonensis*), but the compounds responsible for this activity, to our knowledge, have not been reported. Leishmaniasis is a global health problem, coupled with drug resistance and the side effects caused by current drugs, which makes it necessary to redouble efforts to continue investigating other medicinal species in order to find active compounds that contribute to the treatment of the disease or that serve as prototype molecules to develop drugs with different mechanism of actions from those currently employed.

Conflicts of interest

All authors have read and approved the final version of the manuscript. The authors declare that they have no competing interests.

Ethical responsibilities concerning the protection of people and animals

This manuscript is a bibliographic review and no persons or animals were used.

Confidentiality of data

This review does not describe patient data.

Author details

María Adelina Jiménez-Arellanes* and Rosalba León-Díaz

*Address all correspondence to: adelinajim08@prodigy.net.mx

Unidad de Investigación Médica en Farmacología, Hospital de Especialidades, Centro Médico Nacional Siglo XXI (CMN Siglo XXI), Instituto Mexicano del Seguro Social (IMSS), Ciudad de México, Mexico

References

[1] Domínguez-Carmona DB, Escalante-Erosa F, García-Sosa K, Ruiz-Pinell G, Gutiérrez-Yapu D, Chan-Bacab MJ, et al. Antiprotozoal activity of betulinic acid derivatives. Phytomedicine. 2010;**17**(5):379-382

[2] Getti G, Durgadoss O, Domínguez-Carmona D, Martín-Quintal Z, Peraza-Sánchez S, Peña-Rodriguez LM, et al. Leishmanicidal activity of Yucatecan medicinal plants on *Leishmania* species responsible for cutaneous leishmaniasis. The Journal of Parasitology. 2009;**95**(2):456-460

[3] Oryan A, Akbar M. Worldwide risk factors in leishmaniasis. Asian Pacific Journal of Tropical Medicine. 2016;**9**(10):925-932

[4] World Health Organization. Fact Sheet No. 375, update September 2016. Available from: http://www.who.int/mediacentre/factsheets/fs375/en/ [Accessed: November 3, 2016]

[5] Arjona-Jiménez G, Villegas N, López-Céspedes A, Marín C, Longoni SS, Bolio-González ME, et al. Prevalence of antibodies against three species of Leishmania (*L. mexicana*, *L. braziliensis*, *L. infantum*) and possible associated factors in dogs from Mérida, Yucatán, Mexico. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2012;**106**(4):252-258. DOI: 10.1016/j.trstmh.2011.12.003

[6] Bero J, Hannaert V, Chataigné G, Hérent MF, Quetin-Leclercq J. *In vitro* antitrypanosomal and antileishmanial activity of plants used in Benin in traditional medicine and bio-guided fractionation of the most active extract. Journal of Ethnopharmacology. 2011;**137**(2):998-1002. DOI: 10.1016/j.jep.2011.07.022

[7] Varela-M RE, Villa-Pulgarín JA, Yepes E, Müller I, Modolell M, Muñoz DL, et al. *In vitro* and *in vivo* efficacy of ether lipid edelfosine against *Leishmania spp*. and SbV-resistant parasites. PLoS Neglected Tropical Diseases. 2012;**6**(4):e1612. DOI: 10.1371/journal.pntd.0001612

[8] Wink M. Medicinal plants: A source of antiparasitic secondary metabolites. Molecules. 2012;**17**(11):12771-12791. DOI: 10.3390/molecules171112771

[9] Hernández-Rivera MP, Hernández-Montes O, Chiñas-Pérez A, Batiza-Avelar JM, Sánchez-Tejeda G, Wong-Ramírez C, et al. Study of cutaneous leishmaniasis in the state of Campeche (Yucatan peninsula), Mexico, over a period of two years. Salud Pública de México. 2015;**57**(1):58-65

[10] WHO Resources 2017 consulted November 9, 2017. Available from: http://www.who.int/leishmaniasis/resources/MEXICO.pdf

[11] Bhattacharya P, Ali N. Treatment of visceral leishmaniasis: Anomalous pricing and distribution of AmBisome and emergence of an indigenous liposomal amphotericin B. fungisome. Journal of Parasitic Diseases. 2015;**40**(3):1094-1095. DOI: 10.1007/s12639-014-0607-3

[12] Wasunna M, Njenga S, Balasegaram M, Alexander N, Omollo R, Edwards T, Dorlo TP, et al. Efficacy and safety of AmBisome in combination with sodium stibogluconate or Miltefosine and Miltefosine monotherapy for African visceral leishmaniasis: Phase II randomized trial. PLoS Neglected Tropical Diseases. 2016;**10**(9):e0004880. DOI: 10.1371/journal.pntd.0004880

[13] Pérez-Franco JE, Cruz-Barrera ML, Robayo ML, López MC, Daza CD, Bedoya A, et al. Clinical and parasitological features of patients with American cutaneous leishmaniasis that did not respond to treatment with meglumine antimoniate. PLoS Neglected Tropical Diseases. 2016;**10**(5):e0004739. DOI: 10.1371/journal.pntd.0004739

[14] Basselin M, Denise H, Coombs GH, Barrett MP. Resistance to pentamidine in *Leishmania mexicana* involves exclusion of the drug from the mitochondrion. Antimicrobial Agents and Chemotherapy. 2002;**46**(12):3731-3738

[15] Chow LM, Volkman SK. Plasmodium and leishmania: The role of MDR genes in mediating drug resistance. Experimental Parasitology. 1998;**90**(1):135-141

[16] Carneiro SM, Carvalho FA, Santana LC, Sousa AP, Neto JM, Chaves MH. The cytotoxic and antileishmanial activity of extracts and fractions of leaves and fruits of *Azadirachta indica* (a Juss). Biological Research. 2012;**45**(2):111-116

[17] Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. Journal of Natural Products. 2007;**70**(3):461-477

[18] Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sciences. 2005; **78**(5):431-441

[19] Chin YM, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. The AAPS Journal. 2006;**8**(2):E239-E253

[20] Félix MB, de Souza ER, de Lima M, Do C, Frade DK, de Serafim VL, Rodrigues KA, et al. Antileishmanial activity of new thiophene-indole hybrids: Design, synthesis, biological and cytotoxic evaluation, and chemometric studies. Bioorganic and Medicinal Chemistry. 2016;**24**(18):3972-3977. DOI: 10.1016/j.bmc.2016.04.057

[21] Galdo A. A propósito de un caso de Botón de Oriente en primera infancia. Med Rev Mex. 1934;**14**:388-396

[22] Chan-Bacab MJ, Peña-Rodríguez LM. Plant natural products with leishmanicidal activity. Natural Product Reports. 2001;**18**(6):674-688

[23] Sundar S, Singh A. Recent developments and future prospects in the treatment of visceral leishmaniasis. Therapeutic Advances in Infectious Disease. 2016;**3**(3-4):98-109. DOI: 10.1177/2049936116646063

[24] Chacko A, Joseph M, Feltis T, Morris SK. Successful treatment of cutaneous leishmaniasis with topical paramomycin in a child after treatment failure with systemic fluconazole. The American Journal of Tropical Medicine and Hygiene. 2016;**95**(4):793-794

[25] Torres-Santos EC, Lopes D, Oliveira RR, Carauta JP, Falcao CA, Kaplan MA, et al. Antileishmanial activity of isolated triterpenoids from *Pourouma guianensis*. Phytomedicine. 2004;**11**(2-3):114-120

[26] Mishra BB, Kale RR, Singh RK, Tiwari VK. Alkaloids: Future prospective to combat leishmaniasis. Fitoterapia. 2009;**80**(2):81-90. DOI: 10.1016/j.fitote.2008.10.009

[27] Polonio T, Efferth T. Leishmaniasis drug resistance and natural products (review). International Journal of Molecular Medicine. 2008;**22**(3):277-286

[28] Singh N, Mishra BB, Bajpai S, Singh RK, Tiwari VK. Natural product based leads to fight against leishmaniasis. Bioorganic and Medicinal Chemistry. 2014;**22**(1):18-45. DOI: 10.1016/j.bmc.2013.11.048

[29] Mishra BB, Singh RK, Srivastava A, Tripathi VJ, Tiwari VK. Fighting against leishmaniasis: Search of alkaloids as future true potential anti-leishmanial agents. Mini-Reviews in Medicinal Chemistry. 2009;**9**(1):107-123

[30] Fournet A, Barrios AA, Muñoz V, Hocquemiller R, Roblot F, Cavé A. Antileishmanial activity of a tetralone isolated from *Ampelocera edentula*, a Bolivian plant used as a treatment for cutaneous leishmaniasis. Planta Medica. 1993;**60**(1):8-12

[31] Eltayeb A, Ibrahim K. Potential antileishmanial effect of three medicinal plants. Indian Journal of Pharmaceutical Sciences. 2012;**74**(2):171-174. DOI: 10.4103/0250-474X.103856

[32] Torres-Santos EC, Rodrigues JM Jr, Moreira DL, Kaplan MA, Rossi-Bergmann B. Improvement of *in vitro* and *in vivo* antileishmanial activities of 2′,6′-dihydroxy-4′-methoxychalcone by entrapment in poly(D,L-lactide) nanoparticles. Antimicrobial Agents and Chemotherapy. 1999;**43**(7):1776-1778

[33] Medda S, Mukhopadhyay S, Basu BK. Evaluation of the *in vivo* activity and toxicity of amarogentin, an antileishmanial agent, in both liposomal and niosomal forms. The Journal of Antimicrobial Chemotherapy. 1999;**44**(6):791-794

[34] Fournet A, Barrios AA, Muñoz V, Hocquemiller R, Cavé A. Effect of natural naphthoquinones in BALB/c mice infected with *Leishmania amazonensis* and *L. venezuelensis*. Tropical Medicine and Parasitology. 1992;**43**(4):219-222

[35] Fournet A, Angelo A, Muñoz V, Roblot F, Hocquemiller R, Cavé A. Biological and chemical studies of *Pera benensis*, a Bolivian plant used in folk medicine as a treatment of cutaneous leishmaniasis. Journal of Ethnopharmacology. 1992;**37**(2):159-164

[36] Hazra B, Saha AK, Ray R, Roy DK, Sur P, Banerjee A. Antiprotozoal activity of diospyrin towards *Leishmania donovani* promastigotes *in vitro*. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1987;**81**(5):738-741

[37] Awasthi BP, Kathuria M, Pant G, Kumari N, Mitra K. Plumbagin, a plant-derived naphthoquinone metabolite induces mitochondria mediated apoptosis-like cell death in *Leishmania donovani*: An ultrastructural and physiological study. Apoptosis. 2016; **21**(8):941-953. DOI: 10.1007/s10495-016-1259-9

[38] Ray S, Hazra B, Mittra B, Das A, Majumder HK. Diospyrin, a bisnaphthoquinone a novel inhibitor of type I DNA topoisomerase of *Leishmania donovani*. Molecular Pharmacology. 1998;**54**(6):994-999

[39] Lall N, Meyer JJ. Inhibition of drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis* by diospyrin, isolated from *Euclea natalensis*. Journal of Ethnopharmacology. 2001;**78**(2-3):213-216

[40] López-Carvajal L, Cardona-Árias JA, Zapata-Cardona MI, Sánchez-Giraldo V, Vélez ID. Efficacy of cryotherapy for the treatment of cutaneous leishmaniasis: Meta-analyses of clinical trials. BMC Infectious Diseases. 2016;**16**:360. DOI: 10.1186/s12879-016-1663-3

[41] Chan-Bacab MJ, Balanza E, Deharo E, Muñoz V, García RD, Peña-Rodríguez LM. Variation of leishmanial activity in four population of *Urechites andrieuxii*. Journal of Ethnopharmacology. 2003;**86**(3):243-247

[42] Peraza-Sánchez SR, Cen-Pacheco F, Noh-Chimal A, May-Pat F, Simá-Polanco P, Dumonteil E, et al. Leishmanicidal evaluation of extracts from native plants of the Yucatan peninsula. Fitoterapia. 2007;**78**(4):315-318

[43] Martín-Quintal Z, Moo-Puc R, González-Salazar F, Chan-Bacab MJ, Torres-Tapia LW, Peraza-Sánchéz SR. *In vitro* activity of *Tridax procumbens* against promastigotes of *Leishmania mexicana*. Journal of Ethnopharmacology. 2009;**122**(3):463-467. DOI: 10.1016/j.jep.2009.01.037

[44] Martín-Quintal Z, García-Miss Del R, Mut-Martín M, Matus-Moo A, Torres-Tapia LW, Peraza-Sánchez SR. The leishmanicidal effect of (3S)-16,17-didehydrofalcarinol, an oxylipin isolated from *Tridax procumbens*, is independent of NO production. Phytotherapy Research. 2010;**24**(7):1004-1008. DOI: 10.1002/ptr.3052

[45] Gamboa-León R, Vera-Ku M, Peraza-Sánchez SR, Ku-Chulim C, Horta-Baas A, Rosado-Vallado M. Antileishmanial activity of a mixture of *Tridax procumbens* and *Allium sativum* in mice. Parasite. 2014;**21**(15):1-7. DOI: 10.1051/parasite/2014016

[46] Viscencio GS, Tamay PM, Issac AP, Lezama CM. Toxicidad in vitro de extractos de Urechites andrieuxii Muell-Arg, en contra de *L. mexicana*. Memorias de la III Reunión de Investigación Química en el Sureste de México, Mérida, Yucatán. 1995;**1**:93

[47] Adebayo OL, Suleman D, Samson AA. Natural products in antileishmanial drug discovery: A review. Journal of Asian Scientific Research. 2013;**3**(2):157-173

[48] Lezama-Dávila CM, Pan L, Isaac-Márquez AP, Terrazas C, Oghumu S, Isaac-Márquez R, et al. *Pentalinon andrieuxii* root extract is effective in the topical treatment of cutaneous leishmaniasis caused by *Leishmania mexicana*. Phytotherapy Research. 2014;**28**(6):909-916. DOI: 10.1002/ptr.5079

[49] Pan L, Lezama-Dávila CM, Isaac-Márquez AP, Calomeni EP, Fuchs JR, Satoskar AR, et al. Sterols with antileishmanial activity isolated from the roots of *Pentalinon andrieuxii*. Phytochemistry. 2012;**82**:128-135. DOI: 10.1016/j.phytochem.2012.06.012

[50] Gupta G, Peine KJ, Abdelhamid D, Snider D, Shelton AB, Rao L, et al. A novel sterol isolated from a plant used by Mayan traditional healers is effective in treatment of visceral leishmaniasis caused by *Leishmania donovani*. ACS Infectious Diseases. 2015;**1**(10):497-506. DOI: 10.1021/acsinfecdis.5b00081

[51] Lezama-Dávila CM, Isaac-Márquez AP, Zamora-Crescencio P, Uc-Encalada MDR, Justiniano-Apolinar SY, del Ángel-robles L, et al. Leishmanicidal activity of *Pentalinon andrieuxii*. Fitoterapia. 2007; **78**(3): 255-257

[52] Borges-Argáez R, Balnbury L, Flowers A, Giménez-Turba A, Ruiz G, Waterman PG, et al. Cytotoxic and antiprotozoal activity of flavonoids from *Lonchocarpus* spp. Phytomedicine. 2007;**14**(7-8):530-533

[53] Borges-Argáez R, Vela-Catzín T, Yam-Puc A, Chan-Bacab MJ, Moo-Puc RE, Cáceres-Farfán M. Antiprotozoal and cytotoxic studies on some Isocordoin derivatives. Planta Medica. 2009;**75**(12):1336-1338. DOI: 10.1055/s-0029-1185670

[54] Martínez Ruiz MG, Richard-Greenblatt M, Juárez ZN, Av-Gay Y, Bach H, Hernández LR. Antimicrobial, anti-inflammatory, antiparasitic, and cytotoxic activities of *Laennecia confusa*. Scientific World Journal. 2012;**2012**: ID: 263572:1-8. DOI: 10.1100/2012/263572

[55] Cruz Paredes C, Bolívar-Balbás P, Gómez-Velasco A, Juárez ZN, Sánchez-Arreola E, et al. Antimicrobial, antiparasitic, anti-inflammatory, and cytotoxic activities of *Lopezia racemosa*. Scientific World Journal. 2013;**2013**: ID 237438:1-6. DOI: 10.1155/2013/237438

[56] Bolívar P, Cruz-Paredes C, Hernández LR, Juárez ZN, Sánchez-Arreola E, Av-Gay Y, Bach H. Antimicrobial, anti-inflammatory, antiparasitic, and cytotoxic activities of *Gallium mexicanum*. Journal of Ethnopharmacology. 2011;**137**(1):141-147. DOI: 10.1016/j.jep.2011.04.069

[57] Martínez-Ruiz MG, Gómez-Velasco A, Juárez ZN, Hernández LR, Bach H. Exploring the biological activities of *Echeveria leucotricha*. Natural Product Research. 2013;**27**(12): 1123-1126. DOI: 10.1080/14786419.2012.708662

[58] Delgado-Altamirano R, Monzote L, Piñón-Tápanes A, Vibrans H, Rivero-Cruz JF, Ibarra-Alvarado C, Rojas-Molina A. In vitro antileishmanial activity of Mexican medicinal plants. Heliyon. 2017;**3**:e00394. DOI: 10.1016/j.heliyon.2017. e00394

[59] Alamilla-Fonseca LN, Delgado-Domínguez J, Zamora-Chimal J, Cervantes-Sarabia RB, Jiménez-Arellanes A, Rivero-Cruz JF, Becker I. *Leishmania mexicana* cell death achieved by *Cleoserrata serrata* (Jacq.) Iltis: Learning from Maya healers. Journal of Ethnopharmacology. 2018;**211**:180-187

Section 7

Nanomedicines for Anti-leishmanial Therapy

Chapter 11

Nanomedicines for Cutaneous Leishmaniasis

Ariane Sousa-Batista and Bartira Rossi-Bergmann

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75750

Abstract

Leishmaniasis is a vector-borne disease caused by *Leishmania* parasites, which cause a range of clinical manifestations in man. These are didactically classified into cutaneous leishmaniasis (CL), the most common form of the disease, and visceral leishmaniasis (VL), the life-threatening form. There are so far no vaccines approved for humans. Conventional drugs pose limitations ranging from low efficacy and high cost to systemic toxicity. Low efficacy derives in part from difficult drug access to the parasites, which rides themselves inside macrophage phagosomes. This prompts to high dosage, with consequent increased toxicity. Difficult intracellular drug access can be overcome with nanomedicines such as biocompatible lipid and polymeric nanoparticles that can be phagocytosed by the infected macrophages. Besides cell membranes, appropriate drug nanostructuring may allow tissue barrier penetration and drug administration through higher compliance routes such as skin and intestine, in contrast to the usual intravenous and intramuscular routes. In general, CL and VL are both treated with toxic systemic injections, disregard of disease severity. This chapter will review and discuss studies with nanomedicines that have reached the market such as liposomal amphotericin B for intravenous administration, and innovative preclinical studies aiming at developing effective cutaneous and oral drugs with focus on CL.

Keywords: *Leishmania*, cutaneous leishmaniasis, chemotherapy, drug delivery systems, nanodrugs, liposomes, solid lipid nanoparticles, polymeric nanoparticles, nanoemulsions

1. Introduction

Leishmaniasis is a complex of neglected tropical diseases (NTDs) caused by intracellular protozoans of the genus *Leishmania*, transmitted to humans and other animals by the bite of infected female phlebotomine sand flies. Once in the vertebrate skin, the flagellated

promastigote forms are phagocytosed by local macrophages. Once inside macrophage phagolysosomes, the parasites survive enzyme digestion, transform into amastigote forms and multiply. Dermotropic parasite species causing cutaneous leishmaniasis (CL) remain in the skin, whereas viscerotropic species causing visceral leishmaniasis (VL) migrate to deeper macrophage-rich organs such as liver, spleen, and bone marrow.

Although not fatal as VL, CL is the most common form of leishmaniasis and a serious public health problem. According to the World Health Organization (WHO) estimates, CL is endemic in 87 countries, with almost 200,000 new cases reported in 2015 [1]. From 2005 to 2013, CL-associated morbidity increased by 175% of disability-adjusted life years (DALYs) [2]. The impact of CL may be much greater considering the high under-reported cases and estimation that one fourth of the world population (1.7 billion people) live in area at risk of infection [3]. In addition, inadequate disease control may promote the progression of CL to more morbid and undefined subforms, such as diffuse CL and mucosal leishmaniasis (ML).

In the great majority (>90%) of cases worldwide, CL is of the uncomplicated type, with 1–4 localized skin ulcers, not larger than 3–4 cm diameter, with a raised border and central depression [4]. Even with localized manifestation, current treatment is normally based in the daily administration of intramuscular or intravenous injections with antimonials, pentamidine, or amphotericin B for 20–30 days. Besides limited to few drugs, and occurrence of drug resistance, available CL treatment produces unacceptable systemic toxicity [5].

Ideally, CL chemotherapy as proposed by Drugs for Neglected Diseases initiative (DNDi) should be efficacious against all species, compatible in combination therapy, safe in pregnant and breastfeeding women, and administered by oral or topical route [6]. However, oral and topical therapies have shown limited efficacy.

The major challenge in CL treatment is the preferred intracellular parasite location in macrophage phagolysosomes. That hinders drug access, making treatment with conventional formulations especially difficult [7].

Thus, the search for new drugs with different mechanisms of action and innovative forms of drug delivery systems appropriate for the effective treatment of CL is urgently needed. In that context, nanotechnology has emerged as an interesting strategy to increase drug potency and reduce toxicity.

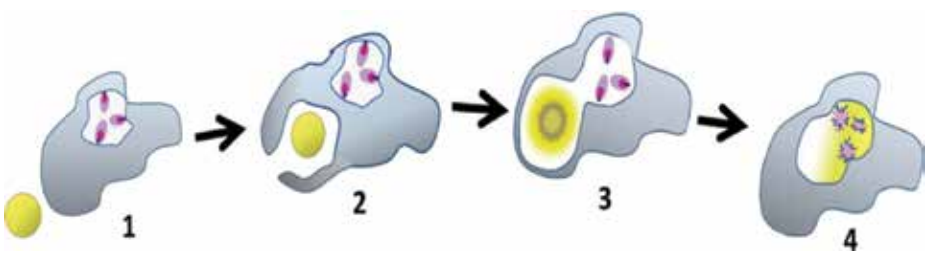


Figure 1. Nanoparticle drug delivery to intracellular parasites. A drug-loaded lipid or polymeric nanoparticle (Np, yellow) reaches the *Leishmania*-infected macrophage (1). The Np is actively phagocytosed by the infected macrophage (2). The Np-containing phagolysosome fuses with the amastigote-containing parasitophorous vacuole (3). Drug is released from digested Np to kill amastigotes (4).

Nanotechnology consists of the development of systems, structures, or devices in the nanometric scale, presenting at least one novel/superior characteristic or property over the original [8]. The use of nanostructured particles for drug delivery is a promising strategy due to their versatility. Besides, they may: (i) protect the drug against physical, chemical, and/or enzymatic degradation, (ii) enhance the pharmacokinetic properties, and (iii) improve bioavailability. They may also be functionalized for drug release at a specific site and thereby reduce systemic toxicity [7]. Furthermore, leishmaniasis is a particularly interesting disease to be treated with drug-loaded nanoparticles since the parasites almost exclusively infect the highly phagocytic macrophages. In this way, the infected cells of the skin (CL) or deep organs (VL) take up the nanoparticulated drug, which will reach the parasitophorous vacuole and act directly on the parasite (**Figure 1**). This allows the drug to reach an effective intracellular concentration, allowing dose and toxicity reduction. Particle uptake may be further increased with surface functionalization with receptor-binding ligands like mannose or mannan [9].

Interest in designing nanomedicines for CL has grown over the years, as seen by the steady increase in scientific publications. Several nanosystems, such as liposomes [10–20], solid lipid nanoparticles [21, 22], lipid complexes [23, 24], lipid-core nanocapsules [25], polymeric particles [26–31], inorganic nanoparticle [32–35], cyclodextrins complexes [36, 37], and drug nanoparticles [38] have been tested *in vivo* by different routes in experimental mouse and hamster models to improve CL treatment as summarized in **Table 1**. Of those, only liposomal

Routes	Drug	Nanosystem	Parasite	Efficacy	Ref
Parenteral	Amphotericin B	Chitosan and chondroitin sulfate nanoparticles	*L. amazonensis*	Yes	[26]
	Amphotericin B	Poloxamer 407-micelles	*L. amazonensis*	Yes	[27]
	Amphotericin B	PLGA-DMSA nanoparticles	*L. amazonensis*	Yes	[28]
	Amphotericin B	Liposome	*L. tropica*	No	[10]
	Amphotericin B	Liposome (Ambisome®)	*L. major*	Yes	[11]
	Amphotericin B	DSHemsPC-liposome	*L. major*	Yes	[12]
	Amphotericin B	Nanodisks	*L. major*	Yes	[23]
	Amphotericin B	PADRE-derivatized-dendrimer complexed with liposome	*L. major*	Yes	[13]
	Chalcone DMC	PLA Nanoparticles	*L. amazonensis*	Yes	[29]
	Nanoselenium	Inorganic nanoparticle	*L. major*	Yes	[33]
	Paromomycin	Solid lipid nanoparticle	*L. major*	Yes	[21]
	Paromomycin	Solid lipid nanoparticle	*L. tropica*	Yes	[22]
	Pentamidine	Methacrylate nanoparticles	*L. major*	Yes	[30]
	Pentavalent antimonial	Nanohybrid hydrosols	*L. amazonensis*	Yes	[38]
	Sodium stibogluconate	Liposome	*L. mexicana* / *L. major*	Yes	[14]

Routes	Drug	Nanosystem	Parasite	Efficacy	Ref
Oral	Quercetin	Lipid-core nanocapsules	*L. amazonensis*	Yes	[25]
	Meglumine antimoniate	Beta-cyclodextrin	*L. amazonensis*	Yes	[36]
	Meglumine antimoniate	Polarity-sensitive nanocarrier	*L. amazonensis*	Yes	[24]
Topical	Amphotericin B	Liposome	*L. mexicana*	No	[15]
	Amphotericin B	Gamma-cyclodextrin	*L. amazonensis*	Yes	[37]
	Chalcone CH8	Liposome	*L. amazonensis*	Yes	[16]
	Paromomycin	Liposome	*L. major*	Yes	[17]
	Paromomycin	Liposome	*L. major*	Yes	[18]
	Meglumine antimoniate	Liposome	*L. major*	Yes	[19]
	Nanosilver	Inorganic nanoparticles	*L. major*	No	[32]
	Nanosilver	Inorganic nanoparticles	*L. major*	No	[34]
Intralesional	Amphotericin B	Liposome (Ambisome®)	*L. major*	No	[11]
	Chalcone CH8	PLGA microparticles	*L. amazonensis*	Yes	[31]
	Nanosilver	Inorganic nanoparticles	*L. amazonensis*	Yes	[35]
	Meglumine antimoniate	Liposome	*L. major*	No	[20]
	Miltefosine	Liposome	*L. major*	Yes	[20]
	Paromomycin	Liposome	*L. major*	No	[20]
	Paromomycin	Solid lipid nanoparticle	*L. tropica*	Yes	[22]
	Sodium stibogluconate	Liposome	*L. mexicana* / *L. major*	Yes	[14]

Note: Chalcone DMC – 2′,6′-dihydroxy-4′-methoxychalcone; Chalcone CH8 – 3-nitro-2′-hydro-4′,6′-dimethoxychalcone; DMSA – dimercaptosuccinic acid; DSHemsPC – 1,2-distigmasterylhemi-succinoyl-sn-glycero-3-phosphocholine; PADRE – pan DR-binding epitope; PLA – poly(D,L-lactide); PLGA – poly(lactic-co-glycolic acid); UVB – ultraviolet B radiation.

Table 1. Experimental studies using nanosystems for CL treatment.

amphotericin B has been approved for human leishmaniasis so far, but that is restricted to VL and the more severe mucosal form of CL. Additional studies and clinical trials are needed to validate the potential of those experimental nanomedicines in human CL.

Advances and challenges of nanotechnology use in leishmaniasis treatment, especially for VL, have been extensively reviewed recently [7, 39, 40]. Here, we attempted to identify some of the opportunities and challenges of using nanotechnology to improve CL treatment. For that, mainly *in vivo* studies were considered.

http://dx.doi.org/10.5772/intechopen.75750

2. Systemic therapies

2.1. Parenteral treatments

For more than 70 years, injectable pentavalent antimonials such as meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) have been the first-choice drugs in most countries. The paucity of new effective drugs in the market is due to lack of investment/economic interest for the discovery of therapeutic alternatives. The therapeutic regimen consists of intramuscular or intravenous daily injections for 20–30 days. The long period of treatment leads to the accumulation of antimony (Sb) in the tissues, producing myalgia, pancreatitis, pancytopenia, hepatic and cardiotoxicity [41]. Other limiting factors are drug resistance and increased therapeutic failure [42]. In India, its use in VL has been contraindicated due to the appearance of resistant *L. donovani* strains [43].

In Sb-refractory cases, injectable pentamidine, amphotericin B or paromomycin are used. Pentamidine acts on the DNA synthesis of the parasite and has similar efficacy to antimonials, but also produces side effects such as hypoglycemia, diabetes, tachycardia, hypotension, nephrotoxicity and pain at the site of administration [44]. Like antimonials, cases of pentamidine resistance have been increasing, compromising their use in many endemic regions [45].

Amphotericin B is a polyene antibiotic mostly used in VL and in the disfiguring CL form, mucosal leishmaniasis, administered intravenously for 20 days, usually under hospital admission. This is the most efficacious antileishmanial drug, but it produces serious side effects due to its low solubility (nephrotoxicity), and secondary affinity not only for the parasite ergosterol but also for the host cholesterol, causing hypokalemia and cardiotoxicity [5]. Formulations of amphotericin B in lipids have led to a marked improvement in their plasma solubility and bioavailability. Three lipid formulations are commercially available: unilamellar liposomes (Ambisome®), lipid complex (Abelcet®) and colloidal cholesterol suspension (Amphocil®). Among these, Ambisome® has the highest plasma half-life, lowest toxicity, and highest efficacy against VL and CL models [46, 47]. In some countries, Ambisome® is already recommended as the first-choice drug for the treatment of VL and ML difficult cases. However, its high cost, the undefined optimum dosing regimen, toxicity, and the greater uptake of liposomes by the liver make its widespread use in the treatment of CL unfeasible [48].

The interest in the administration of nanosystems by parenteral routes has been increased, mainly for VL, since they increase the drug bioavailability and depending on the charge, size and composition accumulate preferentially in organs such as liver. In addition, nanosystems can be conjugated to biological compounds, such as peptides, antibodies and mannose, favoring their targeting to macrophages [9]. Thus, even with the dose reduction, the encapsulated drugs present greater efficacy and reduction of toxic effects. To date, most experimental studies are conducted parenterally that include chitosan and chondroitin sulfate nanoparticles, Poloxamer 407-micelles, PLGA-DMSA nanoparticles, PADRE-derivatized dendrimer complexed with liposomes, PLA nanoparticles, solid lipid nanoparticles, methacrylate nanoparticles, and liposomes (**Table 1**). Since amphotericin B is currently the most potent antileishmanial

agent, most studies have used it in order to improve its specificity and reduce its adverse effects [10–12, 23, 26–28]. Despite the promising effects of nanomedicines obtained so far, Ambisome® remains the only nanomedicine approved for leishmaniasis parenteral treatment.

Another interesting strategy in the treatment of CL is the use of inorganic nanoparticles, such as nanoselenium, nanosilver and nanotitanium dioxide. Despite the promising efficacy of injected nanosilver [35] and nanoselenium [33] in CL models, the use of nanosilver by topical route was ineffective [32, 34], probably due to the lack of nanoparticle permeation through the infected skin, since those particles were directly active against culture parasites. Nanosilver may act directly on the *Leishmania* parasite by different mechanisms including: (i) increased cell cycle S phase length; (ii) inhibition of trypanothione/trypanothione reductase (TR) redox system; and (iii) cell necrosis [49].

The experimental parenteral routes are normally intravenous or intraperitoneal, the latter not applicable in clinical usage. An important issue to be considered when nanoparticles are intravenously injected is the possibility of thrombosis induction [50]. However, small and submicrometric they may be, larger aggregates can form and clog small veins [51]. Therefore, for safety reasons, intralesional, topical and oral routes should be preferable for CL treatment.

2.2. Oral treatment

The oral route is recommended for both CL and VL due to the ease of administration, high patient compliance, and versatility to increase drug bioavailability. However, systemic adverse effects cannot be precluded.

Miltefosine, a hexadecylphosphocholine previously used to treat cancer, is the only oral drug approved in VL treatment, with good cure rates in India, Nepal, and Bangladesh. However, its teratogenic potential, poor efficacy in patients coinfected with VL and human immunodeficiency virus and recently high rates of clinical failures have increasingly restricted its use in combination therapy [52]. Data on the efficacy of miltefosine in CL treatment are inconclusive, with a large variation depending on the parasite species and geographical area [53].

Another oral drug, allopurinol, an inhibitor of xanthine oxidase, has been explored since 1982 when its activity was demonstrated *in vitro*. Despite the promising results in the oral treatment of CL in Asia, it does not appear to be as effective in Latin America [54]. The azoles act directly on the parasite, blocking the synthesis of ergosterol, and have good pharmacokinetic profile. However, clinical studies with fluconazole, ketoconazole, and itraconazole have shown controversial efficacy, suggesting that the effect is species-dependent [55–57].The main limiting factor for an oral drug is its low intestinal absorption. Nanosystems can overcome this problem by increasing aqueous solubility and epithelial barrier permeation. In addition, nanosystems can protect drugs from physical, chemical, and biological degradation. In this sense, a few studies have attempted to improve miltefosine and amphothericin B oral efficacy in VL models by encapsulation in nanosystems [58, 59]. For example, PLGA nanoparticles have been used to increase the oral bioavailability of the immunomodulator curcumin and the efficacy of miltefosine in hamsters infected with *L. donovani* [60]. However, only a few studies in the literature have used different nanosystems to increase drug efficacy in CL. In

L. amazonensis-infected BALB/c mice, nanoassemblies formed by two different complexes with N-Octanoyl-N-methylglucamide and β-cyclodextrin were used to increase intestinal permeability of a highly water-soluble meglumine antimoniate drug [24, 36]. More recently, quercetin, a poorly water-soluble plant flavonoid with promising antileishmanial activity [61], was successfully encapsulated in lipid-core poly-e-polycaprolactone (PCL) nanocapsules [25]. Nanoparticle encapsulation increased by more than 40-fold drug oral efficacy in BALB/c mice infected with *L. amazonensis*. The enhancing effect was possibly due to quercetin protection against extensive gastric and intestinal degradation [62]. Besides, PCL nanocapsules were shown to be absorbed intact by mouse intestinal epithelia [63] and also taken up by M cells [64]. Whether or not absorbed quercetin-loaded particles reach the circulation [65] and *Leishmania*-infected skin macrophages remained to be determined.

3. Localized skin therapies

Local therapies are the ideal way to treat uncomplicated CL, as they avoid unnecessary systemic side effects. This topic was subdivided in topical and intralesional treatments due to their different delivery approaches.

3.1. Topical treatment

Topical CL treatment may be provided with chemical drugs or physical methods, such as thermotherapy and cryotherapy. Thermotherapy is the application of high temperature (>50°C) at the center and border of each lesion, based on the inability of *Leishmania* to multiply at temperatures higher than 39°C. Its use has been restricted to the Old World, where 70% efficacy in repeated applications was shown to be similar to intramuscular or intralesional antimony [66]. Presently, thermotherapy is under clinical trial in Colombia in combination with a short course of oral miltefosine [67]. Cryotherapy is the application of liquid nitrogen (−195°C) in the center and border of the lesion once or twice a week for 6 weeks. This treatment has also shown ~70% efficacy [4]. Both therapies are well accepted by the patient, but the difficult access to the specific device (Thermomed), liquid nitrogen, and trained personnel for subjective applications limits their use.

Topical drug treatment of CL normally involves administration of drugs in the form of ointments, creams or gels. These should be ideal for uncomplicated CL due to reduced hospital costs, since it can be auto-applied [44]. The most studied topical formulations are paromomycin creams and gels. The low skin permeation of paromomycin requires association with strong permeants, such as methylbenzethonium chloride, urea and surfactant-associated gentamicin (WR-279396), which may produce local burn and skin irritation [68]. To circumvent that, some formulations have used the milder urea permeant; however, clinical efficacy remains variable depending on the parasite species and geographical area [4]. The results with the WR-279396 formulation are also conflicting, showing high efficacy in patients infected with *L. panamensis* in Panama [69], but not in patients infected with *L. major* in Tunisia [70].

Recently, DNDi supported a Phase Ib and II clinical study in Colombia evaluating the safety, pharmacokinetics, and efficacy of Anfoleish, a cream formulation containing 3%

amphotericin B [71]. However, limited efficacy was found after topical application in patients infected with *L. braziliensis* and *L. panamensis*.

For an optimal topical formulation, the drug should be highly effective and have a high permeation through the skin, reaching the parasite in the deep dermal layer in effective concentrations. For the drug to successfully permeate the stratum corneum, it must possess adequate lipophilicity and a molecular size below 500 Da. The failure or partial success of the topical formulations of paromomycin (615 Da) and amphotericin B (924 Da) is directly related to the low permeability of these drugs through the skin, probably due to their high molecular size [72]. In addition, the typical morphology of CL ulcer with necrotic center and high borders influences the permeation of drugs. Although local inflammatory reaction may facilitate the permeation of more hydrophilic drugs [68], infected macrophages are located in the border of the lesions where epidermal thickening occurs, with hyperplasia and increased number of cell layers, which may hamper drug permeation.

Topical liposomes have emerged as an advantageous way to overlay this problem by increasing drug skin permeation. In fact, some studies have shown the efficacy of liposomes loaded with paromomycin [17, 18] or meglumine antimoniate [19] in *L. major*-infected BALB/c mice, although even with the use of liposomes only 1.5% of antimoniate and a range of 4.8 to 15% of paromomycin were able to permeate through the skin. The use of liposomes was also shown to increase, the *in vitro* permeation and activity of amphotericin B in *L. braziliensis* promastigotes and intracellular amastigotes [73]. Nonetheless, *in vivo* another amphotericin B liposomal formulation did not show effectiveness in the topical treatment of CL caused by *L. mexicana* using ulcerated (BALB/c) and non-ulcerated (129SVE) experimental mice models [15]. On the other hand, in two different clinical studies conducted by the same research group in an endemic area for *L. tropica* and *L. major* at Ghaem Hospital in Iran, liposomes loading amphotericin B [74] and azithromycin [75] when administrated topically demonstrated the same efficacy as intralesional meglumine antimoniate.

In the search for new active drugs for leishmaniasis, our group has been studying the chalcone CH8 (3-nitro-2′-hydro-4′,6′-dimethoxychalcone), a nitrosylated derivative of the plant-derived chalcone (DMC – 2′,6′-dihydroxy-4′-methoxychalcone), which demonstrated a high selectivity index (SI = 143) and antileishmanial activity *in vitro* (IC_{50} = 0.7 μM) and *in vivo* against *L. amazonensis* [76]. In addition, the CH8 molecule exhibits physicochemical characteristics favorable to encapsulation with high efficiency in different nanosystems such as liposomes and polymeric particles. Indeed, CH8 loading into cationic liposomes interferes with the lipid structure rendering it more elastic, enhancing formulation permeation through the skin and increasing CH8 topical efficacy in *L. amazonensis* murine model [16].

Notwithstanding, the high phospholipid cost and liposomal instability hinder their use for CL. Thus, other nanosystems such as gamma-cyclodextrin have been studied for amphotericin B skin delivery to improve drug solubility and topical efficacy in *L. amazonensis*-infected golden hamsters [37]. Other interesting nanosystems are solid lipid nanoparticles (SLN), which can improve drug interaction with the stratum corneum facilitating permeation and improving the efficacy of the drug. The better activity of paromomycin entrapped in SLN was already described against *L. major* and *L. tropica* intracellular amastigotes [77]. Despite the

promising results found with the different nanosystems, additional *in vivo* studies are necessary in order to develop an effective topical treatment for CL.

3.2. Intralesional treatments

Intralesional drug administration is an alternative local treatment for CL. This is especially appropriate for patients with uncomplicated localized CL—up to four lesions, each no more than 3 cm in diameter, as well as parenteral medication restrictions due to systemic toxicity. Besides the lesser toxicity, local subcutaneous injections can accelerate clinical cure and reduce hospital costs as less injections are needed [4]. Pentavalent antimonials are the most used drugs, showing 68–100% efficacy in different clinical studies, depending on the size of the lesions [78–81]. Repeated injections are required due to the high solubility that favors rapid absorption into the circulation. Treatment generally consists of 1–5 injections around each lesion per day, twice a week. In addition to the pain inflicted, adverse effects like local hyperpigmentation and anaphylactic shock have been reported [82].

Amphotericin B has also been tested by intralesional route in Iran in patients refractory to antimony therapy, leading to complete lesion remission in 61% of the cases [83]. Due to the necrotizing effect of deoxycholate surfactant in amphotericin B formulation, the amount injected has to be as low as possible, reducing effectiveness. Thus, despite its high potential in CL, intralesional treatments need improvement, particularly as regards dose number reduction.

Intralesional drug-loaded nanoparticles have appeared as interesting drug delivery systems in CL due to direct drug delivery to the infected macrophages. However, for the formulation to be effective, drug chemistry, nanosystem choice, and treatment schedule must be finely adjusted. Lipid systems such as SLN loaded with paromomycin have been tested intralesionally in *L. tropica*-infected BALB/c mice and shown increased drug efficacy by 2-fold [22]. On the other hand, in another study comparing the efficacy of liposomal formulations of Glucantime®, miltefosine and paromomycin in *L. major*-infected BALB/c mice, only liposomal miltefosine was shown to have therapeutic effect compared with control group [20]. Interestingly, intralesional Ambisome® was not effective in *L. major*-infected mice [11]. Additionally, intralesional Pentostam® liposomes were only effective if given at the time of infection with *L. major* or *L. mexicana* in TFW mice [14].

In this context, polymeric particles have emerged as an interesting strategy for CL intralesional and single-dose treatment. The advantage of this system is that particles smaller than 6 μM can be easily phagocytosed by infected macrophages releasing the drug directly into the target, whereas the larger microparticles form a depot slowly releasing the drug into the site, allowing at only one dose the drug to remain in the site of infection for the time needed for healing. The size of the microparticles and their polymer composition ensures retention of the drug in the lesion and determines its release time. In this way, adverse systemic effects are avoided and the effectiveness of the drug is increased. Recently, the safety and efficacy of PLGA microparticles containing chalcone CH8 in the intralesional treatment was demonstrated in *L. amazonensis*-infected BALB/c mice. Even a single subcutaneous injection with CH8-loaded particles was effective in controlling parasite growth, superior than three injections with the free drug or Glucantime®, demonstrating the promising use of these systems in local and single-dose treatment of CL [31].

4. Conclusion

Since nanomedicines can be more efficiently taken up by the infected macrophages than free drugs, and also be designed to cross skin and epithelial barriers, they have emerged as promising strategies to allow novel topical and oral treatments for CL. Noteworthy is the possibility to treat the disease with a single local injection with biodegradable polymeric particles. Despite the promising results obtained with the different nanomedicines in pre-clinical studies, so far none has so far progressed to clinical trials in CL. Therefore, further efforts must be made in order to have them in the near future in the antileishmanial therapy arsenal.

Acknowledgements

A.J. Sousa-Batista is a recipient of Rio de Janeiro State Research Foundation—FAPERJ postdoc grant # E-26/202.401/2017.

Conflict of interest

The authors have no conflict of interest to declare.

Author details

Ariane Sousa-Batista and Bartira Rossi-Bergmann*

*Address all correspondence to: bartira@biof.ufrj.br

Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

References

[1] WHO. Global leishmaniasis update, 2006-2015: A turning point in leishmaniasis surveillance. Weekly Epidemiological Record [Internet]. 2017;**92**:557-572. Available from: http://www.who.int/wer/2017/wer9238/en/ [Accessed: 2017-11-16]

[2] WHO. Leishmaniasis in high-burden countries: An epidemiological update based on data reported in 2014. Weekly Epidemiological Record [Internet]. 2016;**91**:285-296. Available from: http://www.who.int/wer/2016/wer9122/en/ [Accessed: 2017-11-16]

[3] Pigott DM, Bhatt S, Golding N, Duda KA, Battle KE, Brady OJ, et al. Global distribution maps of the leishmaniases. eLife. 2014;**3**:1-21. DOI: 10.7554/eLife.02851.001

[4] Sundar S, Chakravarty J. An update on pharmacotherapy for leishmaniasis. Expert Opinion on Pharmacotherapy. 2014;**16**:1-16. DOI: 10.1517/14656566.2015.973850

[5] Uliana SRB, Trinconi CT, Coelho AC. Chemotherapy of leishmaniasis: Present challenges. Parasitology. 2017;**20**:1-17. DOI:10.1017/S0031182016002523

[6] DNDi. DNDi's Strategy for Cutaneous Leishmaniasis. 2017. Available from: https://www.dndi.org/diseases-projects/leishmaniasis/dndi-strategy-cl/ [Accessed: November 16, 2017]

[7] Gutiérrez V, Seabra AB, Reguera RM, Khandare J, Calderón M. New approaches from nanomedicine for treating leishmaniasis. Chemical Society Reviews. 2016;**45**:152-168. DOI: 10.1039/C5CS00674K

[8] Bawa R, Melethil S, Simmons WJ, Harris D. Nanopharmaceuticals: Patenting issues and FDA regulatory challenges. The SciTech Lawyer. 2008;**5**:6-13

[9] Gustafson HH, Holt-Casper D, Grainger DW, Ghandehari H. Nanoparticle uptake: The phagocyte problem. Nano Today. 2015;**10**:487-510. DOI: 10.1016/j.nantod.2015.06.006

[10] Panosian CB, Barza M, Szoka F, Wyler DJ. Treatment of experimental cutaneous leishmaniasis with liposome-intercalated amphotericin B. Antimicrobial Agents and Chemotherapy. 1984;**25**:655-656

[11] Yardley V, Croft SL. Activity of liposomal amphotericin B against experimental cutaneous leishmaniasis. Antimicrobial Agents and Chemotherapy. 1997;**41**:752-756

[12] Iman M, Huang Z, Alavizadeh SH, Szoka FC, Jaafari MR. Biodistribution and in vivo Antileishmanial activity of 1,2-distigmasterylhemisuccinoyl-sn-glycero-3-phosphocholine liposome-intercalated amphotericin B. Antimicrobial Agents and Chemotherapy. 2017;**61**:1-16. DOI: 10.1128/AAC.02525-16

[13] Daftarian PM, Stone GW, Kovalski L, Kumar M, Vosoughi A, Urbieta M, et al. A targeted and adjuvanted nanocarrier lowers the effective dose of liposomal amphotericin B and enhances adaptive immunity in murine cutaneous leishmaniasis. The Journal of Infectious Diseases. 2013;**208**:1914-1922. DOI: 10.1093/infdis/jit378

[14] New RRC, Chance ML, Heath S. The treatment of experimental cutaneous leishmaniasis with liposome-entrapped Pentostam. Parasitology. 1981;**83**:519-527. DOI: 10.1017/S0031182000080501

[15] Varikuti S, Oghumu S, Saljoughian N, Pioso MS, Sedmak BE, Khamesipour A, et al. Topical treatment with nanoliposomal amphotericin B reduces early lesion growth but fails to induce cure in an experimental model of cutaneous leishmaniasis caused by *Leishmania mexicana*. Acta Tropica. 2017;**173**:102-108. DOI: 10.1016/j.actatropica.2017.06.004

[16] Rossi-Bergmann B, Falcao CAB, Zanchetta B, Bentley MVLB, Santana MHA. Performance of elastic liposomes for topical treatment of cutaneous Leishmaniasis. In: Beck R, Guterres S, Pohlmann A, editors. Nanocosmetics Nanomedicines New Approaches Ski. Care, Berlin, Heidelberg: Springer; 2011. pp. 181-196. DOI: 10.1007/978-3-642-19792-5_9

[17] Carneiro G, Santos DC, Oliveira MC, Fernandes AP, Ferreira LS, Ramaldes GA, et al. Topical delivery and in vivo antileishmanial activity of paromomycin-loaded liposomes for treatment of cutaneous leishmaniasis. Journal of Liposome Research. 2010;**20**:16-23

[18] Jaafari MR, Bavarsad N, Bazzaz BSF, Samiei A, Soroush D, Ghorbani S, et al. Effect of topical liposomes containing paromomycin sulfate in the course of *Leishmania major* infection in susceptible BALB/c mice. Antimicrobial Agents and Chemotherapy. 2009; **53**:2259-2265. DOI: 10.1128/AAC.01319-08

[19] Moosavian Kalat SA, Khamesipour A, Bavarsad N, Fallah M, Khashayarmanesh Z, Feizi E, et al. Use of topical liposomes containing meglumine antimoniate (Glucantime) for the treatment of *L. major* lesion in BALB/c mice. Experimental Parasitology. 2014;**143**:5-10. DOI: 10.1016/j.exppara.2014.04.013

[20] Momeni A, Rasoolian M, Momeni A, Navaei A, Emami S, Shaker Z, et al. Development of liposomes loaded with anti-leishmanial drugs for the treatment of cutaneous leishmaniasis. Journal of Liposome Research. 2013;**23**:134-144. DOI: 10.3109/08982104.2012.762519

[21] Heidari-Kharaji M, Taheri T, Doroud D, Habibzadeh S, Badirzadeh A, Rafati S. Enhanced paromomycin efficacy by solid lipid nanoparticle formulation against *Leishmania* in mice model. Parasite Immunology. 2016;**38**:599-608. DOI: 10.1111/pim.12340

[22] Heidari-Kharaji M, Taheri T, Doroud D, Habibzadeh S, Rafati S. Solid lipid nanoparticle loaded with paromomycin: In vivo efficacy against *Leishmania tropica* infection in BALB/c mice model. Applied Microbiology and Biotechnology. 2016;**100**:7051-7060. DOI: 10.1007/s00253-016-7422-y

[23] Nelson KG, Bishop JV, Ryan RO, Titus R. Nanodisk-associated amphotericin B clears *Leishmania major* cutaneous infection in susceptible BALB/c mice. Antimicrobial Agents and Chemotherapy. 2006;**50**:1238-1244. DOI: 10.1128/AAC.50.4.1238-1244.2006

[24] Lanza JS, Fernandes FR, Corrêa-Júnior JD, Vilela JM, Magalhães-Paniago R, Ferreira LA, et al. Polarity-sensitive nanocarrier for oral delivery of Sb (V) and treatment of cutaneous leishmaniasis. International Journal of Nanomedicine. 2016;**11**:2305-2318. DOI: 10.2147/IJN.S105952

[25] Sousa-Batista AJ, Poletto FS, Philipon CIMS, Guterres SS, Pohlmann AR, Rossi-Bergmann B. Lipid-core nanocapsules increase the oral efficacy of quercetin in cutaneous leishmaniasis. Parasitology. 2017;**144**:1-6. DOI: 10.1017/S003118201700097X

[26] Ribeiro TG, Franca JR, Fuscaldi LL, Santos ML, Duarte MC, Lage PS, et al. An optimized nanoparticle delivery system based on chitosan and chondroitin sulfate molecules reduces the toxicity of amphotericin B and is effective in treating tegumentary leishmaniasis. International Journal of Nanomedicine. 2014;**9**:5341-5353. DOI: 10.2147/IJN.S68966

[27] Mendonça DVC, Lage LMR, Lage DP, Chávez-Fumagalli MA, Ludolf F, Roatt BM, et al. Poloxamer 407 (Pluronic® F127)-based polymeric micelles for amphotericin B: In vitro biological activity, toxicity and in vivo therapeutic efficacy against murine tegumentary leishmaniasis. Experimental Parasitology. 2016;**169**:34-42. DOI: 10.1016/j.exppara.2016.07.005

[28] de Carvalho RF, Ribeiro IF, Miranda-Vilela AL, de Souza Filho J, Martins OP, de Oliveira Cintra e Silva D, et al. Leishmanicidal activity of amphotericin B encapsulated in PLGA-DMSA nanoparticles to treat cutaneous leishmaniasis in C57BL/6 mice. Experimental Parasitology. 2013;**135**:217-222. DOI: 10.1016/j.exppara.2013.07.008

[29] Torres-Santos EC, Rodrigues JM, Moreira DL, Kaplan MAC, Rossi-Bergmann B. Improvement of in vitro and in vivo antileishmanial activities of 2′,6′- dihydroxy-4′-methoxychalcone by entrapment in poly(D,L-lactide) nanoparticles. Antimicrobial Agents and Chemotherapy. 1999;**43**:1776-1778

[30] Fusai T, Deniau M, Durand R, Bories C, Paul M, Rivollet D, et al. Action of pentamidine-bound nanoparticles against *Leishmania* on an in vivo model. Parasite. 1994;**1**:319-324. DOI: 10.1051/parasite/1994014319

[31] Sousa-Batista AJ, Pacienza-Lima W, Arruda-Costa N, Falcão CAB, Rossi-Bergmann B. Depot subcutaneous injection with chalcone CH8-loaded PLGA microspheres aiming at a single-dose treatment of cutaneous leishmaniasis. Antimicrobial Agents Chemotherapy. 2018;**62**: 1-11. DOI: 10.1128/AAC.01822-17

[32] Mohebali M, Rezayat MM, Gilani K, Sarkar S, Akhoundi B, Esmaeili J, et al. Nanosilver in the treatment of localized cutaneous leishmaniasis caused by *Leishmania major* (MRHO/IR/75/ER): An in vitro and in vivo study. Daru. 2009;**17**:285-289

[33] Beheshti N, Soflaei S, Shakibaie M, Yazdi MH, Ghaffarifar F, Dalimi A, et al. Efficacy of biogenic selenium nanoparticles against *Leishmania major*: In vitro and in vivo studies. Journal of Trace Elements in Medicine and Biology. 2013;**27**:203-207. DOI: 10.1016/j.jtemb.2012.11.002

[34] Nilforoushzadeh MA, Shirani-Bidabadi L, Zolfaghari-Baghbaderani A, Jafari R, Heidari-Beni M, Siadat AH, et al. Topical effectiveness of different concentrations of nanosilver solution on *Leishmania major* lesions in Balb/c mice. Journal of Vector Borne Diseases. 2012;**49**:249-253

[35] Rossi-Bergmann B, Pacienza-Lima W, Marcato PD, De Conti R, Duran N. Therapeutic potential of biogenic silver nanoparticles in murine cutaneous leishmaniasis. Journal of Nano Research. 2012;**20**:89-97

[36] Demicheli C, Ochoa R, da Silva JBB, Falcão CA, Rossi-Bergmann B, de Melo AL, et al. Oral delivery of meglumine antimoniate-beta-cyclodextrin complex for treatment of leishmaniasis. Antimicrobial Agents and Chemotherapy. 2004;**48**:100-103. DOI: 10.1128/AAC.48.1.100

[37] Ruiz HK, Serrano DR, Dea-Ayuela MA, Bilbao-Ramos PE, Bolás-Fernández F, Torrado JJ, et al. New amphotericin B-gamma cyclodextrin formulation for topical use with synergistic activity against diverse fungal species and *Leishmania* spp. International Journal of Pharmaceutics. 2014;**473**:148-157. DOI: 10.1016/j.ijpharm.2014.07.004

[38] Franco AMR, Grafova I, Soares FV, Gentile G, Wyrepkowski CDC, Bolson MA, et al. Nanoscaled hydrated antimony (V) oxide as a new approach to first-line antileishmanial drugs. International Journal of Nanomedicine. 2016;**11**:6771-6780. DOI: 10.2147/IJN.S121096

[39] Akbari M, Oryan A, Hatam G. Application of nanotechnology in treatment of leishmaniasis: A review. Acta Tropica. 2017;**172**:86-90. DOI: 10.1016/j.actatropica.2017.04.029

[40] Bruni N, Stella B, Giraudo L, Della Pepa C, Gastaldi D, Dosio F. Nanostructured delivery systems with improved leishmanicidal activity: A critical review. International Journal of Nanomedicine. 2017;**12**:5289-5311. DOI: 10.2147/IJN.S140363

[41] Haldar AK, Sen P, Roy S. Use of antimony in the treatment of leishmaniasis: Current status and future directions. Molecular Biology International. 2011;**2011**:1-24. DOI: 10.4061/2011/571242

[42] Castro MDM, Cossio A, Velasco C, Osorio L. Risk factors for therapeutic failure to meglumine antimoniate and miltefosine in adults and children with cutaneous leishmaniasis in Colombia: A cohort study. PLoS Neglected Tropical Diseases. 2017;**11**:1-14. DOI: 10.1371/journal.pntd.0005515

[43] Sundar S. Drug resistance in Indian visceral leishmaniasis. Tropical Medicine & International Health. 2001;**6**:849-854

[44] Iqbal H, Ishfaq M, Wahab A, Abbas MN, Ahmad I, Rehman A, et al. Therapeutic modalities to combat leishmaniasis: A review. Asian Pacific Journal of Tropical Medicine. 2016; **6**:1-5. DOI:10.1016/S2222-1808(15)60975-6

[45] Basselin M, Denise H, Coombs GH, Barrett MP. Resistance to pentamidine in *Leishmania mexicana* involves exclusion of the drug from the mitochondrion. Antimicrobial Agents and Chemotherapy. 2002;**46**:3731-3738. DOI: 10.1128/AAC.46.12.3731

[46] Yardley V, Croft SL. A comparison of the activities of three amphotericin B lipid formulations against experimental visceral and cutaneous leishmaniasis. International Journal of Antimicrobial Agents. 2000;**13**:243-248. DOI: 10.1016/S0924-8579(99)00133-8

[47] De Almeida L, Fujimura AT, Del Cistia ML, Fonseca-Santos B, Imamura KB, Michels PAM, et al. Nanotechnological strategies for treatment of leishmaniasis – A review. Journal of Biomedical Nanotechnology. 2017;**13**:117-133. DOI: 10.1166/jbn.2017.2349

[48] Wortmann G, Zapor M, Ressner R, Fraser S, Hartzell J, Pierson J, et al. Lipsosomal amphotericin B for treatment of cutaneous leishmaniasis. The American Journal of Tropical Medicine and Hygiene. 2010;**83**:1028-1033. DOI: 10.4269/ajtmh.2010.10-0171

[49] Zahir AA, Chauhan IS, Bagavan A, Kamaraj C, Elango G, Shankar J, et al. Green synthesis of silver and titanium dioxide nanoparticles using Euphorbia prostrata extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in *Leishmania donovani*. Antimicrobial Agents and Chemotherapy. 2015;**59**:4782-4799. DOI: 10.1128/AAC.00098-15

[50] Agallou M, Margaroni M, Athanasiou E, Toubanaki DK, Kontonikola K, Karidi K, et al. Identification of BALB/c immune markers correlated with a partial protection to *Leishmania infantum* after vaccination with a rationally designed multi-epitope cysteine protease a peptide-based nanovaccine. PLoS Neglected Tropical Diseases. 2017;**11**:1-27. DOI: 10.1371/journal.pntd.0005311

[51] Mayer A, Vadon M, Rinner B, Novak A, Wintersteiger R, Fröhlich E. The role of nanoparticle size in hemocompatibility. Toxicology. 2009;**258**:139-147. DOI: 10.1016/j.tox.2009.01.015

[52] Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, et al. Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. Clinical Infectious Diseases. 2012;**55**:543-550. DOI: 10.1093/cid/cis474

[53] Monge-Maillo B, López-Vélez R. Miltefosine for visceral and cutaneous leishmaniasis: Drug characteristics and evidence-based treatment recommendations. Clinical Infectious Diseases. 2015;**60**:1398-1404. DOI: 10.1093/cid/civ004

[54] Vélez I, Agudelo S, Hendrickx E, Puerta J, Grogl M, Modabber F, et al. Inefficacy of allopurinol as monotherapy for Colombian cutaneous leishmaniasis: A randomized, controlled trial. Annals of Internal Medicine. 1997;**126**:232-236

[55] Navin TR, Arana BA, Arana FE, Berman JD, Chajon JF. Placebo-controlled clinical trial of sodium stibogluconate (Pentostam) vs. ketoconazole for teating cutaneous leishmaniasis in Guatemala. The Journal of Infectious Diseases. 1992;**165**:528-534

[56] Alrajhi A, Ibrahim E, De Vol E, Khairat M, Faris R, Maguire J. Fluconazole for the treatment of cutaneous leishmaniasis caused by *Leishmania major*. The New England Journal of Medicine. 2002;**346**:891-895. DOI: 10.1056/NEJM200208013470517

[57] Nassiri-Kashani M, Firooz A, Khamesipour A, Mojtahed F, Nilforoushzadeh M, Hejazi H, et al. A randomized, double-blind, placebo-controlled clinical trial of itraconazole in the treatment of cutaneous leishmaniasis. Journal of the European Academy of Dermatology and Venereology. 2005;**19**:80-83. DOI: 10.1111/j.1468-3083.2004.01133.x

[58] da Gama Bitencourt JJ, Pazin WM, Ito AS, Barioni MB, de Paula Pinto C, dos SMA, et al. Miltefosine-loaded lipid nanoparticles: Improving miltefosine stability and reducing its hemolytic potential toward erythrocytes and its cytotoxic effect on macrophages. Biophysical Chemistry. 2016;**217**:20-31. DOI: 10.1016/j.bpc.2016.07.005

[59] Serrano DR, Lalatsa A. Oral amphotericin B: The journey from bench to market. Journal of Drug Delivery Science and Technology. 2017;**42**:75-83. DOI: 10.1016/j.jddst.2017.04.017

[60] Tiwari B, Pahuja R, Kumar P, Rath SK, Gupta KC, Goyal N. Nanotized curcumin and miltefosine: A potential combination for treatment of experimental visceral leishmaniasis. Antimicrobial Agents and Chemotherapy. 2017;**61**:1-13. DOI: 10.1128/AAC.01169-16

[61] Muzitano MF, Falcão CAB, Cruz EA, Bergonzi MC, Bilia AR, Vincieri FF, et al. Oral metabolism and efficacy of Kalanchoe pinnata flavonoids in a murine model of cutaneous leishmaniasis. Planta Medica. 2009;**75**:307-311. DOI: 10.1055/s-0028-1088382

[62] Weiss-Angeli V, Poletto FS, De Marco SL, Salvador M, Da Silveira NP, Guterres SS, et al. Sustained antioxidant activity of quercetin-loaded lipid-core nanocapsules. Journal of Nanoscience and Nanotechnology. 2012;**12**:2874-2880. DOI: 10.1166/jnn.2012.5770

[63] Rodrigues SF, Fiel LA, Shimada AL, Pereira NR, Guterres SS, Pohlmann AR, et al. Lipid-core nanocapsules act as a drug shuttle through the blood brain barrier and reduce glioblastoma after intravenous or oral administration. Journal of Biomedical Nanotechnology. 2016;**12**:986-1000. DOI: 10.1166/jbn.2016.2215

[64] Lopes M, Abrahim B, Cabral L, Rodrigues C, Seiça R, de Baptista F, et al. Intestinal absorption of insulin nanoparticles: Contribution of M cells. Nanomedicine. 2014;**10**:1139-1151. DOI: 10.1016/j.nano.2014.02.014

[65] Li H, Chen M, Su Z, Sun M, Ping Q. Size-exclusive effect of nanostructured lipid carriers on oral drug delivery. International Journal of Pharmaceutics. 2016;**511**:524-537. DOI: 10.1016/j.ijpharm.2016.07.049

[66] Cardona-Arias JA, Darío Vélez I, López-Carvajal L. Efficacy of thermotherapy to treat cutaneous leishmaniasis: A meta-analysis of controlled clinical trials. PLoS One. 2015; **10**:1-15. DOI: 10.1371/journal.pone.0122569

[67] DNDi D for ND initiative. Thermotherapy + A Short Course of Miltefosine for the Treatment of Uncomplicated Cutaneous Leishmaniasis in the New World. ClinicalTrials.gov. 2017. Available from: https://www.clinicaltrials.gov/ct2/show/NCT02687971 [Accessed: January10, 2018]

[68] Van BK, Yardley V, Murdan S, Croft SL. Drug permeation and barrier damage in *Leishmania*-infected mouse skin. The Journal of Antimicrobial Chemotherapy. 2016;**71**:1578-1585. DOI: 10.1093/jac/dkw012

[69] Sosa N, Capitán Z, Nieto J, Nieto M, Calzada J, Paz H, et al. Randomized, double-blinded, phase 2 trial of WR 279,396 (paromomycin and gentamicin) for cutaneous leishmaniasis in Panama. The American Journal of Tropical Medicine and Hygiene. 2013;**89**:557-563. DOI: 10.4269/ajtmh.12-0736

[70] Ben Salah A, Ben Messaoud N, Guedri E, Zaatour A, Ben Alaya N, Bettaieb J, et al. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. The New England Journal of Medicine. 2013;**368**:524-532. DOI: 10.1056/NEJMoa1202657

[71] DNDi D for ND initiative. Topical 3% Amphotericin B Cream for the Treatment of Cutaneous Leishmaniasis in Colombia (Anfoleish). ClinicalTrials.gov. 2017. Available from: https://clinicaltrials.gov/ct2/show/NCT01845727 [Accessed: January 10, 2018]

[72] Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Experimental Dermatology. 2000;**9**:165-169. DOI: 10.1034/j.1600-0625.2000.009003165.x

[73] Perez AP, Altube MJ, Schilrreff P, Apezteguia G, Celes FS, Zacchino S, et al. Topical amphotericin B in ultradeformable liposomes: Formulation, skin penetration study, antifungal and antileishmanial activity in vitro. Colloids Surfaces B Biointerfaces. 2016; **139**:190-198. DOI: 10.1016/j.colsurfb.2015.12.003

[74] Layegh P, Rajabi O, Jafari MR, Emamgholi Tabar Malekshah P, Moghiman T, Ashraf H, et al. Efficacy of topical liposomal amphotericin B versus intralesional meglumine antimoniate (Glucantime) in the treatment of cutaneous leishmaniasis. Journal of Parasitology Research. 2011;**2011**:1-5. DOI: 10.1155/2011/656523

[75] Rajabi O, Layegh P, Hashemzadeh S, Khoddami M. Topical liposomal azithromycin in the treatment of acute cutaneous leishmaniasis. Dermatologic Therapy. 2016;**29**:358-363. DOI: 10.1111/dth.12357

[76] Boeck P, Bandeira Falcão CA, Leal PC, Yunes RA, Filho VC, Torres-Santos EC, et al. Synthesis of chalcone analogues with increased antileishmanial activity. Bioorganic & Medicinal Chemistry. 2006;**14**:1538-1545. DOI: 10.1016/j.bmc.2005.10.005

[77] Kharaji MH, Doroud D, Taheri T, Rafati S. Drug targeting to macrophages with solid lipid nanoparticles harboring paromomycin: An in vitro evaluation against *L. major* and *L. tropica*. AAPS PharmSciTech. 2016;**17**:1110-1119. DOI: 10.1208/s12249-015-0439-1

[78] Sharquie KE, Al-Talib K, Chu A. Intralesional therapy of cutaneous leishmaniasis with sodium stibogluconate antimony. The British Journal of Dermatology. 1988;**119**:53-57

[79] Tallab TM, K a B, Mirdad S, Johargi H, Mourad MM, Ibrahim K, et al. Cutaneous leishmaniasis: Schedules for intralesional treatment with sodium stibogluconate. International Journal of Dermatology. 1996;**35**:594-597

[80] Oliveira-Neto MP, Schubach A, Mattos M, da Costa SC, Pirmez C. Intralesional therapy of American cutaneous leishmaniasis with pentavalent antimony in Rio de Janeiro, Brazil – An area of *Leishmania* (V.) *braziliensis* transmission. International Journal of Dermatology. 1997;**36**:463-468

[81] Soto J, Rojas E, Guzman M, Verduguez A, Nena W, Maldonado M, et al. Intralesional antimony for single lesions of Bolivian cutaneous leishmaniasis. Clinical Infectious Diseases. 2013;**56**:1255-1260. DOI: 10.1093/cid/cit049

[82] Esfandiarpour I, Farajzadeh S, Rahnama Z, Fathabadi EA, Heshmatkhah A. Adverse effects of intralesional meglumine antimoniate and its influence on clinical laboratory parameters in the treatment of cutaneous leishmaniasis. International Journal of Dermatology. 2012;**51**:1221-1225. DOI: 10.1111/j.1365-4632.2012.05460.x

[83] Goyonlo VM, Vosoughi E, Kiafar B, Nahidi Y, Momenzadeh A, Taheri AR. Efficacy of intralesional amphotericin B for the treatment of cutaneous leishmaniasis. Indian Journal of Dermatology. 2014;**59**:631-636. DOI: 10.4103/0019-5154.143571

Chapter 12

Crossing Biological Barriers for Leishmaniasis Therapy: From Nanomedicinal Targeting Perspective

Gul Shahnaz, Hafiz Shoaib Sarwar and Masoom Yasinzai

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75911

Abstract

Despite past 60 years of extensive research in antileishmanial drug development, the successful therapy of this disease cannot be achieved at full potential. The biological barriers encountered by the therapeutic modalities favor the disseminations of the disease like intramacrophage location of parasite, lack of oral bioavailability, permeability across the cutaneous tissue, and active efflux of the drug. Nanomedicines are specifically engineered nano-sized delivery systems. The goal of designing a nanomedicine is to achieve the specific therapeutic objective via targeting the specific cells and intracellular locations, pharmacological receptors, enzymes and proteins, crossing biological barriers, and navigation through endocytic pathways. This chapter will cover various nanomedicinal approaches like targeting the macrophages, pathological organs, efflux pumps, metabolic enzymes, redox biology of *Leishmania* by using polymeric and metal nanocarriers to overcome all the biological barriers thus providing a successful alternative over the conventional therapies.

Keywords: biological barriers, macrophage targeting, nanocarriers, photodynamic therapy, oral bioavailability, leishmaniasis

1. Introduction

The challenges faced by the current antileishmanial therapy include subtherapeutic efficacy, development of resistance, toxicity, and cost-effectiveness [1]. Despite past 60 years of extensive research in antileishmanial drug development, the successful therapy of this disease cannot be achieved at full potential. There is no vaccine available against *Leishmania* and the treatment relies mainly on the chemotherapy. The classic chemotherapeutic agents cannot control the prevalence of *Leishmania* effectively as they encounter various biological barriers

like the intramacrophage location of *Leishmania* parasite, selective access to the pathological organs, lack of oral bioavailability, permeability across the cutaneous tissue, activity of drug efflux pumps, development of toxicity and serious side effects. *Leishmania* parasite utilizes these barriers in its favor like redox biology of *Leishmania* helps them to survive inside the phagolysosomes of the macrophages, and impermeability of the macrophages for the antileishmanial drugs deprives the free access of drug to the target [2]. The nontargeted nature of current therapeutic modalities results in free circulation of drugs in the blood, and accumulation in the pathological organ at desired concentration cannot be achieved. Permeability glycoprotein (P-gp) efflux pumps present in *Leishmania* actively efflux the drug out of the cell resulting in decreased intracellular accumulation [3]. Lack of oral absorption necessitates the parental formulation of antileishmania drugs, which needs hospitalization of the patients and lack of compliance. These circumstances augment the development of new therapeutic option which can be achieved either by developing the new antileishmanial agents or by changing the drug delivery systems. Traditional new drug development usually takes over 10–12 years and involves extensively high manufacturing cost. Leishmaniasis is a neglected tropical disease and receives very little funding regarding the research and development due to low market turnover [4]. So, switching the research toward the new drug delivery systems such as nanomedicine is a suitable approach in pursuit of successful therapy of leishmaniasis.

Nanomedicine is a specifically engineered nano-sized particulate drug delivery system designed for the improved pharmaceutical formulations. The nanoparticles can achieve the discrete therapeutic objectives which are otherwise impossible with conventional drug delivery systems like targeting a specific cell and organelles, enzymes, proteins, and pharmacological receptors, accumulating in the pathological organs, bypassing the organs prone to the toxic effects, crossing biological membranes, and navigating through endocytic pathways [5]. All these properties of nanoparticles can address the biological barriers encountered in the effective therapy of leishmaniasis. Various polymeric and metal nanocarriers-based strategies like macrophage targeting, organ targeting, improved oral bioavailability, and photodynamic therapy are being explored for their supreme antileishmanial effects.

This chapter will discuss the various biological barriers compromising the effectiveness of antileishmanial therapy and the role of nanomedicine to overcome the problems associated with the conventional therapeutic modalities thus providing a platform for the enhanced antileishmanial therapy.

2. Current medical management of leishmaniasis

For the past six decades, the standard first-line drugs for the treatment of leishmaniasis are antimonial drugs, meglumine antimoniate, and sodium stibogluconate [6]. Antimonial compounds required to be administered IV/IM at the dose of 20 mg/kg of Sb-V for 10 days in case of cutaneous leishmaniasis (CL) and for 28 days in case of visceral leishmaniasis (VL). Antimonial drugs act by inhibiting a thiol metabolic enzyme trypanothione reductase (TR) and thus causing a decreased trypanothione ($T[SH]_2$) levels which results in the decreased ability of the parasite to counteract the oxidative stress [7]. However, the variations in the clinical

response and development of resistance from the past several years are a persistent clinical threat. The activity of aqua glycoproteins, trypanothione reductase/trypanothione (TR/T[SH]$_2$) system, and permeability glycoprotein (P-gp) efflux pumps is involved in the development of resistance resulting in decreased intracellular accumulation of antimony in subtherapeutic concentrations thus jeopardizing the effectiveness [8, 9]. Serious toxic effects associated with antimonial therapy like cardiotoxicity, changes in ECG, renal and liver impairment, muscle pain, and severe fatigue further limit the therapeutic potential of antimonial compounds [10].

Amphotericin B (AmB), a polyene antibiotic, is the second-line standard drug for the leishmaniasis since the 1960s [11]. Whereas, in India, the AmB is the first-line drug approved for VL due to widespread resistance against antimonial compounds. The standard dose of AmB for VL is 1 mg/kg every other day for 20 days via IV route. It has selective activity against the *Leishmania*, *Trypanosoma cruzi*, and fungi due to the presence of ergosterol in the said microbes compared with the mammal cell having cholesterol in their cell membranes. AmB binds with the ergosterol and induces pore formation [12]. Moreover, resistance against the AmB is further related to the change in cell membrane composition and fluidity. *Leishmania donovani*-resistant strains showed a significant change in the sterol profile, in which the ergosterol was replaced by a precursor known as cholesta-5,7,24-trien3β-ol [13, 14]. This is due to the loss in functionality of S-adenosyl-L-methionin-C24Δ-sterol methyltransferase resulting in the impaired C-24 transmethylation [15]. The use of AmB results in nephrotoxicity resulting in renal failure, thrombocytopenia, anemia, anaphylaxis, convulsions, phlebitis, and high fever [16].

Miltefosine (MILT) has been recently tagged as an antileishmanial drug required to be administered at the dose of 50 mg orally three times a day for 28 days. The variation in the clinical response has been observed due to the species variations and the development of resistance [17]. Promastigote-resistant strains of *L. donovani* have been developed in the laboratory that was resistant against the MILT up to 40 μM [18]. The mechanism of resistance was found to be greater than 95% of reduced accumulation indicated by the ^{14}C-labeled MILT. Pérez-Victoria et al. [19] reported the involvement of novel plasma membrane P-type transporters from the aminophospholipid translocase subfamily to be responsible for the reduced accumulation of glycerophospholipid and MILT in the resistant promastigotes [19].

Pentamidine (PTM) is also being used as the second-line therapy against leishmaniasis; however, the use is limited in zoonotic settings. The recommended dose is 2–3 mg/kg, IV or IM once a day for 4–7 doses in case of CL, while for VL, its dose is 2–4 mg/kg administered every other day via IV or IM for up to 15 doses. The use of PTM in the pentavalent antimonial Sb(V) refractory patients in India resulted in decreased efficacy from 95 to 70% within a short duration suggesting the development of resistant against the PTM. Resistance to PTM in *Leishmania* is due to the inhibition of polyamine biosynthetic, and studies suggested that PMT is transported into the cell via polyamine and arginine transporters [20].

From above discussion, it is evident that resistance against the antileishmanial agents is rising, compromising the therapeutic efficacy. Apart from the development of resistance, other factors like associated toxic effects, unavailability of oral dosage forms, longer duration of therapy, and high cost also contributing toward the suboptimal control of leishmaniasis. These limitations arise primarily due to the various biological barriers encountered by the

antileishmanial agents. Considering the development of parasitic resistance and a limited number of effective antileishmanial drugs, there is an imperative demand to revise the standard medical management of leishmaniasis. Administering the available standard drugs with appropriate delivery systems that help to cross the biological barriers seems to be an encouraging strategy, which requires being given serious consideration.

3. Biological barriers to leishmaniasis therapy

3.1. Intramacrophage location

Mononuclear phagocytes (MP; monocytes, macrophages, and dendritic cells) along with eosinophils and neutrophils constitute the first line of defense against the invading pathogens and are involved in detection and elimination of the foreign bodies [21]. When the sand fly takes the blood meal, it inoculates the promastigotes of *Leishmania* along with saliva. The saliva contains immunogenic proteins that trigger the immune response. The promastigotes are immediately taken up by the MP cells like macrophages following a receptor-mediated endocytic event. During initial recognition, the macrophage receptors play a vital role depending upon the *Leishmania* species like scavenger receptors (SRs), mannose receptors (MRs), complement receptors (CRs), and fibronectin receptors (FRs). The binding of the parasite to specific receptors then determines the course of infection [22]. Upon the successful entrapment of *Leishmania* inside macrophages, complex cellular signals are produced like activation of lysosomal enzymes, production of nitric oxide ($NO^{\bullet}$), and initiation of oxidative burst as shown in **Figure 1** [23, 24]. Oxidative burst is a potent antileishmanial response produced by the reactive oxygen species (ROS) namely hydroxyl ion (OH^{-}), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^{-}$), and hypochlorous acid (HOCl) [25]. *Leishmania* employs the various mechanisms

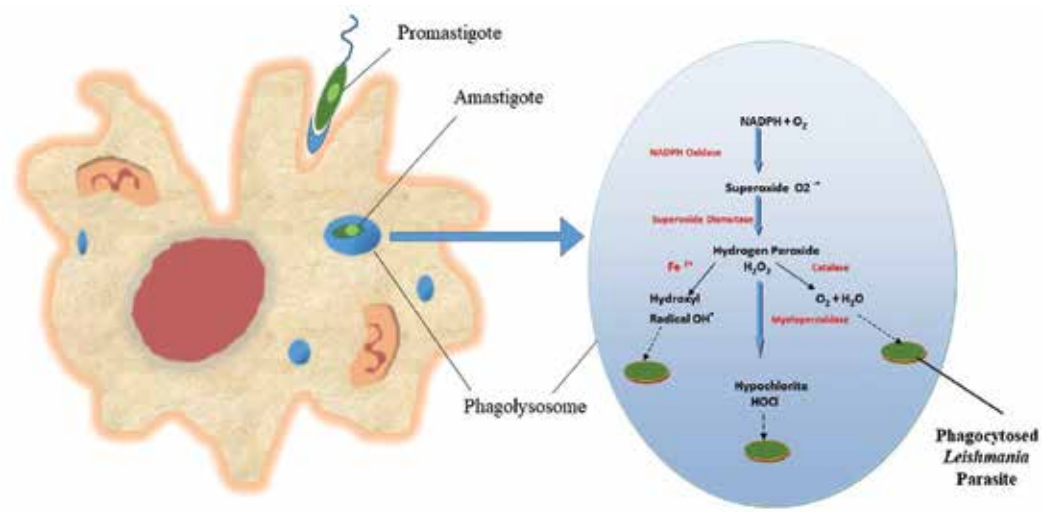


Figure 1. Endocytosis of *Leishmania* parasite and macrophage-induced oxidative burst.

to counteract the ROS-like activation of protein tyrosine phosphatase (PTP) and TR/$T[SH]_2$ system [26, 27]. Thus, *Leishmania* parasite survives the oxidative stress induced by the macrophages owing to its unique redox biology where it replicates and utilizes macrophages as a source of propagation of infection. The macrophage cell membrane is not freely permeable to the antileishmanial agents and acts as a barrier against the intracellular accumulation of chemotherapeutic agents at the concentrations required for optimum therapeutic effectiveness.

3.2. Activity of P-gp efflux pumps

The activity of P-gp efflux pumps presents another major barrier to the antileishmanial therapy [28]. Active efflux of the drug via the efflux pumps is one of the most common mechanisms for developing multidrug resistance (MDR) in the microorganism [29]. In fact, MDR mediated through efflux pumps has been described in various organisms like fungi, bacteria, and protozoa including *Leishmania* [30]. P-gp efflux pumps belong to the ATP-binding cassette (ABC) transporters, acting as the physiological barrier by extruding the toxins and xenobiotics out of the cells. The ABC transporters are the largest superfamily of efflux pumps known; being present in all organisms, from archaebacteria to higher eukaryotes. Various types of drugs with a wide range of chemical structure can be recognized by a single P-gp molecule ranging in molecular weight from 250 g/mol (cimetidine) to 1202 g/mol (cyclosporine). P-gp is primarily found in epithelial cells which have the excretory roles including the apical surface of epithelial cells lining the colon, small intestine, where it is involved in the decreased oral bioavailability of drugs [31]. In *Leishmania*, two types of ABC transporter have been reported to be amplified in the laboratory strains when exposed to different drugs: P-gp and multidrug resistance-related protein (MRP) also known as P-gp A [32]. P-gp A is believed to be involved in the decreased intracellular accumulation of antimonial compounds, the first-line therapy against *Leishmania*, resulting in the subtherapeutic response and emergence of resistance. The gene responsible for the P-gp A has been found to be amplified in the laboratory mutant strains of *Leishmania* that were resistant to the antimonial compounds [33]. However, this transporter is not involved in the efflux of antimonial drugs in the form of Sb-III or SB-V rather it confers resistance by sequestration of Sb-III conjugated with $T[SH]_2$ in the form of Sb-III-$T[SH]_2$ adducts as presented in **Figure 2** [34]. $T[SH]_2$ acts as the main reducing agent and is oxidized into its disulfide form $T[S]_2$ which is reduced back to $T[SH]_2$ by the activity of NADPH-dependent enzyme TR [35]. $T[SH]_2$ exerts its protective effect by the reduction of $NO^{\bullet}$, H_2O_2, and $ONOO^-$. Sb-V are converted to its trivalent form (Sb-III) inside the cell, and Sb-III has the ability to form a complex with the thiol groups of $T[SH]_2$. This Sb-III-$T[SH]_2$ conjugate is sequestrated by the P-gp A pumps. Thus, in *Leishmania,* the P-gp efflux pumps work in coordination with the activity of TR/$T[SH]_2$ system resulting in decreased intracellular accumulation of SB-III [36].

3.3. Lack of oral bioavailability

Oral administration is the most suitable method of delivering the drugs due to the convenience of dosing, noninvasive nature, and high acceptance at patient levels [37]. Most of the therapeutic agents used for systemic and localized GIT effects are administered orally because of the highly absorptive nature of the intestine that provides a large surface of around 300–400 m^2. The oral administration is successful only in the case where the drugs have sufficient bioavailability.

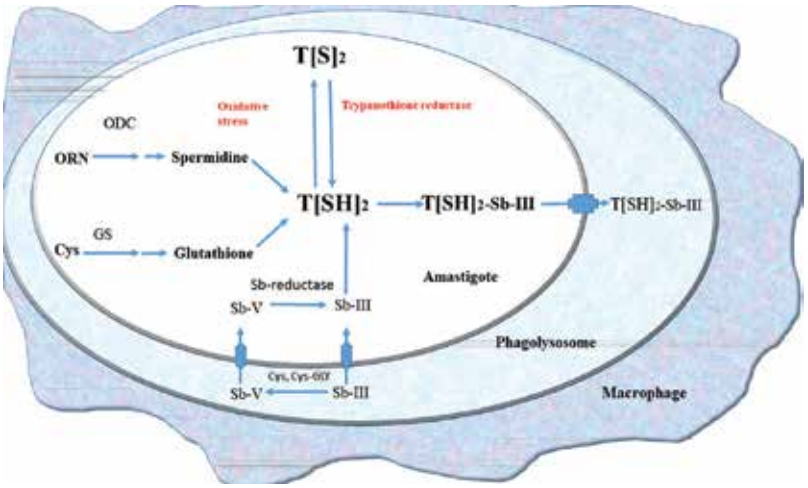


Figure 2. Mechanism of P-gp efflux pumps and TR-mediated drug resistance against antimonial compounds. ORN = ornithine, ODC = ornithine decarboxylase, GS = glutathione synthetase, Cys = cysteine, and Gly = glycine.

Many physiochemical and physiological factors determine the oral bioavailability of drugs like solubility, permeability, a mucus layer, partition coefficient, stability, dissolution, pH, enzymatic degradation, and activity of P-gp efflux pumps. Unfortunately, most of the antileishmanial drugs encounter the above-described barriers and exhibit limited oral bioavailability except for MILT.

In fact, solubility and permeability govern the oral bioavailability. Most of the drugs diffuse across cell membrane via passive transport, and for that purpose, the drug should be lipophilic in nature as the unionized form is better to diffuse across the phospholipid bilayer. However, the drug molecules should not be lipophilic enough to remain soluble in the lipid bilayer suggesting a suitable log P value. To maximize the possibilities of passive diffusion, the ideal log P value is considered to be around 2. The molecular weight of the drug also has a role in the passive diffusion of the drug and molecular mass less than 500 Da is considered to be favorable for the absorption across the small intestine [38]. The effect of solubility, permeability, and molecular weight is better explained in Lipinski' s rule. Lipinski's rule of five is a very useful tool to predict the drug-like characteristics of a compound and is especially applicable to assess whether a drug is orally active or not [39]. Lipinski's rule states that in general, an orally active drug has no more than one violation of the following criteria:

- No more than five hydrogen bond donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds);
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms);
- A molecular mass less than 500 Da;
- An octanol-water partition coefficient log P not greater than 5.

Most of the antileishmanial drugs do not follow the Lipinski's rule of five, therefore, not absorbed orally. According to biopharmaceutics classification system (BCS), AmB is class IV drug with the

Drug	Molecular weight (Da)	Log P	Hydrogen acceptor	Hydrogen donor	Rule of five
Sodium stibogluconate	907.88	−3.4	17	5	No
Amphotericin B	924.079	−0.66	17	12	No
Paromomycin	615.62	−8.3	19	13	No
Pentamidine	340.41	2.32	6	4	Yes
Miltefosine	407.57	2.25	2	0	Yes

Table 1. Physicochemical properties of antileishmanial agents.

aqueous solubility of <1 mg/L at physiological pH, molecular weight of 924 Da, and log P of 0.95, 17 hydrogen bond acceptors and 12 hydrogen bond donors and in this way does not follow the rule of 5. Similarly, sodium stibogluconate possesses a molecular weight of 910.10 Da and log P of −0.34, 17 hydrogen bond acceptors, and 5 hydrogen bond donors, thus violate the rule of 5. The physiochemical properties of the antileishmanial drugs are presented in **Table 1**.

Thus, the lack of oral bioavailability of most of the antileishmanial agents is the major limitation in the cost-effective and optimum therapy of leishmaniasis. Minimal oral absorption below the minimum effective concentration (MEC) necessitates the formulation of antileishmanial drugs for the parenteral administration. The long-term parenteral administration has its own limitations as it requires the patient to be hospitalized, increased cost of the therapy, and patient compliance.

3.4. Skin as barrier to topical therapy

Skin is the largest organ of the body and protects the organism from the external environment. Histologically, the skin is divided into the superficial layer called the epidermis and a deeper layer, the dermis. The several strata make up the epidermis distinguished by the changes in keratinocytes from dermis-epidermis junction to the outer surface of epidermis, the stratum corneum (SC). SC is designated as the main barrier to the transport of substances across the skin and is formed by the corneocytes characterized as densely packed, dead, and keratinized cells. These cells are surrounded by the intracellular lipid matrix composed of nonpolar lipids in lamellar lipid layers, making SC a hydrophobic layer [40]. Although SC is only 10–20 μm in thickness, it acts a barrier and hampers the penetration of microorganisms, drugs, and other chemicals besides being involved in the transepidermal water loss.

Drug delivery across the skin follows three possible pathways: the intracellular route, between the corneocytes sinuously through the lipid layer; the transcellular route, through the corneocytes and lipid matrix; and through the cutaneous appendices (sweat and sebaceous glands, hair follicles). The drug diffusion across the skin is essentially a passive transport; however, it follows either one or combination of the three pathways. However, it must be noted that the intracellular route is considered to be the most suitable for the drug diffusion as it offers less resistance compared to the transcellular route in which the drug molecules have to move

between the intercellular hydrophobic region to intracellular hydrophilic region repeatedly. The skin appendages, although having a small surface area compared to the total skin (0.1%), present an opportunity for the penetration of ions, polar compounds, and large molecules and thus circumvent the low diffusional character of SC [41].

To deliver the drug across the skin, the choice of the adequate molecule in terms of molecular weight and partition coefficient is very important. A molecular weight less than 600 Da, low melting point, and suitable log P are desired. Thus, the drug with high molecular weight and hydrophilic character will face the maximum resistance and their penetration will be limited [40]. The drugs for the CL-like paromomycin (PA), antimonial compounds, face the problem of skin penetration due to their hydrophilicity and high molecular weight. In case of CL, the lesions are developed and parts of epidermis and dermis are lost; therefore, the barriers provided by the SC are absent and almost any type of drug can be absorbed. However, the formation of scar tissues and keratotic nodules during the healing process restores the functionality of SC thus depriving the drug absorption at the end of the treatment, and complete healing of the lesion is difficult [42].

4. Nanodrug delivery system for leishmaniasis

The drug delivery systems are crucial in drug development and design, and many active pharmaceutical ingredients result in serious side effects when administered nonspecifically. The lack of appropriate drug delivery system causes the therapeutic modalities to be accumulated in healthy tissue inciting the adverse effects, lower bioavailability, and inefficient targeting of the desired pathological organs. Most of the latest researches in the field of leishmaniasis are focused on addressing the physiological, biological, and biopharmaceutical aspects of the use of nanotechnology. Nanodrug delivery systems provide an attractive opportunity to resolve the drug delivery problems associated with the therapy of leishmaniasis by crossing the above-demonstrated barriers encountered by the antileishmanial drugs. Examples of nanotechnology progress in pharmaceutical products include liposomes [43], niosomes [44], nanodisks [45], nanoemulsions [46], polymeric nanoparticles [47], solid lipid nanoparticles, and polymer-drug conjugates [48] as described in **Table 2**.

4.1. Liposomes

Liposomes are the lipid bilayer systems described in 1965 and rapidly taken as drug delivery systems [49]. In 1977, Ward and Hanson, first time reported the encapsulation of Sb-V into liposomes for targeted delivery to liver and spleen in VL. After intravenous administration, the Sb levels of liver and spleen were found to be 20-fold higher compared to the free drugs [50]. However, due to the toxic effects in monkeys, the interest in liposomal Sb-V was declined [51]. The same concept was also applied to AmB in order to avoid its toxicity by encapsulating into multilamellar liposomes. The liposomal AmB got a little bit more attention than Sb-V and initiated model for the development of three-lipid-based AmB drug delivery systems licensed for clinical use (Ambisome®, Amphocil®, and Abelcet®) [52]. However, the only true liposomal

Type of nanocarrier	Active moiety	Targeting approach	Strain tested	Model	Ref
Thiolated chitosan NPs	Amphotericin B	Macrophage targeting	*L. donovani*	J774.1 macrophages/BALB/c mice	[69]
Chitosan NPs	Rifampicin		—		[71]
Nanocapsules	Doxorubicin			Wistar rats	[72]
Gelatin nanoparticles	Amphotericin B		*L. donovani*	J774.1 macrophages/ Hamster	[73]
Liposomes	Antimony		*L. chagasi*	Peritoneal macrophages	[74]
GCPQ chitosan	Amphotericin B	Organ targeting via oral route	*L. donovani*		[76]
MT-chitosan	Amphotericin B		*L. donovani*	J774.1 macrophages/BALB/c mice	—
Liposomes	Zinc phthalocyanine	Photodynamic therapy	*L. braziliensis*		[86]
Metal oxide	ZnO		*L. tropica* KHW23		[87]
Liposomes	Paromomycin	Skin permeating nanocarriers	*L. major*	BALB/c mice	[90]
SLN	Amphotericin B		*L. major*		[92]

Table 2. Various types of nanocarriers and different targeting approaches for leishmaniasis.

formulation, Ambisome®, is recommended for treating patients with leishmaniasis who are resistant to antimonials. The efficacy of liposomal AmB was further enhanced by decorating the liposomal surface with specific ligands like polysaccharides, peptides, antibodies, and glycolipids. The decorated liposomes were able to specifically target the macrophages to avoid the exposure of AmB to healthy tissues [53, 54]. The detail of macrophage-targeted liposomes will be discussed under the section macrophage targeting.

4.2. Niosomes

Niosomes are the attractive alternatives over liposomes due to their increased stability, low cost, and biodegradability [55, 56]. Niosomes are the vesicles consisting of nonionic surfactants. Niosomal formulations of sodium stibogluconate were more efficacious compared to the liposomes and free drugs against experimental murine VL [56]. More recently, in vivo studies demonstrated that the niosomes containing autoclaved *L. major* have a significant result in the prevention of CL in BALB/c mice [57]. Niosomes have also found their role in vaccination against leishmaniasis. Purified gp63 entrapped into niosomal formulation provided considerable resistance to the leishmaniasis when used as the subcutaneous vaccine in C57BL/10 mice [58]. Advancement of a commercial antiparasitic vaccine for the human appliance is a central goal that faces modern science. Therefore, further research will be required to investigate immunological pathways, followed after vaccination with *Leishmania* antigens loaded into niosomes, and possible unwanted adverse effects in order to assess the real potential for a vaccination trial in humans.

4.3. Polymeric nanoparticles

Polymeric nanoparticles are very valuable in the treatment of infectious diseases like leishmaniasis owing to the small size and abilities to enhance the cellular uptake, cross the biological barriers, and deliver drugs at the site of infection [59, 60]. The use of polymer for the development of nanocarriers provides us the opportunity of modifying the functional groups with various chemical methods to incorporate the desired ligands for better penetration and enhanced endocytosis by the active or passive targeting. The ability of polymeric nanocarriers to bear the physiological strains and tunable surface properties provides an edge over liposomes and niosomes. While utilizing the polymeric nanoparticles for leishmaniasis, the category of polymers is of considerable importance as the hydrophobicity of the polymer will facilitate the internalization by macrophages, the core target in leishmaniasis. For example, polymethylmethacrylate-based nanoparticles indicated a superior macrophage uptake compared to the polycyanoacrylate [61]. Various studies reported the potential of polymeric nanocarriers in leishmaniasis. Primaquine-loaded polymeric nanoparticles were found to be 21-fold more efficient compared to the free primaquine [62]. β-aescin-loaded polylactide-co-glycolide nanoparticles showed twofold increase in efficacy against J744.1 macrophage-infected *L. donovani* model [63]. In a recent study, PEGylated polylactic acid nanoparticles loaded with bisnaphthalimidopropyl derivatives have been tested against human macrophage and THP-1 murine J744 macrophage model of leishmaniasis [64].

4.4. Polymer drug conjugate

The advances in the field of polymer engineering have opened new dimensions for the drug delivery. One example is the polymer therapeutics in which the drug molecules are attached to the polymer backbone by using a suitable chemical method. In this way, the efficacy of the drug can be increased significantly with the reduction in the toxicity. The hydrophobic drug encounters a problem of free circulation in the blood. The hydrophilicity of these drugs can be increased by conjugation of these hydrophobic drugs with the hydrophilic polymer. These polymer-drug conjugates provide increased plasma half-life and retention in the infectious tissue with the minimum toxicity. The conjugation of AmB with the N-2-(hydroxypropyl) methacrylamide resulted in the increased efficacy as compared to the free drug (fungizone) [65].

4.5. Nanodisks

Nanometer scale, a lipoprotein-stabilized phospholipid bilayer disk complexes termed nanodisks (NDs) are novel transport vehicles different from liposomes because they do not hold an aqueous core and are completely soluble in aqueous phase media [66]. NDs harboring poorly soluble antileishmanial agent AmB-nanodisks demonstrate an effective therapy for experimental CL (*L. major*) infection in BALB/c mice. Surprisingly, AmB-nanodisks were illustrated to have a long-term effect in that parasite burden continued to decrease for more than 100 days subsequent the final treatment. The results shown for intraperitoneal administration are most likely because of the small size of the ND [45].

http://dx.doi.org/10.5772/intechopen.75911

5. Nanomedicinal targeting approaches for leishmaniasis

Paul Ehrlich in 1891 was the first to theorize the concept of "magic bullets" providing the first description of drug targeting paradigm. The aim of drug targeting is delivering the drug at the right concentration at the right time and at the right place. The evolution of this "magic bullet" concept revolutionized the drug delivery systems and provided a vast platform, known as nanomedicine, to achieve the very specific and highly desirable therapeutic outcomes that are otherwise impossible to achieve with conventional drug delivery systems [67]. Their small size at nanoscale dictates the very unique properties like the interaction with the biological entities, penetration across the membrane, intracellular trafficking, accumulation at the target area, improved blood circulation, and biodistribution. For example, in case of VL, the major organs representing the parasitic burden are liver, spleen, and bone marrow, and the drug has to target the parasite inside the macrophage in these organs [68]. In CL, the drug must reach the parasite inside the macrophages at the inner layers of skin by crossing the skin barrier, SC. To maximize the potential of nanocarriers, a suitable strategy is required to target the pathological area via a patient-friendly route of administration while avoiding the healthy tissues. In view of this, various nanomedicinal targeting approaches have been explored for the therapy of leishmaniasis like macrophage targeting, organ targeting via the oral route, use of permeability enhancers, and photodynamic therapy (PDT).

5.1. Macrophage-targeted drug delivery

The niche in which *Leishmania* parasite lives presents challenges to drug delivery, and depending upon the species and the area affected, the drug has to achieve antiparasitic levels at multiple sites. Furthermore, the antileishmanial drug has to cross the multiple membranes before they act on the parasite. Nanoparticle-mediated drug delivery system to overcome the cell membrane barriers, release the drug inside the cells, and specifically target *Leishmania*-infected macrophages is emerged as a promising strategy to overcome resistance [69]. Various phagocytic receptors are expressed on the surface of *Leishmania* like MRs, CRs, SRs, and FRs [22]. These receptors bind with the specific ligands on the surface of *Leishmania* parasite and internalize the parasite. MRs are highly expressed especially on the *Leishmania*-infected macrophages. MRs recognize and bind with mannose and fructose glycoproteins followed by rapid endocytosis of the parasite. The mannose-binding protein belongs to the lectin-like carbohydrate-binding groups and cytoplasmic group that are involved in the remodeling of the cytoskeleton during endocytosis [2]. Scavenger receptors are the glycoproteins and are responsible for recognizing a broad range of ligands like chemically modified proteins, the apoptotic cell, low-density lipoproteins, phosphatidylserine, and various polyanionic molecules [70].

Targeting the macrophages via these receptors with surface-decorated nanocarriers leads to the accumulation of appreciable amounts of drug at the same niche where the parasite resides inside the macrophages. Various studies conducted on the macrophage-targeted drug delivery are summarized in **Table 2**. Recently, our research group utilized the MRs for macrophage-targeted delivery of mannose-anchored thiolated nanocarriers loaded with AmB. The uptake studies by

using the J744.1 macrophages indicated that macrophage-targeted nanocarriers provided AmB concentration of 28.6 ± 1.4 μg/10^6 cells as compared to 0.4 ± 0.01 μg/10^6 cells of the free AmB. These results provided the evidence that macrophage-targeted nanocarriers were 71-fold more efficient than the nontargeted ones. Also, the macrophage-targeted nanocarriers were having superior antileishmanial activities against *L. donovani*-infected macrophage model with 13-fold reduced IC_{50} values compared to the nontargeted ones. *In vivo* efficacy studies against *L. donovani*-infected BALB/c mice model at the dose of 1 mg/kg indicated that mannose-bearing thiolated chitosan (MTC) nanocarriers were significantly more effective in reducing the parasitic burden (89 ± 7%) against the free AmB (17 ± 4%) [69]. In another study, Chaubey and Mishra [71] also targeted the macrophages via mannose-decorated chitosan for the delivery of rifampicin against VL. *Ex vivo* cellular uptake studies indicated 16-fold increased uptake in case of mannosylated chitosan nanoparticles (mCNPs). The pharmacokinetic studies revealed that mCNPs exhibited Cmax of 5.40 ± 1.64 μg/ml with MRT of 58.48 ± 9.1 h compared to Cmax of 279.00 ± 17.71 μg/ml and MRT of 1.82 ± 0.2 h for free rifampicin indicating the long circulating time of the mCNPs. Similarly, very encouraging results were obtained with *in vivo* biodistribution studies conducted at the dose of 12 mg/kg. The maximum accumulation of drug was observed in liver (57.5 ± 1.3%) followed by spleen (14.2 ± 1.5%), achieved with mCNPs when compared with the free rifampicin (6.91 ± 1.3% for liver and 1.1 ± 0.2% for spleen) after 6 h. The drug accumulation is attributed to the fact that macrophage-targeted nanocarriers were rapidly taken up by the MP cells and delivered to the liver, main pathological organ of VL [71]. Similarly, curcumin-loaded mannosylated chitosan-based nanoparticles were reported to be highly efficacious against the free drug. Their study indicated that mannosylated nanoparticles effectively increased the endocytosis with the mean residence time of 39.38 h compared with the 0.30 h of free drug solution [68].

Kansal et al. [72] utilized scavenger receptors for the macrophage-targeted delivery of doxorubicin via phosphatidylserine-decorated nanocapsules (PS-NCs-DOX) for the therapy of leishmaniasis. Flow cytometry analysis indicated 1.75-fold increased uptake of PS-NCs-DOX compared with nonmodified nanocarriers (NCs-DOX). PS-NCs-DOX also accumulated in liver and spleen at higher concentration against NCs-DOX confirmed via *in vivo* biodistribution analysis in Wistar rats. Highly significant antileishmanial activities were observed in *Leishmania*-infected hamster model. PS-NCs-DOX exhibited 85.23 ± 4.49% inhibition of splenic parasitic burden compared with 72.88 ± 3.87 and 42.85 ± 2.11% parasite inhibition for NCs-DOX and free DOX, respectively [72]. Another study reported the development of 1, 2-diacyl-sn-glycero-3-phospho-l-serine (PS)-coated gelatin nanoparticles (GNPs) bearing amphotericin B for the enhanced *in vitro in vivo* antileishmanial efficacy in VL. The nanocarriers decorated with 1, 2-diacyl-sn-glycero-3-phospho-l-serine (PS-AmB-GNPs) were more efficient in terms of uptake by J774A.1 macrophages analyzed via flow cytometry [73]. Also, PS-AmB-GNPs exhibited a very significant reduction in parasitic burden providing 85.3 ± 7.89% inhibition compared to 50.5 ± 5.12% of free AmB in *Leishmania*-infected hamster model. Antimony-loaded liposomes have also been modified with phosphatidylserine (Sb-LP) for the enhanced uptake of macrophages via scavenger receptors. Sb-LP was 16-fold more effective than free drug against *L. chagasi*-infected macrophage model [74].

5.2. Organ targeting via oral route

One of the limitations associated with the conventional antileishmanial therapy is the free systemic circulation of the drug and distribution into different body organs including pathological

http://dx.doi.org/10.5772/intechopen.75911

and nonpathological. The exposure of nonpathological organs to the drugs is associated with the severe toxicity of the antileishmanial drugs thus limiting its therapeutic potential [17]. In this regard, the specific organ targeting is a promising strategy that reduces the toxic effects by minimizing the exposure to nondesired organs and improves therapeutic efficacy by increasing the drug accumulation at the desired organs. One such example of the nanoliposomal formulation of AmB is Ambisome® for VL, when administered is taken up by MP cells and transported to the liver and spleen via passive targeting [52, 75]. Although this strategy greatly improves the safety of AmB, the macrophage-targeted nanocarriers described above can be of more potential in this regard. The surface modification with the ligands actively targets the infected macrophages because of the high expression of endocytic receptors like MRs. However, one factor, the parenteral delivery of these systems, limits their wide application and acceptance at the patient level due to the hazards and high cost associated with and needs to be addressed yet. In pursuit of the solution to this limitation, Serrano et al. [76] provided the concept of organ targeting via the oral route and introduced nanomedicine in which nanoparticles were taken up by the intestinal epithelia and are transported to liver, spleen, and lungs as shown in **Figure 3**, thus enhancing the bioavailability of these pathological organs of VL and bypassing the organs of potential toxicity [76]. This technique utilized specifically engineered polymeric excipients with the potential to interact with specific proteins in the intestinal epithelium thus enhancing the permeation and absorption of the constituted nanocarriers.

Serrano et al. [76] illustrated this concept by utilizing N-palmitoyl-N-methyl N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan (GCPQ) nanoparticles loaded with AmB. Such modification of chitosan will provide the mucoadhesive character to the nanocarriers. As the mucus is a negatively charged glycoprotein, the positively charged polymer will provide increased electrostatic

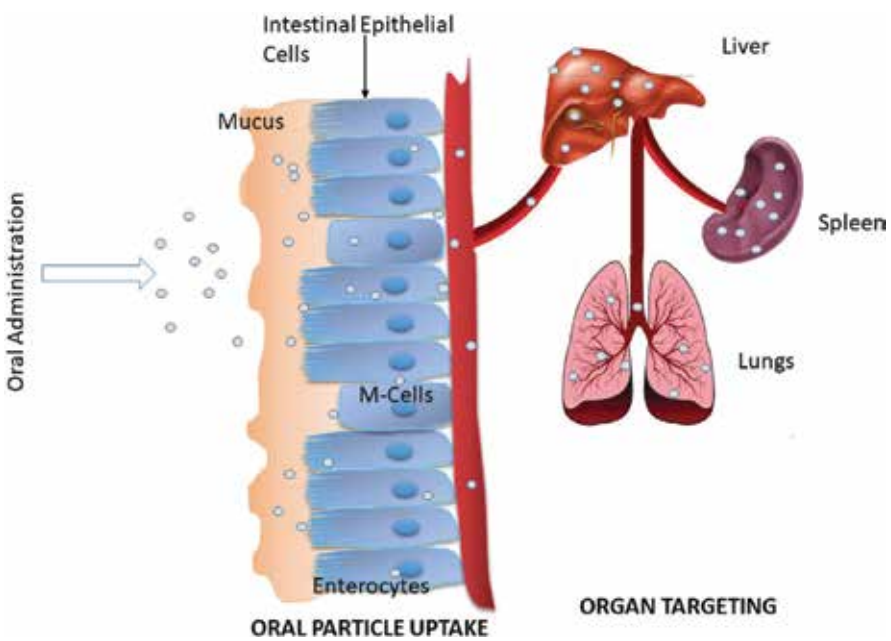


Figure 3. Uptake of nanoparticles via oral route and accumulation in liver, spleen, and lungs.

interaction and bind with proteins thus better chances to be taken up by the enterocytes. Single-dose oral pharmacokinetic studies in CD-1 mice were carried out by utilizing AmB-GCPQ, Amb-sodium deoxycholate (Amb-d), and AmB in dextrose solution at the dose of 5 mg/kg. The nanoparticulate formulations, AmB-GCPQ, and Amb-d exhibited higher plasma drug levels compared to the AmB in dextrose. These results indicate that the particulate formulations were able to cross the intestinal membrane. Furthermore, significantly higher levels of Amb-GCPQ were found in target organs, i.e., liver and spleen as compared to the Amb-d. The target organ to kidney ratio was also determined and provided very encouraging results. As AmB is a nephrotoxic drug, target organ:kidney ratios are crucial. Lung:kidney AUC0-24 ratios for AmB-GCPQ and Amb-d were 1.44 and 0.86, respectively, while the corresponding spleen:kidney ratios were 1.22 and 0.81, respectively, and the corresponding liver:kidney ratios were 0.88 and 0.40, respectively. These data demonstrate that when compared to the deoxycholate micelles, GCPQ nanoparticles delivered relatively more drug to the target organs (liver, lung, and spleen) rather than kidney. These findings were also supported by the low urinary excretion of Amb-GCPQ, while AmB in dextrose delivered most of the drug to the kidney, a fact that contributes to the nephrotoxicity associated with AmB and reduced drug levels in target organs. Also, the oral particle location to major organs was studied by coherent anti-Stokes Raman spectroscopy. The results located the GCPQ nanocarriers within the hepatocytes in the liver, intracellular spaces in the hepatocytes. The reason for their location in the hepatocytes and lungs is their uptake by the intestinal villi from where they are transported to the liver via the hepatic portal vein. GCPQ nanocarriers were also taken up by the M cells of Peyer's patches from where they are carried to the systemic circulation via the lymphatic system. The *in vivo* efficacy studies were carried out in VL murine model. The data indicated oral GCPQ nanocarriers were equal in efficacy to the parenterally administered AmB [76].

Recently, our research group utilized thiolated polymer-based mannose-anchored nanocarriers to target the visceral organs via oral route for the delivery of AmB against VL (unpublished data). Thiolated polymers, the so-called thiomers, are well known for their mucoadhesion, permeation enhancing, and P-gp inhibition properties with great impact on the nanodrug delivery. Thiomer contains thiol group (—SH) covalently attached to the polymer chain, and by the virtue of –SH, the thiolated polymer can interact with the proteins and receptors via disulfide bond formation (—S—S—) in disulfide exchange mechanism [77]. Mucus in the intestine acts as the physical barrier for the diffusion of drugs across the intestinal membrane. The structure of mucus is complex, which arises from the properties of mucins. Mucins are large glycoproteins composed of more than 800 amino acids, also containing cysteine- and disulfide-rich domains. Mucins have long flexible proline, threonine, and serine (PTS) domains that are glycosylated. The glycans terminate with negatively charged carboxylic groups. Diffusion in the mucus structure depends on the charge of the molecules. Mucus contains pores that are 200–400 nm in diameter, thus allowing diffusion of many APIs [78, 79]. If APIs are encapsulated in nano- or microcarriers, the size of the carrier can preclude diffusion in mucus. Thiomer-based nanocarriers will remain adhered to the mucus by making disulfide bond with the cysteine-rich units of mucin, and by the virtue of small size of nanocarriers, they can easily pass through the pores in the mucus. After crossing the mucus barrier, they are taken up by the enterocytes, M-cells of Peyer's patches and also cross the membrane via paracellular route owing to permeation-enhancing capabilities of thiomers. The primary mechanism of the permeation enhancing by thiomers is the inhibition of PTP. The inhibition of PTP is

accomplished by the disulfide (—S—S) bond formation by thiomer with cysteine-rich units and consequently increased phosphorylation of membrane proteins thus leading to the opening of tight junction [80]. Furthermore, the mannose anchoring to the thiolated polymer enables the nanoparticles to target the macrophages via mannose receptors. Thus, the combined effect of mucoadhesion, permeation enhancing, and macrophage targeting successfully target the pathological organs of VL, i.e., liver, spleen, and lungs via the oral route.

5.3. Photodynamic therapy

The survival of *Leishmania* inside macrophages is dependent upon its unique redox biology that neutralizes the ROS produced by the macrophages [81]. PDT targets the redox biology of *Leishmania* by producing the ROS that supersedes neutralizing capabilities of the parasite. The increased ROS thus exert the antileishmanial effects by jeopardizing the reducing potential [82]. PDT involves the delivery of special drug called photosensitizers (PS) to the infectious tissue with nanocarriers and subsequent exposure to a light of specific wavelength. The photosensitizers absorb the light and then transfer the energy to the molecular oxygen which is converted to the free oxygen [$O^{\bullet}$] or $OH^{\bullet}$. These ROS are responsible for the killing of the cells or tissue where the PS is localized. The photooxidation of biomolecules changes the structure and functions of cells. The generation ROS can be classified into two types of reactions. Type I reactions called electron transfer reactions and are responsible for producing various free radicals including highly reactive $OH^{\bullet}$. The type II reaction involves the energy transfer via molecular oxygen leading to the formation [$O^{\bullet}$] [83]. However, the clinical application of PDT is limited to the easily accessible areas where the direct exposure of LASER or incoherent light can be provided. Consequently, the PDT finds its application in the treatment of CL [84]. The most commonly used photosensitizer against CL is a porphyrin precursor, 5-aminolevulinic acid (ALA). ALA is a substrate for heme synthesis and its exogenous delivery results in the accumulation of protoporphyrin IX (PpIX). When exposed to the light of a suitable wavelength like the red or blue light after some specific intervals, cell death occurs due to the apoptosis and necrosis caused by the activated PpIX generating ROS [85]. Zinc phthalocyanine (ZnPc) is another commonly used PS for the PDT. However, one major concern with applying the PS to the skin is that they cannot penetrate to the deeper layers of skin because of the SC barrier of the skin. Due to which their efficacy at full potential is hampered.

Nanoparticles have been extensively explored to improve the efficacy of PDT against CL, due to the ability to penetrate the skin by crossing SC barrier and also protect the PS from aggregation and subsequent inactivation. The current PDT against the CL involves the indirect destruction of the parasites either by enhancing the immune response or by killing the macrophages. Montanari et al. [86] conducted a study, in which they delivered ZnPc, loaded in liposomes, to treat the infection induced by *L. braziliensis*. They indicated ZnPc alone has 20% activity against the promastigotes and amastigotes; however, when incorporated into the liposomes the antileishmanial activity increased up to 100% for promastigotes and 80% for amastigote. Moreover, the penetration studies indicated liposomal ZnPc showed eightfold increased penetration and sevenfold increased accumulation of ZnPc into the deeper layers of skin as compared to the ZnPc alone. Their study provided the proof that targeted PDT with nanocarriers greatly enhances the penetration and accumulation of PS into deeper layers of skin thus enhanced antileishmanial efficacy [86].

Metallic nanoparticles have found their application in the PDT due to their surface localized plasmon response, and they enhance the effectiveness of PDT by producing ROS. Also, they are not involved in the immune system activation. Several studies have been reported in which the effectiveness of the metal nanoparticles in the PDT has been established. PEGylated silver-doped zinc oxide nanoparticles (DSNs) for the PDT of leishmaniasis have been reported by the Nadhman et al. [87]. They indicated DSNs were highly efficacious in providing the photodynamic effect than nondoped zinc oxide nanocarriers (NDSNs). Doping of zinc oxide with silver enhanced the band gap and thus excitation at the visible light source. The IC_{50} of DSNs was in the range of 0.009 (±0.0012) to 0.02 μg/ml (±0.0023), while that of NDSN was 0.1 μg/ml (±0.016). The DSNs were 10 times more active than the NDSN. Free radical scavenger studies indicated 77–83% cell death occurs due to singlet oxygen, while 18–27% due to the production of hydroxyl ions [87].

5.4. Skin-permeating nanocarriers

The role of the skin as barriers to the drug delivery has been discussed above in detail. The nanomedicine is a promising strategy to cross the skin barrier since they offer several advantages over the conventional drug delivery systems, and skin permeation and follicular targeting are the most significant regarding the topical treatment of CL. The nanoparticles larger than 20 nm and lesser than 200 nm can be accumulated in the hair follicles where they are retained for longer period of time for up to 10 days, thus providing the continuous supply of the drug for the absorption [88, 89]. Various types of nanocarriers have been utilized for this purpose but the lipid-based nanocarriers such as liposomes, solid lipid nanoparticles, and nanoemulsions are most extensively studied for skin permeation.

Ferriera et al. [90] first time reported the encapsulation of PA into liposomes and evaluated their permeation across the stripped and intact mouse skin. The results exhibited significantly increased PA penetration into and across the intact skin compared to the PA in solution. However, this model was based on the hairless skin and cannot be extrapolated for human due to the presence of hairs. Topical treatment of *L. major* infected BALB/c mice resulted in a decrease in lesion size in animals treated with PA-loaded liposomes and free PA gel. However, local relapse, characterized by the reappearance of ulcers, occurred faster in animals treated with free PA than in those treated with liposomes. These findings suggest that liposomes represent a promising alternative for the topical treatment of CL using PA [90]. Jaafari et al. [42] reported the efficacy of liposomes loaded with PA at 10 and 15%. Both types of liposomal formulations indicated high retention and permeation profile of PA in the mouse skin. These formulations exhibited 3–4 times better efficacy against *L. major* amastigotes compared to simple PA solution. Significant reduction in the lesion size and parasitic burden in liver and spleen was observed in *L. major*-infected BALB/c mice with topical PA liposomal formulations compared to the control mice. However, in this study, comparison of topical treatment with free PA was not reported [42].

Frankenburg et al. [93] evaluated the effectiveness of AmB-based lipid nanoformulations applied topically to *L. major* experimentally infected mice. The three evaluated formulations (Amphocil, Fungizone, and Abelcet) were ineffective when applied topically, except when Amphocil and Abelcet were dispersed in 5% ethanol. No relapse was observed during the follow-up period after treatment [91]. Subsequently, Amphocil dispersed in 5% ethanol was tested in *L. major*-infected patients in a prospective placebo-controlled study. The results

provided the significant reduction in the lesion size against the placebo-treated lesions. This treatment exhibited complete healing lesions with no evidence of relapse on follow-up visits [92]. This modality was also used for the topical treatment of an infant patient who had not responded to the topical application of a PA ointment, resulting in resolution of the skin lesions and absence of local or systemic side effects [93].

6. Conclusion

The conventional therapy of leishmaniasis failed to provide the satisfactory control over the progression of disease due to the involvement of certain biological barriers. *Leishmania* parasite resides inside macrophages providing a barrier of macrophage cell membrane permeability for the drugs. The activity of P-gp efflux pumps directly related to the decreased intracellular accumulation of antimonial compounds. The physiochemical properties of antileishmanial drugs limit their oral bioavailability making it necessary to deliver drug via IV route. SC provides another barrier to delivery of drugs in CL. The biological barriers encountered by the chemotherapeutic agents leads to the development of resistance, lack of effectiveness and toxicity, thus are the factors jeopardizing the full therapeutic potential of antileishmanial drugs. These biological barriers cannot be tackled with the conventional drug delivery systems, and lack of therapeutic choices necessitates the development of new drug delivery system with the better therapeutic profile. In this area, nanotechnology is a great hope that provided real breakthrough over conventional formulations. The nanotechnology-based pharmaceutical formulations can easily navigate through the biological barriers and enhance the therapeutic effectiveness of antileishmanial drugs. Various types of nanocarriers, like liposomes, niosomes, polymeric nanocarriers, and metal oxide nanoparticles provided very encouraging results regarding leishmaniasis therapy. One of the very promising aspects of nanotechnology is the targeted delivery of nanocarriers to a very specific organ of pathology and avoiding the healthy tissues. In this regard, the targeting approaches like receptor-mediated macrophage targeting, organ targeting via oral route, photodynamic therapy provided a platform for successful therapy of the disease. Various nanotechnology-based formulations of antileishmanial drugs are in different phases of clinical trial. However, a lot of efforts from the scientific community are required to further investigate the targeted delivery of antileishmanial agents to translate the nanomedicinal concepts into first-line gold standard therapy.

Author details

Gul Shahnaz[1], Hafiz Shoaib Sarwar[1] and Masoom Yasinzai[2]*

*Address all correspondence to: rector@iiu.edu.pk

1 Department of Pharmacy, Faculty of Biological Sciences, Quad-I-Azam University, Islamabad, Pakistan

2 Centre for Interdisciplinary Research in Basic Sciences, International Islamic University, Islamabad, Pakistan

References

[1] Croft S, Olliaro P. Leishmaniasis chemotherapy—Challenges and opportunities. Clinical Microbiology and Infection. 2011;**17**(10):1478-1483

[2] Sarwar HS, Akhtar S, Sohail MF, Naveed Z, Rafay M, Nadhman A, et al. Redox biology of Leishmania and macrophage-targeted nanoparticles for therapy. Nanomedicine. 2017;**12**(14):1713-1725

[3] Maltezou HC. Drug resistance in visceral leishmaniasis. BioMed Research International. 2009;**2010**. DOI: 10.1155/2010/617521

[4] Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;**7**(5):e35671

[5] M Rabanel J, Aoun V, Elkin I, Mokhtar M, Hildgen P. Drug-loaded nanocarriers: Passive targeting and crossing of biological barriers. Current Medicinal Chemistry. 2012;**19**(19):3070-3102

[6] Jeddi F, Piarroux R, Mary C. Antimony resistance in leishmania, focusing on experimental research. Journal of Tropical Medicine. 2011;**2011**. DOI: 10.1155/2011/695382

[7] Krauth-Siegel RL, Comini MA. Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. Biochimica et Biophysica Acta (BBA)—General Subjects. 2008;**1780**(11):1236-1248

[8] Yasinzai M, Khan M, Nadhman A, Shahnaz G. Drug resistance in leishmaniasis: Current drug-delivery systems and future perspectives. Future Medicinal Chemistry. 2013;**5**(15):1877-1888

[9] dos Santos Ferreira C, Martins PS, Demicheli C, Brochu C, Ouellette M, Frézard F. Thiol-induced reduction of antimony (V) into antimony (III): A comparative study with trypanothione, cysteinyl-glycine, cysteine and glutathione. Biometals. 2003;**16**(3):441-446

[10] Sundar S, Chakravarty J. Antimony toxicity. International Journal of Environmental Research and Public Health. 2010;**7**(12):4267-4277

[11] Saravolatz LD, Bern C, Adler-Moore J, Berenguer J, Boelaert M, den Boer M, et al. Liposomal amphotericin B for the treatment of visceral leishmaniasis. Clinical Infectious Diseases. 2006;**43**(7):917-924

[12] Purkait B, Kumar A, Nandi N, Sardar AH, Das S, Kumar S, et al. Mechanism of amphotericin B resistance in clinical isolates of *Leishmania donovani*. Antimicrobial Agents and Chemotherapy. 2012;**56**(2):1031-1041

[13] Mbongo N, Loiseau PM, Billion MA, Robert-Gero M. Mechanism of amphotericin B resistance in *Leishmania donovani* promastigotes. Antimicrobial Agents and Chemotherapy. 1998;**42**(2):352-357

[14] Chattopadhyay A, Jafurulla M. A novel mechanism for an old drug: Amphotericin B in the treatment of visceral leishmaniasis. Biochemical and Biophysical Research Communications. 2011;**416**(1):7-12

[15] Kelly SL, Lamb DC, Taylor M, Corran AJ, Baldwin BC, Powderly WG. Resistance to amphotericin B associated with defective sterol Δ8 → 7 isomerase in a *Cryptococcus neoformans* strain from an AIDS patient. FEMS Microbiology Letters. 1994;**122**(1-2):39-42

[16] Laniado-Laborín R, Cabrales-Vargas MN. Amphotericin B: Side effects and toxicity. Revista Iberoamericana de Micología. 2009;**26**(4):223-227

[17] Croft SL, Coombs GH. Leishmaniasis—Current chemotherapy and recent advances in the search for novel drugs. Trends in Parasitology. 2003;**19**(11):502-508

[18] Seifert K, Matu S, Perez-Victoria FJ, Castanys S, Gamarro F, Croft SL. Characterisation of *Leishmania donovani* promastigotes resistant to hexadecylphosphocholine (miltefosine). International Journal of Antimicrobial Agents. 2003;**22**(4):380-387

[19] Pérez-Victoria FJ, Castanys S, Gamarro F. *Leishmania donovani* resistance to miltefosine involves a defective inward translocation of the drug. Antimicrobial Agents and Chemotherapy. 2003;**47**(8):2397-2403

[20] Bray PG, Barrett MP, Ward SA, de Koning HP. Pentamidine uptake and resistance in pathogenic protozoa: Past, present and future. Trends in Parasitology. 2003;**19**(5):232-239

[21] Unanue EL, Allen PM. The basis for the immunoregulatory role of macrophages and other accessory cells. Science. 1987;**236**:551-558

[22] Handman E, Bullen DV. Interaction of Leishmania with the host macrophage. Trends in Parasitology. 2002;**18**(8):332-334

[23] Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, et al. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. Journal of Biological Chemistry. 1994;**269**(42):26066-26075

[24] Smith RM, Connor JA, Chen LM, Babior BM. The cytosolic subunit p67phox contains an NADPH-binding site that participates in catalysis by the leukocyte NADPH oxidase. Journal of Clinical Investigation. 1996;**98**(4):977

[25] Millar TM, Kanczler JM, Bodamyali T, Blake DR, Stevens CR. Xanthine oxidase is a peroxynitrite synthase: Newly identified roles for a very old enzyme. Redox Report. 2002;**7**(2):65-70

[26] Forget G, Gregory DJ, Whitcombe LA, Olivier M. Role of host protein tyrosine phosphatase SHP-1 in *Leishmania donovani*-induced inhibition of nitric oxide production. Infection and Immunity. 2006;**74**(11):6272-6279

[27] Trujillo M, Budde H, Piñeyro MD, Stehr M, Robello C, Flohé L, et al. *Trypanosoma brucei* and *Trypanosoma cruzi* tryparedoxin peroxidases catalytically detoxify peroxynitrite via oxidation of fast reacting thiols. Journal of Biological Chemistry. 2004;**279**(33):34175-34182

[28] Rai S, Goel SK, Dwivedi UN, Sundar S, Goyal N. Role of efflux pumps and intracellular thiols in natural antimony resistant isolates of *Leishmania donovani*. PLoS One. 2013;**8**(9):e74862

[29] Lage H. ABC-transporters: Implications on drug resistance from microorganisms to human cancers. International Journal of Antimicrobial Agents. 2003;**22**(3):188-199

[30] Kourtesi C, Ball AR, Huang Y-Y, Jachak SM, Vera DMA, Khondkar P, et al. Suppl 1: Microbial efflux systems and inhibitors: Approaches to drug discovery and the challenge of clinical implementation. The Open Microbiology Journal. 2013;**7**:34

[31] Li X. Oral Bioavailability: Basic Principles, Advanced Concepts, and Applications. John Wiley & Sons; 2011

[32] Leandro C, Campino L. Leishmaniasis: Efflux pumps and chemoresistance. International Journal of Antimicrobial Agents. 2003;**22**(3):352-357

[33] Ouellette M, Légaré D, Papadopoulou B. Multidrug resistance and ABC transporters in parasitic protozoa. Journal of Molecular Microbiology and Biotechnology. 2001;**3**(2): 201-206

[34] Rosen BP. Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2002;**133**(3):689-693

[35] Romão P, Tovar J, Fonseca S, Moraes R, Cruz A, Hothersall J, et al. Glutathione and the redox control system trypanothione/trypanothione reductase are involved in the protection of *Leishmania* spp. against nitrosothiol-induced cytotoxicity. Brazilian Journal of Medical and Biological Research. 2006;**39**(3):355-363

[36] Mukhopadhyay R, Dey S, Xu N, Gage D, Lightbody J, Ouellette M, et al. Trypanothione overproduction and resistance to antimonials and arsenicals in Leishmania. Proceedings of the National Academy of Sciences. 1996;**93**(19):10383-10387

[37] Demicheli C, Ochoa R, da Silva JB, Falcão CA, Rossi-Bergmann B, de Melo AL, et al. Oral delivery of meglumine antimoniate-β-cyclodextrin complex for treatment of leishmaniasis. Antimicrobial Agents and Chemotherapy. 2004;**48**(1):100-103

[38] Lagarce F, Roger E. Transport of therapeutics across gastrointestinal epithelium. In: Drug Delivery Across Physiological Barriers. 2016. p. 181

[39] Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. Journal of Pharmacological and Toxicological Methods. 2000;**44**(1):235-249

[40] Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nature Reviews Drug Discovery. 2004;**3**(2):115-124

[41] Cullander C, Guy RH. (D) Routes of delivery: Case studies: (6) Transdermal delivery of peptides and proteins. Advanced Drug Delivery Reviews. 1992;**8**(2-3):291-329

[42] Jaafari MR, Bavarsad N, Bazzaz BS, Samiei A, Soroush D, Ghorbani S, Heravi MM, Khamesipour A. Effect of topical liposomes containing paromomycin sulfate in the course of Leishmania major infection in susceptible BALB/c mice. Antimicrobial agents and chemotherapy. 2009;**53**(6):2259-2265

[43] Alving CR, Steck EA, Chapman WL, Waits VB, Hendricks LD, Swartz GM, et al. Therapy of leishmaniasis: Superior efficacies of liposome-encapsulated drugs. Proceedings of the National Academy of Sciences. 1978;**75**(6):2959-2963

[44] Hunter C, Dolan T, Coombs G, Baillie A. Vesicular systems (niosomes and liposomes) for delivery of sodium stibogluconate in experimental murine visceral leishmaniasis. Journal of Pharmacy and Pharmacology. 1988;**40**(3):161-165

[45] Nelson KG, Bishop JV, Ryan RO, Titus R. Nanodisk-associated amphotericin B clears Leishmania major cutaneous infection in susceptible BALB/c mice. Antimicrobial Agents and Chemotherapy. 2006;**50**(4):1238-1244

[46] Gupta S, Dube A, Vyas SP. Antileishmanial efficacy of amphotericin B bearing emulsomes against experimental visceral leishmaniasis. Journal of Drug Targeting. 2007;**15**(6): 437-444

[47] Shahnaz G, Vetter A, Barthelmes J, Rahmat D, Laffleur F, Iqbal J, et al. Thiolated chitosan nanoparticles for the nasal administration of leuprolide: Bioavailability and pharmacokinetic characterization. International Journal of Pharmaceutics. 2012;**428**(1):164-170

[48] Nan A, Croft SL, Yardley V, Ghandehari H. Targetable water-soluble polymer-drug conjugates for the treatment of visceral leishmaniasis. Journal of Controlled Release. 2004;**94**(1):115-127

[49] Lian T, Ho RJ. Trends and developments in liposome drug delivery systems. Journal of Pharmaceutical Sciences. 2001;**90**(6):667-680

[50] New R, Chance M, Thomas S, Peters W. Antileishmanial activity of antimonials entrapped in liposomes. Nature. 1978;**272**(5648):55-56

[51] New R, Chance M, Heath S. The treatment of experimental cutaneous leishmaniasis with liposome-entrapped Pentostam. Parasitology. 1981;**83**(3):519-527

[52] Adler-Moore J, Proffitt RT. AmBisome: Liposomal formulation, structure, mechanism of action and pre-clinical experience. Journal of Antimicrobial Chemotherapy. 2002;**49**(suppl 1):21-30

[53] Torchilin V. Liposomes as targetable drug carriers. Critical Reviews in Therapeutic Drug Carrier Systems. 1985;**2**(1):65-115

[54] Agrawal AK, Agrawal A, Pal A, Guru P, Gupta C. Superior chemotherapeutic efficacy of amphotericin B in tuftsin-bearing liposomes against *Leishmania donovani* infection in hamsters. Journal of Drug Targeting. 2002;**10**(1):41-45

[55] Hu C, Rhodes DG. Proniosomes: A novel drug carrier preparation. International Journal of Pharmaceutics. 1999;**185**(1):23-35

[56] Carter K, Dolan T, Alexander J, Baillie A, McColgan C. Visceral leishmaniasis: Drug carrier system characteristics and the ability to clear parasites from the liver, spleen and bone marrow in *Leishmania donovani* infected BALB/c mice. Journal of Pharmacy and Pharmacology. 1989;**41**(2):87-91

[57] Pardakhty A, Shakibaie M, Daneshvar H, Khamesipour A, Mohammadi-Khorsand T, Forootanfar H. Preparation and evaluation of niosomes containing autoclaved *Leishmania major*: A preliminary study. Journal of Microencapsulation. 2012;**29**(3):219-224

[58] LezamaDávila CM. Vaccination of C57BL/10 mice against cutaneous leishmaniasis. Use of purified gp63 encapsulated into niosomes surfactants vesicles: A novel approach. Memórias do Instituto Oswaldo Cruz. 1999;**94**(1):67-70

[59] Couvreur P, Vauthier C. Nanotechnology: Intelligent design to treat complex disease. Pharmaceutical Research. 2006;**23**(7):1417-1450

[60] Lockman P, Mumper R, Khan M, Allen D. Nanoparticle technology for drug delivery across the blood-brain barrier. Drug Development and Industrial Pharmacy. 2002;**28**(1): 1-13

[61] Gaspar R, Préat V, Opperdoes FR, Roland M. Macrophage activation by polymeric nanoparticles of polyalkylcyanoacrylates: Activity against intracellular *Leishmania donovani* associated with hydrogen peroxide production. Pharmaceutical Research. 1992;**9**(6):782-787

[62] Rodrigues J Jr, Croft S, Fessi H, Bories C, Devissaguet JP. The activity and ultrastructural localization of primaquine-loaded poly(D, L-lactide) nanoparticles in *Leishmania donovani* infected mice. Tropical Medicine and Parasitology: Official Organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ). 1994;**45**(3):223-228

[63] Van de Ven H, Vermeersch M, Vandenbroucke R, Matheeussen A, Apers S, Weyenberg W, et al. Intracellular drug delivery in Leishmania-infected macrophages: Evaluation of saponin-loaded PLGA nanoparticles. Journal of Drug Targeting. 2012;**20**(2):142-154

[64] Basu MK, Lala S. Macrophage specific drug delivery in experimental leishmaniasis. Current Molecular Medicine. 2004;**4**(6):681-689

[65] Nicoletti S, Seifert K, Gilbert IH. N-(2-hydroxypropyl) methacrylamide–amphotericin B (HPMA–AmB) copolymer conjugates as antileishmanial agents. International Journal of Antimicrobial Agents. 2009;**33**(5):441-448

[66] Romero EL, Morilla MJ. Drug delivery systems against leishmaniasis? Still an open question. Expert Opinion on Drug Delivery. 2008;**5**(7):805-823

[67] Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. Nature Reviews Cancer. 2008;**8**(6):473-480

[68] Nahar M, Dubey V, Mishra D, Mishra PK, Dube A, Jain NK. In vitro evaluation of surface functionalized gelatin nanoparticles for macrophage targeting in the therapy of visceral leishmaniasis. Journal of Drug Targeting. 2010;**18**(2):93-105

[69] Shahnaz G, Edagwa BJ, McMillan J, Akhtar S, Raza A, Qureshi NA, et al. Development of mannose-anchored thiolated amphotericin B nanocarriers for treatment of visceral leishmaniasis. Nanomedicine. 2017;**12**(2):99-115

[70] Chaudhuri G. Scavenger receptor-mediated delivery of antisense mini-exon phosphorothioate oligonucleotide to Leishmania-infected macrophages: Selective and efficient elimination of the parasite. Biochemical Pharmacology. 1997;**53**(3):385-391

[71] Chaubey P, Mishra B. Mannose-conjugated chitosan nanoparticles loaded with rifampicin for the treatment of visceral leishmaniasis. Carbohydrate Polymers. 2014;**101**:1101-1108

[72] Kansal S, Tandon R, Dwivedi P, Misra P, Verma P, Dube A, et al. Development of nanocapsules bearing doxorubicin for macrophage targeting through the phosphatidylserine ligand: A system for intervention in visceral leishmaniasis. Journal of Antimicrobial Chemotherapy. 2012;**67**(11):2650-2660

[73] Khatik R, Dwivedi P, Khare P, Kansal S, Dube A, Mishra PR, et al. Development of targeted 1, 2-diacyl-sn-glycero-3-phospho-l-serine-coated gelatin nanoparticles loaded with amphotericin B for improved in vitro and in vivo effect in leishmaniasis. Expert Opinion on Drug Delivery. 2014;**11**(5):633-646

[74] Tempone AG, Perez D, Rath S, Vilarinho AL, Mortara RA, de Andrade HF Jr. Targeting *Leishmania* (L.) *chagasi* amastigotes through macrophage scavenger receptors: The use of drugs entrapped in liposomes containing phosphatidylserine. Journal of Antimicrobial Chemotherapy. 2004;**54**(1):60-68

[75] Davidson R, Martino LD, Gradoni L, Giacchino R, Russo R, Gaeta G, et al. Liposomal amphotericin B (AmBisome) in Mediterranean visceral leishmaniasis: A multi-centre trial. QJM: An International Journal of Medicine. 1994;**87**(2):75-81

[76] Serrano DR, Lalatsa A, Dea-Ayuela MA, Bilbao-Ramos PE, Garrett NL, Moger J, et al. Oral particle uptake and organ targeting drives the activity of amphotericin B nanoparticles. Molecular Pharmaceutics. 2015;**12**(2):420-431

[77] Bonengel S, Bernkop-Schnürch A. Thiomers—From bench to market. Journal of Controlled Release. 2014;**195**:120-129

[78] Cone RA. Barrier properties of mucus. Advanced Drug Delivery Reviews. 2009;**61**(2):75-85

[79] Lai SK, Wang Y-Y, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Advanced Drug Delivery Reviews. 2009;**61**(2):158-171

[80] Bernkop-Schnürch A, Kast C, Guggi D. Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: Thiomer/GSH systems. Journal of Controlled Release. 2003;**93**(2):95-103

[81] Van Assche T, Deschacht M, da Luz RAI, Maes L, Cos P. Leishmania–macrophage interactions: Insights into the redox biology. Free Radical Biology and Medicine. 2011;**51**(2):337-351

[82] Dai T, Huang Y-Y, Hamblin MR. Photodynamic therapy for localized infections—State of the art. Photodiagnosis and Photodynamic Therapy. 2009;**6**(3):170-188

[83] Foote CS. Definition of type I and type II photosensitized oxidation. Photochemistry and Photobiology. 1991;**54**(5):659

[84] Van der Snoek E, Robinson D, Van Hellemond J, Neumann H. A review of photodynamic therapy in cutaneous leishmaniasis. Journal of the European Academy of Dermatology and Venereology. 2008;**22**(8):918-922

[85] Fang Y-P, Wu P-C, Tsai Y-H, Huang Y-B. Physicochemical and safety evaluation of 5-aminolevulinic acid in novel liposomes as carrier for skin delivery. Journal of Liposome Research. 2008;**18**(1):31-45

[86] Montanari J, Maidana C, Esteva MI, Salomon C, Morilla MJ, Romero EL. Sunlight triggered photodynamic ultradeformable liposomes against *Leishmania braziliensis* are also leishmanicidal in the dark. Journal of Controlled Release. 2010;**147**(3):368-376

[87] Nadhman A, Nazir S, Khan MI, Arooj S, Bakhtiar M, Shahnaz G, et al. PEGylated silver doped zinc oxide nanoparticles as novel photosensitizers for photodynamic therapy against Leishmania. Free Radical Biology and Medicine. 2014;**77**:230-238

[88] Contri RV, Fiel LA, Pohlmann AR, Guterres SS, Beck RC. Transport of substances and nanoparticles across the skin and in vitro models to evaluate skin permeation and/or penetration. In: Nanocosmetics and Nanomedicines. Springer; 2011. pp. 3-35

[89] Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, et al. Nanoparticles and microparticles for skin drug delivery. Advanced Drug Delivery Reviews. 2011;**63**(6):470-491

[90] Ferreira LS, Ramaldes GA, Nunan EA, Ferreira LA. In vitro skin permeation and retention of paromomycin from liposomes for topical treatment of the cutaneous leishmaniasis. Drug Development and Industrial Pharmacy. 2004;**30**(3):289-296

[91] Frankenburg S, Glick D, Klaus S, Barenholz Y. Efficacious topical treatment for murine cutaneous leishmaniasis with ethanolic formulations of amphotericin B. Antimicrobial Agents and Chemotherapy. 1998;**42**(12):3092-3096

[92] Vardy D, Barenholz Y, Naftoliev N, Klaus S, Gilead L, Frankenburg S. Efficacious topical treatment for human cutaneous leishmaniasis with ethanolic lipid amphotericin B. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2001;**95**(2):184-186

[93] Zvulunov A, Cagnano E, Frankenburg S, Barenholz Y, Vardy D. Topical treatment of persistent cutaneous leishmaniasis with ethanolic lipid amphotericin B. The Pediatric Infectious Disease Journal. 2003;**22**(6):567-569

Edited by Farhat Afrin and Hassan Hemeg

Leishmania parasites plague the mammalian host causing high morbidity and mortality. The parasites persist in the hostile milieu, crippling its defensive arsenal. In the face of mounting resistance to an antiquated drug arsenal, new approaches are urgently desired to keep the infection at bay. Furthermore, to strengthen the leishmaniasis elimination drive, particular emphasis has to be laid on identification of new targets and vaccination strategies.

This book gives a brief glimpse of the epidemiology of leishmaniasis, immune evasion, vaccination, and therapeutic modalities that may work by untangling the immunological cross-wires of pathogenic cross-talk. The Conventional treatment and its drawbacks, the prospects of phytotherapy and nanomedicines, are also discussed. The identification of drug targets with the aim of designing inhibitors is also exemplified.

Published in London, UK

IntechOpen

ISBN 978-1-83881-521-9