IntechOpen

# Anticoagulant Drugs

*Edited by Mojca Božič-Mijovski*

# ANTICOAGULANT DRUGS

Edited by **Mojca Božič-Mijovski**

**Anticoagulant Drugs**
http://dx.doi.org/10.5772/intechopen.70971
Edited by Mojca Božič-Mijovski

**Contributors**

Flavia Zacconi, Bhadran Bose, Quoc Tran, Kamal Sud, Bassim Jebeili, Alfredo Vannacci, Niccolò Lombardi, Giada Crescioli, Maja Tomicic, Ana Hecimovic, Irena Jukic, Ana Marisa Chudzinski-Tavassi, Fernanda Faria, Miryam Paola Alvarez Flores, Nina Vene, Alenka Mavri



**Notice**



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

Anticoagulant Drugs
Edited by Mojca Božič-Mijovski

p. cm.

Print ISBN 978-1-78923-622-4

Online ISBN 978-1-78923-623-1

eBook (PDF) ISBN 978-1-83881-498-4

# Meet the editor

Mojca Božič Mijovski is currently the head of Laboratory for Haemostasis and Atherothrombosis at the University Medical Centre Ljubljana, Slovenia, and assistant professor in Haemostasis at the University of Ljubljana, Faculty of Pharmacy. She received her PhD in Biochemistry and Molecular Biology at the Medical Faculty (University of Ljubljana) and a specialist degree in Medical Biochemistry at the Slovenian Ministry of Health. She is a member of the Scientific Board of the European and Mediterranean League against Thrombotic Diseases, a member of the International Society of Thrombosis and Haemostasis and the president of the Commission for Continuous Education at the Slovene Chamber of Laboratory Medicine. Her research in the field of thrombosis and haemostasis has resulted in over 30 articles published in peer-reviewed journals.

# Contents

# Preface

Two types of anticoagulant drugs, heparin and vitamin K antagonists, were widely used during the last century and were among the most frequently prescribed drugs in everyday clinical practice. In the past decades, several new direct oral anticoagulants were developed that changed the anticoagulant therapy landscape considerably. On the one hand, this book provides an extensive overview of all the known anticoagulants, including exotic anticoagulants found in different animal species that can be used for studying different aspects of the haemostatic system or as a starting point for new drug development. On the other hand, it is also a valuable tool for clinicians providing a description of the mode of action and management of therapy for anticoagulant drugs used in everyday clinical practice in different clinical settings, including direct oral anticoagulants dabigatran, rivaroxaban, apixaban and edoxaban. Despite the wide range of anticoagulant drugs available today, the ideal anticoagulant drug that would effectively prevent thrombotic events, but would not expose patients to increased risk of bleeding, is yet to be discovered.

**Mojca Božič Mijovski**
Department of Vascular Diseases
University Medical Centre Ljubljana, Slovenia
Slovenia

Chapter 1

# An Overview of the Anticoagulant Drugs Used in Routine Clinical Practice

Nina Vene and Alenka Mavri

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76206

**Abstract**

Anticoagulant drugs directly or indirectly influence coagulation factors preventing fibrin formation thus preventing blot clotting. They are classified into two groups according to the mode of application, namely parenteral and oral drugs. Among the latter, vitamin K antagonists (most often warfarin) were the only available oral drugs and were widely used for almost a century. In the recent years, new oral anticoagulant drugs became available that directly target either factor IIa or Xa. This chapter provides an overview of both parenteral and oral anticoagulant drugs used in clinical practice with description of the mode of action and management of therapy in different clinical settings.

**Keywords:** anticoagulant drugs, indications, therapy

## 1. Introduction

Anticoagulant drugs directly or indirectly influence coagulation factors and thus inhibit the initiation and progress of coagulation and fibrin-clot formation. They are classified into two groups according to the mode of application, namely parenteral and oral drugs. Among the latter, vitamin K antagonists (most often warfarin) were the only available oral anticoagulants and were widely used for almost a century. In recent years, new oral anticoagulant drugs became available that directly target either factor IIa or Xa [1].

This chapter provides an overview of both parenteral and oral anticoagulant drugs used in clinical practice with a description of the mode of action and management of therapy in different clinical settings.

## 2. Parenteral anticoagulant drugs

### 2.1. Unfractionated heparin

Unfractionated heparin (UFH) binds antithrombin—a physiological inhibitor of coagulation—and accelerates its inhibitory action against coagulation factors II and X and in minor degrees also factors IX, XI and XII [2, 3]. UFH is active in a parenteral form only and therefore administered by intravenous (i.v.) infusion [2]. It is used for the treatment of acute thromboembolic events. One of the major disadvantages of UFH is its binding to plasma proteins and endothelial cells making its anticoagulant effect unpredictable [2, 3]. Treatment with UFH must, therefore, be regularly monitored with activated partial thromboplastin time (APTT). Due to different sensitivities of APTT reagents the therapeutic APTT range must be determined by each laboratory and must correspond to heparin anti-factor Xa activity between 0.3 and 0.7 IU/mL [4–6]. Treatment is initiated with UFH bolus of 80 U/kg i.v. and continued with continuous infusion of 18 U/kg body mass/h [7]. Dosage must be adjusted according to the APTT result. At the beginning of treatment, laboratory monitoring is needed several times a day, the first one 6 h after UFH initiation. The two most important non-hemorrhagic side effects of UFH treatment are osteoporosis and thrombocytopenia [2].

### 2.2. Low-molecular weight heparin

Low-molecular weight heparin (LMWH) is obtained by various methods of fractionation or depolymerization of polymeric UFH [8]. Because LMWHs differ in molecular mass, they also differ in pharmacological characteristics and anticoagulant effects [9]. All LMWHs inhibit coagulation factors II and X. Among the most commonly used LMWHs for treatment and the prevention of acute thromboembolic events are dalteparin (Fragmin®), enoxaparin (Clexane®) and nadroparin (Fraxiparine® and Fraxiparine forte®) in the form of subcutaneous injections. They can also be used as a bridging therapy in patients with high thromboembolic risk during a period when these patients cannot receive oral anticoagulants. Therapeutic dose is determined according to the patient's body weight [2] (**Table 1**).

For prevention of venous thromboembolism (VTE), lower (prophylactic) doses of LMWH are used [2] (**Table 2**). The adequate LMWH dose is selected according to the risk. Prophylactic doses are used in some patients during the interim cessation of oral anticoagulant therapy above all in the first days after large interventions.

The most important advantage of LMWH over UFH is the lower degree of binding to plasma proteins and endothelial cells making their pharmacokinetics and anticoagulant effects predictable [10, 11]. Regular laboratory monitoring with coagulation tests is therefore not needed, except in patients with kidney disease and patients with very low (under 45 kg) or very high (above 120 kg) body weight [2]. Although APTT may be mildly prolonged during LMWH therapy it cannot be used for monitoring. The chromogenic anti-Xa is the test of choice for the determination of plasma LMWH concentration [12]. The LMWH dose should be adjusted to

| | **Therapeutic dose** | |
|---|---|---|
| LMWH | Twice daily | Once daily |
| Dalteparin (Fragmin®) | 100 IU/kg BW/12 h sc | 200 IU/kg BW/24 h sc |
| 46–56 kg | 5.000 IU/12 h sc | 10.000 IU/24 h sc |
| 57–68 kg | 6.000 IU/12 h sc | 12.500 IU/24 h sc |
| 69–82 kg | 7.500 IU/12 h sc | 15.000 IU/24 h sc |
| 82–120 kg | 100 IU/kgBW/12 h sc | 18.000 IU/24 h sc |
| Enoksaparin (Clexane®) | 1 mg/kg BW/12 h sc | 1.5 mg/kg BW/24 h sc |
| 45–54 kg | 50 mg/12 h sc | 80 mg/24 h sc |
| 55–64 kg | 60 mg/12 h sc | 90 mg/24 h sc |
| 65–74 kg | 70 mg/12 h sc | 100 mg/24 h sc |
| 75–84 kg | 80 mg/12 h sc | 120 mg/24 h sc |
| 85–94 kg | 90 mg/12 h sc | 135 mg/24 h sc |
| 94–120 kg | 100 mg/12 h sc | 150 mg/24 h sc |
| Nadroparin | (Fraxiparine®) 0.1 ml/10 kg BW /12 h sc | (Fraxiparine FORTE®) 0.1 ml/10 kg BW/24 h sc |
| 50–59 kg | 0.5 ml/12 h sc | 0.5 ml/24 h sc |
| 60–69 kg | 0.6 ml/12 h sc | 0.6 ml/24 h sc |
| 70–79 kg | 0.7 ml/12 h sc | 0.7 ml/24 h sc |
| 80–89 kg | 0.8 ml/12 h sc | 0.8 ml/24 h sc |
| 90–120 kg | 0.9 ml/12 h sc | 0.9 ml/24 h sc |

IU: International Units, BW: body weight, sc: subcutaneously.

**Table 1.** LMWH therapeutic doses according to body weight.

| **LMWH** | **Low prophylactic dose (moderate VTE risk)** | **High prophylactic dose (high VTE risk)** |
|---|---|---|
| Dalteparin | 2500 IU/24 h sc | 5000 IU/24 h sc |
| Enoxaparin | 20 mg/24 h sc | 40 mg/24 h sc |
| Nadroparin | 0.3 ml/24 h sc | 0.4 ml/24 h sc at BW ≤ 70 kg<br>0.6 ml/24 h sc at BW > 70 kg |

IU: International Units, BW: body weight, sc: subcutaneously, VTE: venous thromboembolism.

**Table 2.** Prophylactic doses of LMWH.

0.5–1.0 IU/mL 4 h after the last LMWH dose when administered twice daily or to 1.0–2.0 IU/mL 5–6 h after the last dose when administered once daily [13, 14]. The two main non-hemorrhagic side effects of LMWH therapy are osteopenia and thrombocytopenia; however, both these side effects are considerably rarer compared to UFH therapy [2].

### 2.3. Fondaparin

Fondaparin (Arixtra®) is a synthetic pentasaccharide that closely resembles the pentasaccharide naturally occurring in the UFH and LMWH. It is an antithrombin-mediated factor Xa inhibitor that is devoid of any anti-factor IIa (thrombin) activity [15]. It is used for treating patients with acute coronary syndrome and heparin-induced thrombocytopenia. It is indicated also for certain patients with thrombophlebitis in a fixed dose of 2.5 mg daily s.c. Laboratory monitoring is not needed; however, if necessary fondaparin levels should only be determined using assays that use known fondaparin concentrations to generate their calibration curve. The use of fondaparin in patients with creatinine clearance below 30 mL/min is contraindicated [2].

### 2.4. Hirudin

Hirudin is a naturally occurring peptide in the salivary glands of medicinal leeches that irreversibly inhibits thrombin. Lepirudin, a recombinant hirudin derived from yeast cells, was used in clinical practice but is no longer available. Instead, the synthetic analog—bivalirudin (Angiox®)—with a short half-life is used at percutaneous coronary interventions and for treating patients with heparin-induced thrombocytopenia. The use of bivalirudin in patients with creatinine clearance below 30 mL/min is contraindicated [16].

### 2.5. Argatroban

Argatroban (Argatra®) is a synthetic reversible direct thrombin inhibitor. It is metabolized solely in the liver and is, therefore, suitable for patients with renal failure. It is used in patients with heparin-induced thrombocytopenia. Treatment with argatroban requires laboratory monitoring with activated partial thromboplastin time (APTT) and the dose adjusted to reach 1.5–3.0 times prolonged baseline APTT, but should not exceed 100 s [17].

## 3. Oral anticoagulants

### 3.1. Vitamin K antagonists

The vitamin K-dependent coagulation factors II, VII, IX and X require γ-carboxylation for their procoagulant activity. Treatment with vitamin K antagonists results in the hepatic production of partially carboxylated and decarboxylated proteins with reduced coagulant activity. Among the most commonly used vitamin K antagonists are warfarin and acenocoumarol. Although vitamin K antagonists are absorbed quickly their full effect develops after about 5 days when the activity of all vitamin K-dependent coagulation factors is reduced [1].

Warfarin therapy requires regular laboratory monitoring with prothrombin time (PT). Due to different sensitivities of thromboplastin reagents used for PT measurement the results are expressed as the International Normalized Ratio (INR). For the majority of indications the target INR range falls between 2.0 and 3.0. In certain patient populations, for example, in patients with mechanical heart valves, the target range is 2.5–3.5 INR. A rare non-hemorrhagic

side effect of vitamin K antagonist therapy is skin necrosis that develops at therapy initiation and is a consequence of acute thrombosis of subcutaneous venules and capillaries [1].

### 3.2. Direct oral anticoagulants

#### *3.2.1. Dabigatran*

Dabigatran etexilate (Pradaxa®) is a low-molecular weight prodrug that exhibits no pharmacological activity. After oral administration, dabigatran etexilate is converted to its active form, dabigatran, a potent, competitive and reversible direct thrombin inhibitor [18]. The binding of dabigatran to thrombin is specific and selective and includes both free and thrombus-bound thrombin. Maximal blood concentration of dabigatran is reached after 1–3 h after the intake [18]. About 35% of the drug is bound to plasma proteins. Eighty percent of dabigatran is excreted through the kidneys [18]. Dabigatran half-life is 14–17 h [18]. It is given in fixed doses of either 150 or 110 mg twice daily in patients with atrial fibrillation and 150 mg twice daily in patients with VTE [19, 20]. Prophylactic doses after total hip or knee replacement are 220 or 150 mg once daily with only half the dose given as the first dose after surgery [21].

The anticoagulant effect of dabigatran is predictive and, therefore, requires no regular laboratory monitoring. During dabigatran therapy, APTT and thrombin time (TT) are prolonged, but these two tests can only offer a rough approximation of dabigatran blood concentration. In certain situations when dabigatran concentration needs to be assessed, a specific test must be used, such as modified thrombin time or a chromogenic assay [22, 23].

#### *3.2.2. Rivaroxaban*

Rivaroxaban (Xarelto®) directly inhibits factor Xa. It selectively binds both free and prothrombin complex bound factor Xa and in this way inhibits thrombin and clot formation. Peak blood concentration is achieved after 1–3 h after drug ingestion. As much as 95% of the drug is bound to plasma protein. One-third of the drug is excreted through kidneys, the other two-thirds are metabolized in the liver. The drug half-life is 8–13 h [1, 24]. Therapeutic doses are 20 and 15 mg once daily for patients with atrial fibrillation [25]. Patients with VTE are treated with 15 mg twice daily for the first 3 weeks, followed by 20 mg once daily [26, 27]. The drug must always be ingested with food. The prophylactic dose for patients with total hip or knee replacement is 10 mg once daily [28, 29].

No laboratory monitoring of therapy is needed due to the predictive effect of the drug. Rivaroxaban prolongs PT; however, when an assessment of the drug blood level is needed, an anti-Xa test calibrated to rivaroxaban should be used [30].

#### *3.2.3. Apixaban*

Apixaban (Eliquis®) directly and reversibly inhibits factor Xa. Maximal blood concentration of the drug is achieved 3–4 h after ingestion. As much as 87% of the drug is bound to blood protein. Twenty-seven percent of the drug is excreted through kidneys and the remainder through the liver. The drug half-life is 12 h [31]. Patients with atrial fibrillation are treated with

5 or 2.5 mg twice daily [32]. Patients with VTE are treated with 10 mg twice daily for the first 7 days followed by 5 mg daily [33]. The prophylactic dose for patients with total hip or knee replacement is 2.5 mg twice daily [34].

No laboratory monitoring of therapy is needed due to the predictive effect of the drug. Apixaban unreliably prolongs APTT and PT. When an assessment of the drug blood level is needed, an anti-Xa test calibrated to apixaban should be used [35].

*3.2.4. Edoxaban*

Edoxaban directly inhibits factor Xa. Maximal blood concentration of the drug is achieved 1–2 h after the ingestion. About 40–59% of the drug is bound to plasma protein. Roughly 35% of the drug is excreted through the kidneys and the remainder through the liver. The drug half-life is 9–14 h. Therapeutic doses are 60 and 30 mg daily for patients with atrial fibrillation and VTE. The prophylactic dose for patients with total hip or knee replacement is 30 mg once daily. Edoxaban prolongs APTT and PT, but for a quantitative assessment of the drug level, an anti-Xa test calibrated to edoxaban must be utilized [36, 37].

## Author details

Nina Vene[1] and Alenka Mavri[1,2]*

*Address all correspondence to: alenka.mavri@kclj.si

1 Department of Vascular Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

2 Division of Internal Medicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

## References

[1] Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012; **141**(2):E44s-E88s

[2] Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;**141**(2):E24s-E43s

[3] Hirsh J, Raschke R, Warkentin TE, Dalen JE, Deykin D, Poller L. Heparin: Mechanism of action, pharmacokinetics, dosing considerations, monitoring, efficacy, and safety. Chest. 1995;**108**(4):258S-275S

[4] Kitchen S, Jennings I, Woods TAL, Preston FE. Wide variability in the sensitivity of APTT reagents for monitoring of heparin dosage. Journal of Clinical Pathology. 1996;**49**(1):10-14

[5] Manzato F, Mengoni A, Grilenzoni A, Lippi G. Evaluation of the activated partial thromboplastin time (APTT) sensitivity to heparin using five commercial reagents: Implications for therapeutic monitoring. Clinical Chemistry and Laboratory Medicine. 1998;**36**(12): 975-980

[6] Toulon P, Boutiere B, Horellou M-H, Trzeciak MC. Monitoring heparin therapy using activated partial thromboplastin time—Results of a multicenter trial establishing the therapeutic range for SILIMAT, a reagent with high sensitivity to heparin. Thrombosis and Haemostasis. 1998;**80**:104-108

[7] Hirsh J, Warkentin TE, Shaughnessy SG, Anand SS, Halperin JL, Raschke R, Granger C, Ohman EM, Dalen JE. Heparin and low-molecular-weight heparin mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. Chest. 2001;**119**(1):64S-94S

[8] Heit JA. Low-molecular-weight heparin: Biochemistry, pharmacology, and concurrent drug precautions. Regional Anesthesia and Pain Medicine. 1998;**23**(6 Suppl 2):135-139

[9] Collignon F, Frydman A, Caplain H, Ozoux ML, Le Roux Y, Bouthier J, Thébault JJ. Comparison of the pharmacokinetic profiles of three low molecular mass heparins—dalteparin, enoxaparin and nadroparin—administered subcutaneously in healthy volunteers (doses for prevention of thromboembolism). Thrombosis and Haemostasis. 1995; **73**(4):630-640

[10] Young E, Wells P, Holloway S, Weitz J, Hirsh J. Ex-vivo and in-vitro evidence that low molecular weight heparins exhibit less binding to plasma proteins than unfractionated heparin. Thrombosis and Haemostasis. 1994;**71**(3):300-304

[11] Boneu B. Low molecular weight heparin therapy: Is monitoring needed? Thrombosis and Haemostasis. 1994;**72**(3):330-334

[12] Douxfils J, Tamigniau A, Chatelain B, Goffinet C, Dogne JM, Mullier F. Measurement of non-VKA oral anticoagulants versus classic ones: The appropriate use of hemostasis assays. Thrombosis Journal. 2014;**12**:24

[13] Bates SM, Weitz JI. Coagulation assays. Circulation. 2005;**112**(4):e53-e60

[14] Peternel P, Terbižan M, Tratar G, Božič M, Horvat D, Salobir B, Stegnar M. Markers of hemostatic system activation during treatment of deep vein thrombosis with subcutaneous unfractionated or low-molecular weight heparin. Thrombosis Research. 2002;**105**:241-245

[15] Bauer KA, Hawkins DW, Peters PC, Petitou M, Herbert JM, van Boeckel CAA, Meuleman DG. Fondaparinux, a synthetic pentasaccharide: The first in a new class of antithrombotic agents—the selective factor Xa inhibitors. Cardiovascular Drug Reviews. 2002;**20**(1):37-52

[16] Hirsh J, Bauer KA, Donati MB, Gould M, Samama MM, Weitz JI. Parenteral anticoagulants: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th edition). Chest. 2008;**133**(6 Suppl):141S-159S

[17] Lewis BE, Wallis DE, Berkowitz SD, Matthai WH, Fareed J, Walenga JM, Bartholomew J, Sham R, Lerner RG, Zeigler ZR, Rustagi PK, Jang IK, Rifkin SD, Moran J, Hursting MJ, Kelton JG, Investigators ARGS. Argatroban anticoagulant therapy in patients with heparin-induced thrombocytopenia. Circulation. 2001;**103**(14):1838-1843

[18] Hankey GJ, Eikelboom JW. Dabigatran etexilate: A new oral thrombin inhibitor. Circulation. 2011;**123**(13):1436-1450

[19] Schulman S, Kearon C, Kakkar AK, Mismetti P, Schellong S, Eriksson H, Baanstra D, Schnee J, Goldhaber SZ, R.-C.S. Group. Dabigatran versus warfarin in the treatment of acute venous thromboembolism. The New England Journal of Medicine. 2009;**361**(24): 2342-2352

[20] Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S, Alings M, Xavier D, Zhu J, Diaz R, Lewis BS, Darius H, Diener HC, Joyner CD, Wallentin L, R.-L.S. Committee, Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. The New England Journal of Medicine. 2009;**361**(12):1139-1151

[21] Eriksson BI, Dahl OE, Rosencher N, Kurth AA, van Dijk CN, Frostick SP, Kalebo P, Christiansen AV, Hantel S, Hettiarachchi R, Schnee J, Buller HR, R.-M.S. Group. Oral dabigatran etexilate vs. subcutaneous enoxaparin for the prevention of venous thromboembolism after total knee replacement: The RE-MODEL randomized trial. Journal of Thrombosis and Haemostasis. 2007;**5**(11):2178-2185

[22] Bozic-Mijovski M, Malmstrom RE, Malovrh P, Antovic JP, Vene N, Sinigoj P, Mavri A. Diluted thrombin time reliably measures low to intermediate plasma dabigatran concentrations. Annals of Clinical Biochemistry. 2015;**53**(4):446-451

[23] Gosselin R, Hawes E, Moll S, Adcock D. Performance of various laboratory assays in the measurement of dabigatran in patients receiving therapeutic doses: A prospective study based on peak and trough plasma levels. American Journal of Clinical Pathology. 2014; **141**(2):262-267

[24] Abdulsattar Y, Bhambri R, Nogid A. Rivaroxaban (Xarelto) for the prevention of thromboembolic disease: An inside look at the oral direct factor Xa inhibitor. P T. 2009;**34**(5): 238-244

[25] Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, Breithardt G, Halperin JL, Hankey GJ, Piccini JP, Becker RC, Nessel CC, Paolini JF, Berkowitz SD, Fox KA, Califf RM, Investigators RA. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. The New England Journal of Medicine. 2011;**365**(10):883-891

[26] Investigators E, Bauersachs R, Berkowitz SD, Brenner B, Buller HR, Decousus H, Gallus AS, Lensing AW, Misselwitz F, Prins MH, Raskob GE, Segers A, Verhamme P, Wells P, Agnelli G, Bounameaux H, Cohen A, Davidson BL, Piovella F, Schellong S. Oral rivaroxaban for symptomatic venous thromboembolism. The New England Journal of Medicine. 2010;**363**(26):2499-2510

[27] Investigators E-P, Buller HR, Prins MH, Lensin AW, Decousus H, Jacobson BF, Minar E, Chlumsky J, Verhamme P, Wells P, Agnelli G, Cohen A, Berkowitz SD, Bounameaux H, Davidson BL, Misselwitz F, Gallus AS, Raskob GE, Schellong S, Segers A. Oral rivaroxaban for the treatment of symptomatic pulmonary embolism. The New England Journal of Medicine. 2012;**366**(14):1287-1297

[28] Eriksson BI, Borris L, Dahl OE, Haas S, Huisman MV, Kakkar AK, Misselwitz F, Kalebo P, Investigators OD-HS. Oral, direct factor Xa inhibition with BAY 59-7939 for the prevention of venous thromboembolism after total hip replacement. Journal of Thrombosis and Haemostasis. 2006;**4**(1):121-128

[29] Turpie AG, Fisher WD, Bauer KA, Kwong LM, Irwin MW, Kalebo P, Misselwitz F, Gent M, OdiXa-Knee Study G. BAY 59-7939: An oral, direct factor Xa inhibitor for the prevention of venous thromboembolism in patients after total knee replacement. A phase II dose-ranging study. Journal of Thrombosis and Haemostasis. 2005;**3**(11):2479-2486

[30] Lindhoff-Last E, Samama MM, Ortel TL, Weitz JI, Spiro TE. Assays for measuring rivaroxaban: Their suitability and limitations. Therapeutic Drug Monitoring. 2010;**32**(6):673-679

[31] Byon W, Sweeney K, Frost C, Boyd RA. Population pharmacokinetics, pharmacodynamics, and exploratory exposure-response analyses of apixaban in subjects treated for venous thromboembolism. CPT: Pharmacometrics & Systems Pharmacology. 2017;**6**(5):340-349

[32] Connolly SJ, Eikelboom J, Joyner C, Diener HC, Hart R, Golitsyn S, Flaker G, Avezum A, Hohnloser SH, Diaz R, Talajic M, Zhu J, Pais P, Budaj A, Parkhomenko A, Jansky P, Commerford P, Tan RS, Sim KH, Lewis BS, Van Mieghem W, Lip GY, Kim JH, Lanas-Zanetti F, Gonzalez-Hermosillo A, Dans AL, Munawar M, O'Donnell M, Lawrence J, Lewis G, Afzal R, Yusuf S, A.S. Committee, Investigators. Apixaban in patients with atrial fibrillation. The New England Journal of Medicine. 2011;**364**(9):806-817

[33] Agnelli G, Buller HR, Cohen A, Curto M, Gallus AS, Johnson M, Masiukiewicz U, Pak R, Thompson J, Raskob GE, Weitz JI, Investigators A. Oral apixaban for the treatment of acute venous thromboembolism. The New England Journal of Medicine. 2013;**369**(9): 799-808

[34] Raskob GE, Gallus AS, Pineo GF, Chen D, Ramirez LM, Wright RT, Lassen MR. Apixaban versus enoxaparin for thromboprophylaxis after hip or knee replacement: Pooled analysis of major venous thromboembolism and bleeding in 8464 patients from the ADVANCE-2 and ADVANCE-3 trials. Journal of Bone and Joint Surgery. British Volume (London). 2012; **94**(2):257-264

[35] Gouin-Thibault I, Flaujac C, Delavenne X, Quenet S, Horellou MH, Laporte S, Siguret V, Lecompte T. Assessment of apixaban plasma levels by laboratory tests: Suitability of three anti-Xa assays. A multicentre French GEHT study. Thrombosis and Haemostasis. 2014; **111**(2):240-248

[36] Giugliano RP, Ruff CT, Braunwald E, Murphy SA, Wiviott SD, Halperin JL, Waldo AL, Ezekowitz MD, Weitz JI, Spinar J, Ruzyllo W, Ruda M, Koretsune Y, Betcher J, Shi M, Grip LT,

Patel SP, Patel I, Hanyok JJ, Mercuri M, Antman EM, Investigators EA-T. Edoxaban versus warfarin in patients with atrial fibrillation. The New England Journal of Medicine. 2013; **369**(22):2093-2104

[37] Ruff CT, Giugliano RP, Braunwald E, Morrow DA, Murphy SA, Kuder JF, Deenadayalu N, Jarolim P, Betcher J, Shi MG, Brown K, Patel I, Mercuri M, Antman EM. Association between edoxaban dose, concentration, anti-factor Xa activity, and outcomes: An analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. Lancet. 2015; **385**(9984):2288-2295

Chapter 2

# FXa Direct Synthetic Inhibitors

Flavia C. Zacconi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76518

**Abstract**

Factor Xa (FXa) is an enzyme belonging to the serine protease family which plays a vital role in hemostasis, being an essential part in the blood-clotting cascade by catalyzing the thrombin and clot production, and wound closure. Moreover, the improvement of new anticoagulants drugs is essential to prevent cardiovascular thrombotic and pathologies. FXa has been a main target for the design of new drugs with important antithrombotic action; nevertheless direct FXa inhibitors that are available still have side effects and drawbacks. This chapter describes the FXa function in the blood-clotting cascade, the molecular and structural characteristics of this essential enzyme, and the novel FXa synthetic drug characteristics. This chapter highlights the importance of continuing the efforts towards searching and designing novel and safer anticoagulant drugs.

**Keywords:** FXa, coagulation cascade, anticoagulants, synthetic inhibitors, direct FXa inhibitors

## 1. Introduction

### 1.1. Primary and secondary hemostasis

Hemostasis is the human body's physiological response to blood vessel injury and subsequent prevention of hemorrhage [1–3]. This significant three-step biological process involves a concerted coordination between blood clotting proteins and platelets with the consequent formation of a clot (repair of a damaged vascular tissue) or thrombus (clot in a healthy blood vessel). According to the cell-based coagulation model (**Figure 1**), this process involves: primary and secondary hemostasis, and fibrinolysis [4].

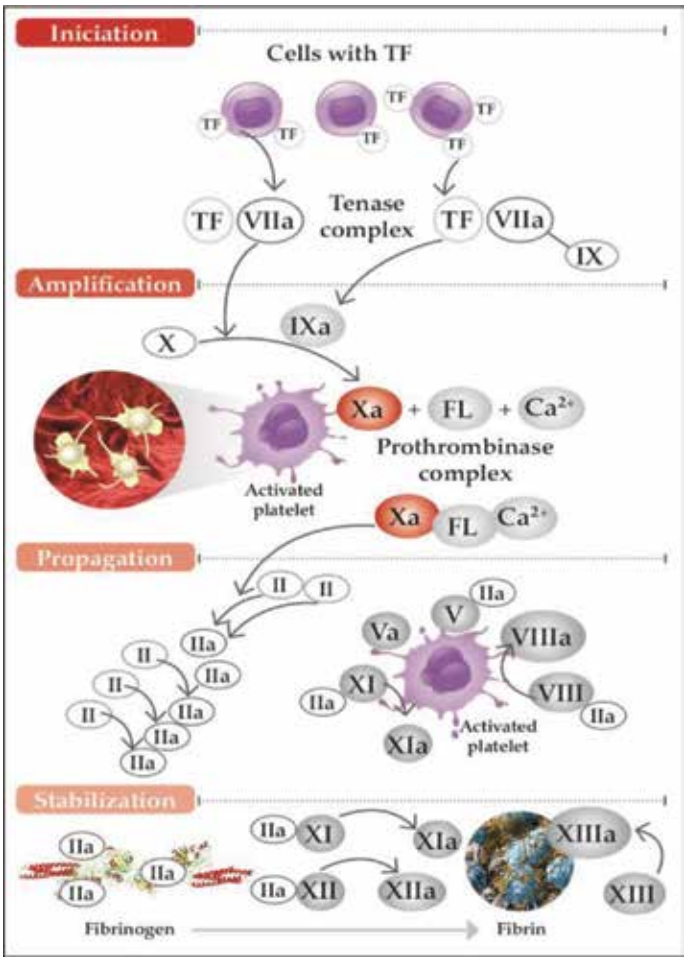


**Figure 1.** Cell-based coagulation model. Figure adapted from Vojacek [8].

*Primary hemostasis* causes local vasoconstriction which diminishes blood flow at the injury site and platelet plug formation. *Secondary hemostasis* implicates a series of enzymatic reactions between coagulation factors and cellular activity. These enzymatic reactions convert fibrinogen to fibrin, an insoluble strand, which together with platelets forms a thrombus.

Lastly, *fibrinolysis* is the biological mechanism which disperses the clot after the blood vessel has healed.

The cell-based model includes the interactions between cells, platelets, and coagulation factors. This model postulates a three-phase process:

- *Initiation*: occurs after vascular injury and leads to the production of a small amount of thrombin. Tissue factor (TF) localized to the cell membrane is activated by non-coagulation and coagulation proteases (blood clotting proteins or factors). The produced FVIIa/TF

complex activates **Factor X to FXa**, and FXa combines with FVa to produce small amounts of thrombin, which subsequently activate platelets during the amplification phase.

- *Amplification*: produces small amounts of thrombin activated platelets increasing platelet adhesion and promoting **FXa and cofactors to catalyze the production of more than 1000 thrombin molecules from each FXa unit.**
- *Propagation*: the protein complexes are assembled on the platelet surface resulting in large-scale thrombin generation. After that, fibrin production starts with the clot formation. Finally, stabilization contributes to the formation of a thrombus, thus producing the world-wide pathology called thrombosis [5–7].

### 1.2. Thrombosis

Over the past few decades, the fact that cardiovascular syndromes are a leading cause of heart problems and rising death rates in the US and Europe has been gradually accepted. With more than 24,000 deaths annually, cerebrovascular accidents (CVA) represent almost a third of all deaths [8–12].


**Figure 2.** Thrombus production on blood vessels.

In 2012, the World Health Assembly (WHA) set a global target to reduce premature deaths from non-infectious disease, including cardiovascular disease, by 25% by 2025. Later, in May 2015, the International Society on Thrombosis and Hemostasis (ISTH) and the World Thrombosis Day (WTD) committee appealed for increased attention to thrombosis in a message to the Assembly of the World Health Organization (WHO) [13].

A thrombus formation, which obstructs arterial circulation, can end in acute myocardial infarction (AMI) or ischemic stroke. In venous circulation, deep vein thrombosis (DVT) can cause chronic leg pain, edema, and ulcers [14–16].

A thrombus can partly or completely block blood vessels, which may deprive tissues of a supply of oxygen and nutrients. An embolus (stroke) is a dislodged thrombus that moves through the bloodstream and obstructs another vessel (**Figure 2**). The thrombus is formed by aggregations of activated platelets, red blood cells, and cross-linked fibrin protein.

Thrombosis is a common causal pathology for three prevalent cardiovascular disorders: stroke, acute coronary syndrome (ACS), and venous thromboembolism (VTE) [17, 18]. Additionally, the latest statistical study from the Global Burden of Diseases, Injuries, and Risk Factors (GBD) shows that 25% of the people around the world die from thrombosis-related events. As an example of this statistic, a recent study carried out in Chile found the incidence risk rate for thromboembolic diseases among patients under general surgery is 55%, and the main cause of death in Chile is cardiovascular disease [9, 10].

Besides, it is important to point out that these diseases have a harsh effect on these people's quality of life and health care costs [19]. Clearly, the high prevalence of thrombosis and its serious implications create an urgent need for safe and reliable prophylaxis and treatment.

## 2. Nowadays antithrombotic therapy

Antithrombotic drugs are used for prevention and treatment of thrombosis. Targeting the thrombi components and the pathology, these agents include *antiplatelet* drugs, *anticoagulants*, and *fibrinolytic* agents [20]. The two first agents act to prevent the thrombus formation during the primary and secondary hemostasis process, while the fibrinolytic agents act when the thrombus is already formed (**Figure 3**) [21].

There is a great variety of commercial drugs for the treatment of antithrombotic pathologies with a wide range of disadvantages and side effects as summarized in **Table 1**.

Various clinical trials have verified the value of standard antiplatelet and anticoagulant agents, which include aspirin (antiplatelet), vitamin K antagonists (VKA) (warfarin), FXa indirect inhibitors (fondaparinux sodium), DTI (argatroban), UFH, LMWH, and TII for wide-ranging prevention and treatment of arterial and venous thromboembolic diseases and cardiovascular pathologies [21–30].

It is evident that, given the prevalence and implications of serious thrombosis, there exists a strong necessity for effective prophylaxis and treatment, and the use of oral anticoagulants is widespread.

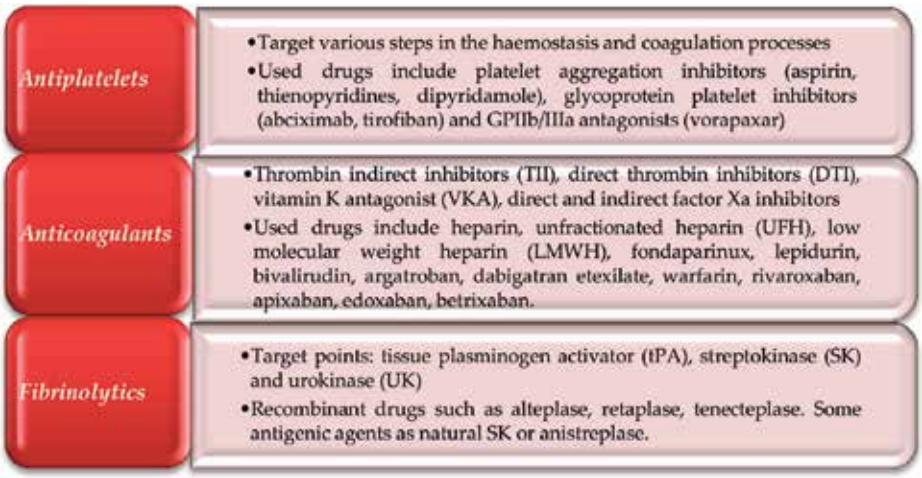


**Figure 3.** Antithrombotic agents' classification, target points, and commercial drugs [20–27].

Several clinical trials have confirmed the efficacy of classic anticoagulants, including vitamin K antagonists (VKA), unfractionated heparin (UFH), and low molecular weight heparins (LMWH weight heparin with reduced activity towards thrombin versus UFH) in prevention and treatment of a wide range of arterial and venous thromboembolic disease prevention [21]. Many approaches have been explored in the development of antithrombotic agents which inhibit enzymes in the coagulation pathways [29, 30, 32].

Unfractionated heparin (UFH) was discovered in 1916 and it targets multiple factors in the coagulation cascade, but it has a number of disadvantages, including a parenteral route of administration, frequent laboratory monitoring of coagulation activity, and the risk for patients to develop mortal heparin-induced thrombocytopenia. Low-molecular-weight heparins (LMWHs), developed in the 1980s, promote the inactivation of both thrombin (factor IIa) and factor Xa. LMWHs have largely replaced UFH due to its lower risk of causing bleeding, lower levels of plasma protein binding, good bioavailability and superior pharmacokinetic properties in comparison with UFH.

However, its use remains limited owing to the need for parenteral administration to patients who will eventually need either to be trained to self-inject or to find assistance from a trained nurse. These anticoagulants all have the limitations mentioned above that restrict their use in the clinic and have created the need for new treatments (**Figure 4**) [33–35].

Warfarin, which was discovered in 1941, is the prototype vitamin K antagonist (VKA, **Figure 5a** and **b**). Its use and other VKAs' uses are especially problematic, albeit these anticoagulants offer the convenience of oral administration. Until recently, the VKAs were the only available oral anticoagulants and the most commonly prescribed. However, VKAs have a number of well-documented drawbacks, including a slow onset and offset of action, unpredictable pharmacokinetics and pharmacodynamics, variability in response to the dosage, and multiple food-drug and drug–drug interactions. Furthermore, regular monitoring of coagulation and dose adjustments are required to maintain patients in the target international normalized

| Antithrombotic agent | Commercial agents disadvantages and side effects |
|---|---|
| Antiplatelet drugs | • *Aspirin:* gastrointestinal complaints, allergy, hepatic and renal pathologies, aspirin resistance |
| | • *Thienopyridines:* gastrointestinal complaints, hematologic side effects |
| | • *Dipyridamole:* gastrointestinal complaints, headache, facial flushing, dizziness, hypotension, caution in patients with coronary artery diseases |
| | • *GPIIb/IIIA receptor antagonists*: bleeding, thrombocytopenia. |
| Anticoagulants | • *Heparin, UFH, and LMWH:* parenteral administration, bleeding, thrombocytopenia, increasing level of bilirubin, osteoporosis. |
| | • *Fondaparinux:* bleeding, there is no antidote |
| | • *Lepirudin, bivalirudin, argatroban:* parenteral administration, serious bleeding, hepatic insufficiency |
| | • *Dabigatran etexilate:* bleeding, renal excretion |
| | • *Warfarin or acenocoumarol:* only 3% of administered warfarin is biologically active due to its binding to albumin, narrow therapeutic window, frequent monitoring, drug-food and drug–drug interactions, interference with the synthesis of vitamin K-dependent clotting proteins (FII, FVII, FIX and FX), bleeding, skin necrosis, and fetal abnormalities. |
| | • *Rivaroxaban:* expensive, renal excretion |
| | • *Apixaban:* expensive, only for major orthopedic surgery, anemia, hemorrhage, nausea |
| | • *Betrixaban:* expensive, anemia, skin rash, drug–drug interactions |
| Fibrinolytics | • *Streptokinase:* expensive, hypotension, rash, fever, chills and rigors, blurred vision, confusion, dizziness, faintness, unusual tiredness or weakness, drug–drug interactions. |
| | • *Urokinase:* bleeding gums, difficulty with breathing or swallowing, headache, increased menstrual flow or vaginal bleeding, nosebleeds, paralysis, prolonged bleeding from cuts |
| | • *Anistreplase, alteplase, tenecteplase, reteplase:* hemorrhage or hematoma formation at the site of venipuncture, gastrointestinal and genitourinary tract hemorrhage, blood in urine |

**Table 1.** Disadvantages and side effects of commercial antithrombotic agents [31].

ratio (INR) range. Monitoring of warfarin therapy is critical due to the variability and relatively narrow therapeutic index, which frequently leads to a higher risk of thromboembolism or excessive anticoagulation with subsequent increased risk of bleeding [36, 37].

Other drugs available for short-term anticoagulation include UFH, LMWHs, fondaparinux (**Figure 5c**) as an indirect FXa inhibitor, and direct thrombin inhibitors (DTIs) such as argatroban, bivalirudin, and hirudin. All these anticoagulants require parenteral administration with their consequent disadvantage. However, LMWH and VKA are the basis for contemporary thromboprophylaxis and treatment in Chile as it is all around the world. The difficulties and inadequacies around the practical and medical aspects of these anticoagulants have encouraged the development of novel drugs that are less expensive for the patient and the health care system [38–41].

Despite the accumulated understanding of the clotting system, its complexity has provided a considerable number of obstacles to the discovery and development of potent anticoagulants that are simultaneously effective and safe.

http://dx.doi.org/10.5772/intechopen.76518

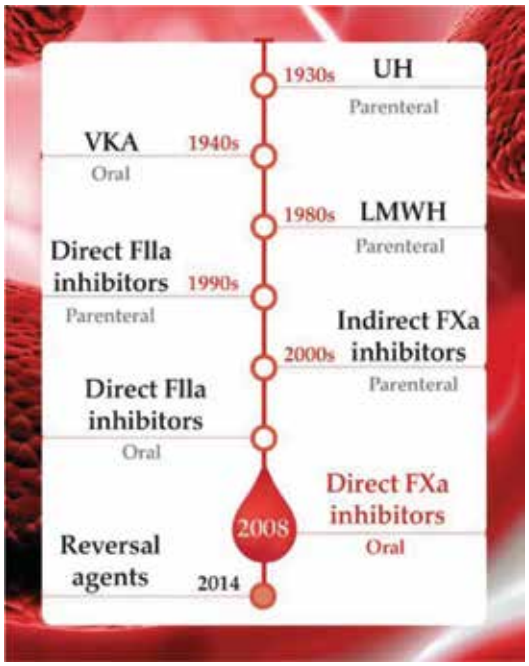


**Figure 4.** Chronological development of anticoagulants.

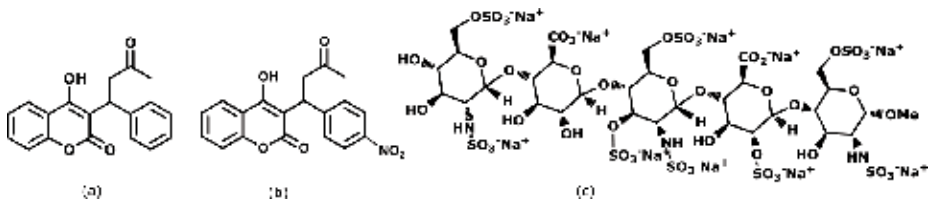

**Figure 5.** (a-b) VKA. (c) Indirect FXa inhibitor.

In recent years, investigation has been focused on novel classes of anticoagulants (small molecules) which target a specific enzyme or coagulation step in the coagulation cascade, including complex inhibitor of factor VIIa-tissue factor, factor IXa inhibitors, and factor XIa, direct thrombin inhibitors, and synthetic direct and indirect inhibitors of Factor Xa (activated Factor X). All of the features mentioned have led to the development of new anticoagulants, including direct FXa inhibitors [42–44].

## 3. Clotting cascade: factor Xa function

Factor X or Stuart-Prower Factor named after the male patient named Stuart in 1957 and a female patient named Prower with FX deficiency [45, 46]. Moreover, Factor X has long been known to have a key role in hemostasis and plays a central part in the blood-clotting cascade by catalyzing the production of thrombin, which leads to clot formation and wound closure [33, 47].

An ideal anticoagulant would prevent thrombosis without inducing systemic hypocoagulation, and would thereby prevent undesired bleeding complications. Thus, a factor Xa inhibitor could potentially have the properties of a desirable anticoagulant. In the search for new drugs, anticoagulant serine protease activated factor Xa is a particularly promising target and has attracted a strong interest in the last 5 years.

FXa plays an important role in first and secondary hemostasis. It produces the core catalyzing reaction that results in thrombin enzyme formation by means of the blood coagulation cascade which results in clot formation and wound closure [33, 47]. Moreover, FXa was found to play a central role in the coagulation process leading to hemostasis in the original extrinsic/intrinsic model [33] as well as in the newly proposed cell-based model. Factor X can be activated through either the intrinsic or extrinsic pathway. Initiation of both pathways activates the inactive precursor FX to FXa. Considering that one molecule of FXa catalyzes the formation of 1000 thrombin molecules, this amplification step can be substantial. Moreover, both pathways lead to the propagation and amplification of coagulation through the activation of FX.

The perfect antithrombotic agent would not induce systemic hypocoagulation and thus provides equilibrium between clot formation and secondary problems such as bleeding. The investigation into finding new anticoagulant agents reveals that serine protease FXa is an important validated pharmaceutical achievement whose use has grown remarkably since the beginning of the twenty-first century [42–44]. Thus, an FXa inhibitor combined with an antiplatelet moiety could possibly provide the features of an effective drug, thus preventing the platelet aggregation during the hemostasis process, avoiding the thrombus formation and inhibiting the catalyzing FXa reaction [33, 48–53].

As explained above, FXa performs a crucial function in the coagulation process. Thus, FXa provides a specific target for novel anticoagulant agents. The synthesis of direct FXa inhibitors that are able to effectively inhibit prothrombinase-associated and clot-bound FXa, and therefore provide greater potential anticoagulant activity, is therefore a significantly important advance. There is enough evidence to imply that inhibition earlier during primary hemostasis in the coagulation cascade at the FXa level could provide higher antithrombotic potential by using inhibition of platelet adhesion drugs. Furthermore, preclinical studies indicate that FXa inhibitors possibly possess a broader therapeutic index. Therefore, there is a significant number of pharmaceutical companies, which are working to discover new anticoagulant drugs, and have finally decided to focus on small molecules such as direct FXa inhibitors [54–59].

FXa is a serine protease which catalyzes the production of 1000 thrombin molecules involving the interaction on the platelets surface, $Ca^{2+}$ ions, and FVa called the prothrombinase complex. The prothrombinase complex acts on the natural substrate producing the catalytic coagulation process.

Structurally FXa, like trypsin, belongs among the family of serine proteases within the catalytic domain, which is formed by two antiparallel β-barrel folds that act in tandem to produce the catalytic triad and the substrate binding site. Schechter and Berger (**Figure 6**) have provided a nomenclature adopted by scientists, which describes the prototypical binding site of a serine protease. Consequently, each protein subsite, labeled Si, binds its related amino acid substrate, labeled Pi [60].

As the discovery of small-molecule protease inhibitors has progressed, this convention has been amplified to denote drug substructures that similarly bind to substrate amino acids (**Figure 6**) [54].

In the 1980s, early attempts to identify FXa inhibitors were prompted by prior thrombin inhibitor discoveries such as the compounds illustrated in **Figure 7** (**a** and **b**), which are examples of early [61] and most recent direct FXa inhibitors rivaroxaban (**Figure 7c**) and apixaban (**Figure 7d**) chemical structures.

Development of rivaroxaban was a major breakthrough in anticoagulation drug discovery and was the first approved orally active direct FXa inhibitor. However, recently studies have shown that rivaroxaban and apixaban discontinuation could result in thromboembolic events, and the use of rivaroxaban associated with warfarin increases the risk of major bleeding in non-valvular atrial fibrillation patients [62–64]. Through the study of the chemical structures of these inhibitors illustrated below, it also became evident that various substitutes could be accepted in both the S1 and S4 regions [45, 65–73].

In spite of extensive knowledge about the clotting mechanism, its complexity poses a considerable challenge to the research and development of powerful anticoagulants that are both safe and effective.

### 3.1. FXa structural target points

As it was exposed before, FXa plays a critical role in coagulation. Together with FVa and calcium ions on a phospholipid surface, FXa forms the prothrombinase complex, which is responsible for the conversion of prothrombin to thrombin, the final effector of coagulation (**Figure 1**).

Oral anticoagulant drug discovery efforts initially focused on the development of small-molecule anticoagulants that target thrombin directly—the oral DTIs. But, there is some evidence to suggest that inhibition earlier in the coagulation cascade at the level of FXa may have greater antithrombotic potential. In addition preclinical studies suggest that FXa inhibitors may possess a wider therapeutic index than DTIs. Thus, it is understandable that a great number of pharmaceutical companies dedicated to the discovery of this oral anticoagulant drug have finally and determinedly concentrated on small-molecule, direct FXa inhibitors [33, 56, 57, 74–78].

It is worth considering briefly some of the significant molecular characteristics of the target protein. FXa belongs to the family of serine proteases such as trypsin; the catalytic domain consists of two antiparallel β-barrel folds that together form the catalytic triad and the substrate binding site. Accordingly to the Schetcher and Berger nomenclature each protein subsite (Si) binds the amino acid (Pi) residue [79]. Specifically, FXa is composed by four principal subsites S1, S2, S3, and S4.

S1 is an anionic pocket—hydrophobic and deep cleft—formed by Tyr228, Ser195, and Asp189; and S4 subsite has three domains to link with the ligand: one hydrophobic pocket defined by Tyr99, Trp215, and Phe174, one cationic hole formed by Glu97 and Lys96, and a water pocket where the natural substrate is trapped under the following amino acids: Thr98, Ile175, and

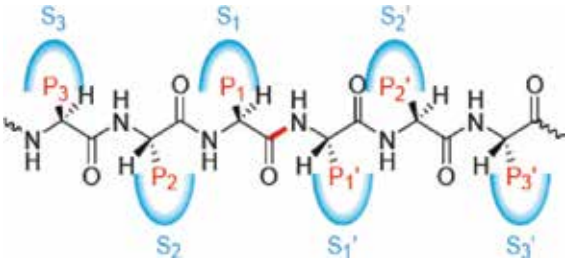


**Figure 6.** Nomenclature based on the Schechter and Berger convention. Figure adapted from Berg et al [67].

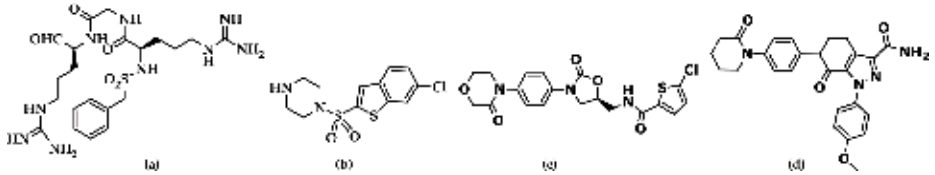

**Figure 7.** Early and more recent direct FXa inhibitors.

Tyr175. Besides, the S2 subsite is not well defined, has a slightly profound pocket, and is fused with the S4 subsite (**Figure 8**). Finally, the S3 pocket is exposed to the solvent, and it is situated at the borderline of the S1 subsite region [78, 80].

All reported data indicates that small-molecule serine protease inhibitors bind one or more of the subsites. **Figure 5** shows the most important serine protease subsites responsible for the design molecules recognition and binding characteristics.

In view of the small size of these synthetic inhibitors, they allow inhibiting both bound prothrombinase and free FXa. In addition, these drugs are able to penetrate the blood clot and inhibit FXa [61, 81, 82].

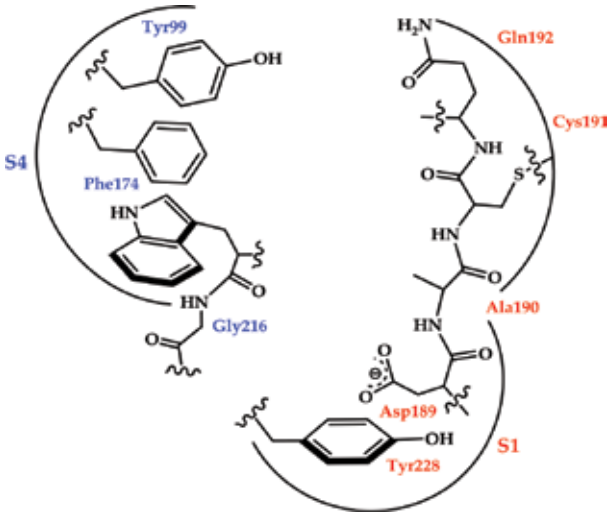


**Figure 8.** Most important amino acid residues in S1 and S4 pockets.

http://dx.doi.org/10.5772/intechopen.76518

## 4. Direct FXa inhibitors development

In spite of the fact that as early as the beginnings of the 1980s, the factor Xa had already been recognized as an auspicious target for the development of new anticoagulants, its viability inhibition was not tested until the late 1980s. It was in 1987 that the first factor Xa inhibitor—naturally occurring compound antistasin—was extracted from the salivary glands of the Mexican leech *Haementeria officinalis*. Antistasin is a 119 amino-acid polypeptide; kinetic studies revealed that it is a slow, tight-binding, potent factor Xa inhibitor [83–85]. Similar properties show another factor Xa inhibitor—the tick anticoagulant peptide (tAP) [86]. This anticoagulant peptide is a single-chain, amino-acid peptide which was isolated in 1990 from extracts of the soft tick *Ornithodoros moubata*. The antithrombotic effects of these compounds were compared with those of direct thrombin inhibitors, and of indirect thrombin and factor Xa inhibitors in animal models of thrombosis.

The discovery of FXa's role in the clotting cascade produced an increasing interest in this enzyme due to its pharmacological target for the treatment of a diverse number of hemostatic pathologies.

The first FXa inhibitory studies were carried by using natural anticoagulants obtained from ticks (antistatin), leeches (Yagin), and bats (Draculin). These natural anticoagulant proteins had indirect activity on FXa. Antistatin produce a slow-release FXa-complex which reduces the cascade amplification. Besides, Draculin directly inhibits FXa without activity on thrombin [87].

Several investigation groups started designing and synthesizing novel small molecules for the treatment of thrombotic-related pathologies such as deep vein thrombus (DVT), acute coronary syndrome (ACS), stroke, as well as for the prevention of clot production during surgeries. Moreover, pharmaceuticals companies have been financing strongly in the research and development of new synthetic oral FXa inhibitors.

For example, fondaparinux (**Figure 5c**), is selective for FXa but acts indirectly via binding to antithrombin and has demonstrated similar clinical benefit over LMWHs in venous thrombotic indications. The safety and efficacy of one provided the first clinical proof of the principle that targeting FXa would be an important advancement in the area of anticoagulation therapy [79, 88–91].

A second generation of synthetic derivatives, idrabiotaparinux, is in late-stage clinical trials for treatment of VTE and for stroke prevention in patients with AF [92]. Early efforts to identify inhibitors of FXa stemmed from the prior discoveries of thrombin inhibitors such as compounds showing in **Figure 6** are examples of early FXa inhibitors. Because of the success of indirect dual factor Xa and thrombin inhibitors, such as LMWHs, indirect inhibitors of factor Xa with greater selectivity, such as fondaparinux, were developed in parallel with oral direct factor Xa inhibitors, such as rivaroxaban [93] (**Figure 7c**) and apixaban [94] (**Figure 7d**).

These last two small molecules have been demonstrated to have the best pharmacokinetics characteristics, and they have a fully complete preclinical characterization as an oral direct FXa inhibitor. Furthermore, rivaroxaban was approved in Canada, Europe, and other countries for the prevention of VTE in adults undergoing hip and knee surgery, and it has a predictable anticoagulant response avoiding the need for monitoring. Moreover, apixaban was

approved by the Food and Drug Administration (FDA) in December 2012 with an indication of reducing the risk of stroke and dangerous blood clots (systemic embolism) in patients with atrial fibrillation (AF) [95–98].

Based on these discoveries, in the mid-1990s, it was assumed that small-molecule, direct factor Xa inhibitors could most likely become a better option than the antithrombotic therapies used in those days [99].

### 4.1. Direct synthetic FXa inhibitors

Indirect FXa inhibitor development led to the advance of direct oral FXa inhibitors, such as rivaroxaban [100] (**Figure 7c**) and apixaban [98] (**Figure 7d**). Interestingly, the "Xa" suffix comes from FXa and "ban" indicating inhibition [101].

These latest FXa direct oral inhibitors comprised a group of small molecules. Taking into account the chronological order, these new direct oral anticoagulants (DOACs) are razaxaban, rivaroxaban, apixaban, darexaban, edoxaban, and betrixaban; however, some of them did not obtain the approval during clinical trials and they are not commercialized [48].

#### *4.1.1. Razaxaban*

This novel FXa synthetic and orally active compound was developed by Bristol Myers Squibb in 2004. The production of razaxaban was carried out through a seven synthetic pathway (**Figure 9**) [56]. It acts as a selective and reversible direct FXa inhibitor which was the first synthetic direct FXa inhibitor developed. Moreover, razaxaban has demonstrated FXa selectivity in venous thrombosis in human beings and arterial thrombosis prevention in animal models [94].

The razaxaban structural L shape allowed it to fit within the FXa S1 pocket where the nitrogen atom of the benzoisoxazole moiety interacts with Ala190 and Asp189 (**Figure 10**) [48, 100]. Moreover, the development was discontinued in Phase II at the end of 2004.

#### *4.1.2. Rivaroxaban*

Rivaroxaban (Xarelto®), was the first direct oral FXa inhibitor developed by Bayer Schering Pharma AG and it obtained the clinical approved in 2008 [102]. At the beginning, the production of rivaroxaban was carried out through a nine synthetic pathway [68]. In recent years, a

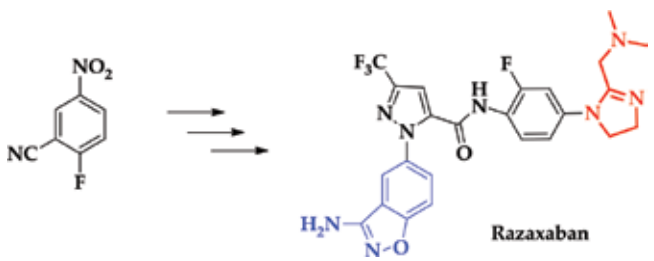


**Figure 9.** Chemical structures: Commercial starting material and Razaxaban. The moiety that interacts with S1, is shown in blue, and the portion involved in the S4 interaction is shown in red [50].

http://dx.doi.org/10.5772/intechopen.76518

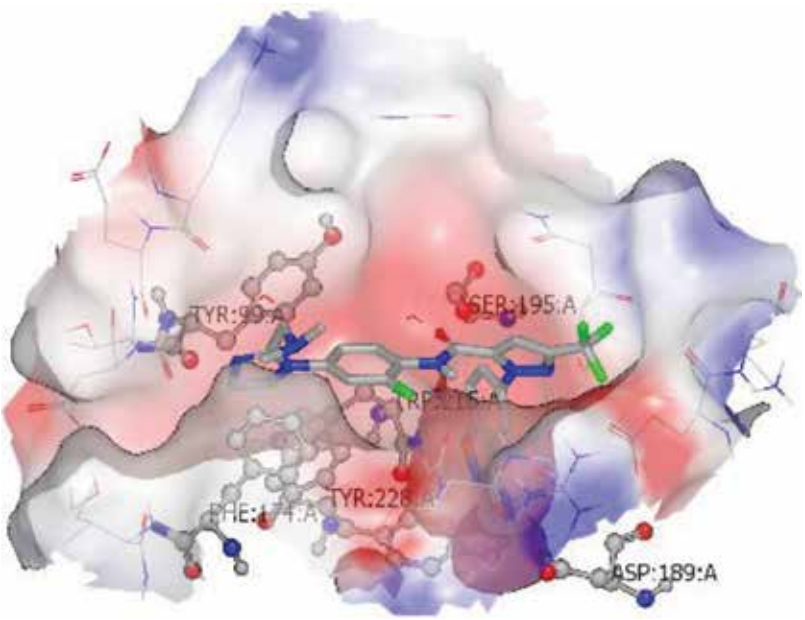


**Figure 10.** Razaxaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

novel synthetic route was design by using only a seven-step procedure diminishing the environmental impact and increasing the reaction yields (**Figure 11**) [103]. This novel DOAC is a selective and reversible FXa inhibitor which shows 100-fold greater selectivity for FXa over any other serine protease. Rivaroxaban inhibits the complex between FXa and prothrombinase with an $IC_{50}$ 2.1 nM; moreover, it shows nanomolar inhibitory constant [Ki = 0.4 nM] [33, 93, 104].

As it is shown in **Figure 12**, the two-ringed moiety, including the morpholinone and bencenic moieties, produced S4 hydrophobic interactions with Phe174 and Tyr99 residues. Moreover, the oxazolidone ring interacts with Gly219 through hydrogen bonds and the chlorothiophene moiety produces necessary interactions with Asp189, Ala190 and Tyr228 in the profound S1 site (**Figure 12**) [33, 68, 82].

This first commercially available DOAC doesn't show food interactions and it is prescribed as one dose-per-day drug after heart attack or stroke [105, 106]. Furthermore, rivaroxaban was approved for prophylaxis after knee or hip surgery [96].

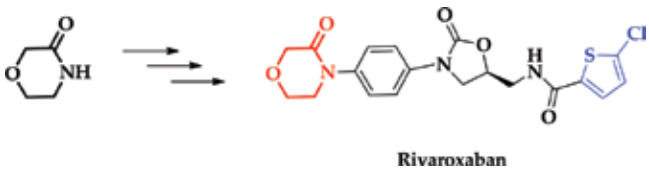


**Figure 11.** Chemical structures: Commercial starting material and rivaroxaban. The moiety that interacts with S1 is shown in blue and the portion involved in the S4 interaction is shown in red [594].

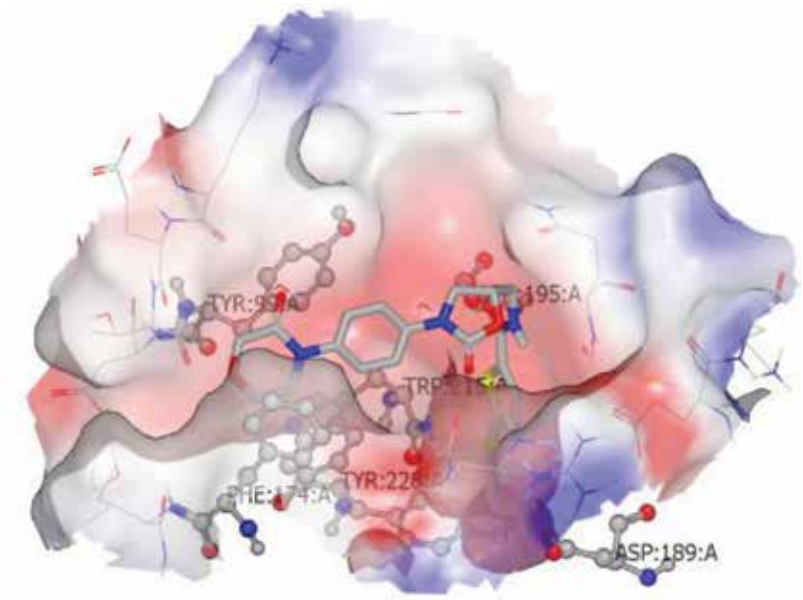


**Figure 12.** Rivaroxaban bound to FXa (PDB ID 2w26) [68]. The binding site is shown in surface mode. Specificity sites and important residues are labeled.

*4.1.3. Apixaban*

Apixaban (Eliquis®) was the second FXa oral inhibitor approved by the European Medicines Agency (EMA) in 2011 and by the Food and Drug Administration (FDA) in 2012 [98]. This novel molecule is a design evolution of razaxaban, and it was developed by Bristol Myers Squibb [53]. Moreover, apixaban is a reversible and selective FXa inhibitor which is prescribed in thromboembolic prophylaxis events such as preventing thrombus production and strokes in persons with atrial fibrillation. Furthermore, apixaban is prescribed to prevent blood clots in deep vein thrombosis (DVT) and pulmonary embolus formation according to the United States regulations [52, 107, 108].

Currently, apixaban has FXa inhibitory activity showing a Ki = 0.08 nM with 50% oral bioavailability [104, 107]. In addition, its action showed selectivity for clot-bound ($IC_{50}$ 1.3 nM) vs. free FXa ($IC_{50}$ 7.6 nM) [107]. Besides, this DOAC induces hepatotoxicity as its adverse effect (**Figure 13**) [109].

The characteristic FXa inhibitors L shape is produced by the peptide bond present between the two ring pyrazole linked to a phenyl piperidinone (**Figure 14**). Apixaban shows the same interactions than Rivaroxaban in the S1 pocket by using the methoxyphenyl portion at the bottom of the S1 pocket [100].

*4.1.4. Darexaban*

Darexaban was designed by Astellas Pharma in 2007 for venous and arterial thromboembolic disease prophylaxis such as venous thrombosis, myocardial infarction, and ischemic stroke [110].

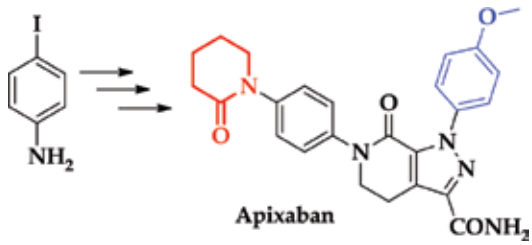


**Figure 13.** Chemical structures: Commercial starting material and apixaban. The moiety that interacts with S1 is shown in blue, and the portion involved in the S4 interaction is shown in red [53].

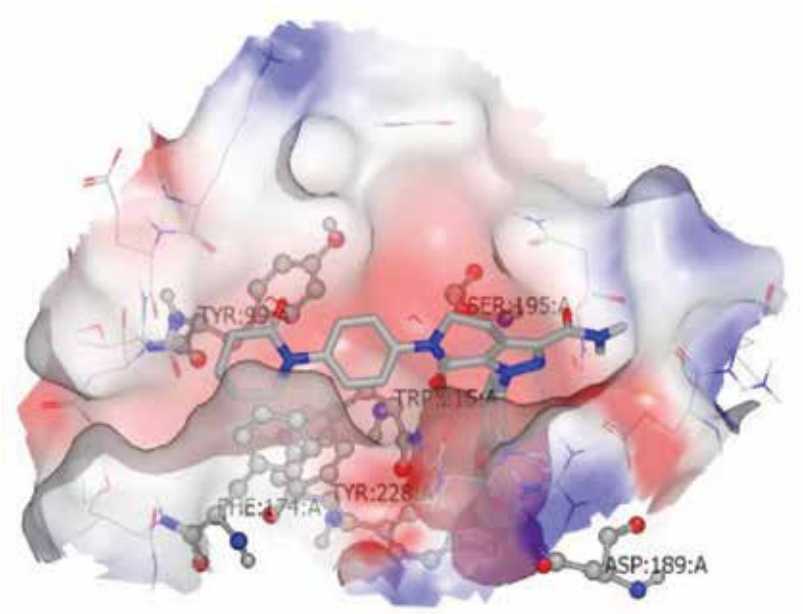


**Figure 14.** Apixaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

It showed an inhibition constant Ki 0.031 μM and $IC_{50}$ 40 nM for free FXa and $IC_{50}$ 80 nM for blood clots [111]. However, darexaban development was discontinued in September 2011, after a phase II in Australia, Canada, and the European Union (EU) because the clinical trial showed that the combination of darexaban with an antiplatelet agent such as acetyl salicylic acid (ASA) caused a fourfold increase in bleeding rates and had no effect on acute coronary syndrome (ACS) (**Figure 15**) [112].

Darexaban establishes the same interactions with Asp189, Ala190, and Tyr228 in the S1 pocket as other DOACs (**Figure 16**) [100].

*4.1.5. Edoxaban*

Edoxaban (Savaysa® in USA and Lixiana ® in Canada and outside the USA) was developed by Daiichi Sankyo and it was approved in Japan (2011) and by the FDA (2015) [71, 113]. This

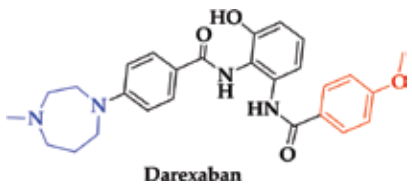


**Figure 15.** Darexaban chemical structure. The moiety that interacts with S1 is shown in blue, and the portion involved in the S4 interaction is shown in red.

novel DOAC is used for stroke and VTE prophylaxis in patients with atrial fibrillation [114]. Edoxaban was synthesized through a twelve-step procedure (**Figure 17**) [113].

Edoxaban bioavailability is 62%, and it is prescribed at 15–150 mg daily. It has been shown a nanomolar value for its Ki (0.56 nM) and $IC_{50}$ (3 nM) [115–117]. Currently, Daiichi Sankyo is developing a phase III trial for cardiovascular disorders during February 2018.

This DOAC interacts with the same amino acidic residues in the S1 serine enzyme pocket as the other FXa direct inhibitors (**Figure 18**). The chloropyridine moiety is responsible for the S1 pocket interaction meanwhile the tetrahydrothiazolo-pyridine moiety interacts with the S4 pocket [100].

*4.1.6. Betrixaban*

Betrixaban (Bevyxxa®) is the newest DOAC developed by Portola Pharmaceuticals and it was designed through structure activity relationship (SAR) studies [80, 118]. It was approved by the FDA in June 2017 for prevention of venous thromboembolism in acute hospitalized medical adult patients by using an initial single dose of 80 mg (**Figure 19**) [119].

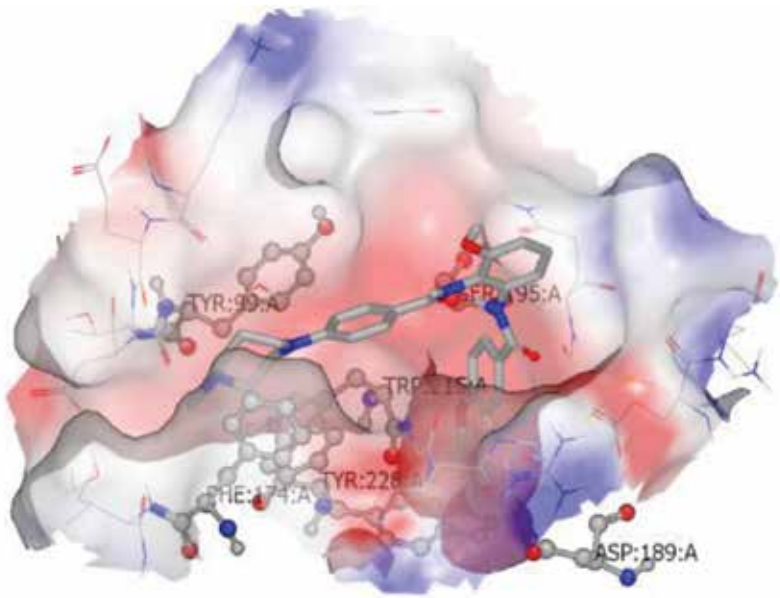


**Figure 16.** Darexaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

http://dx.doi.org/10.5772/intechopen.76518

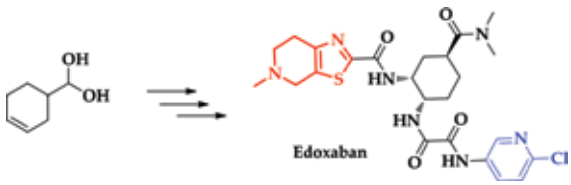


**Figure 17.** Chemical structures: commercial starting material and Edoxaban. The moiety that interacts with S1 is shown in blue, and the portion involved in the S4 interaction is shown in red [113].

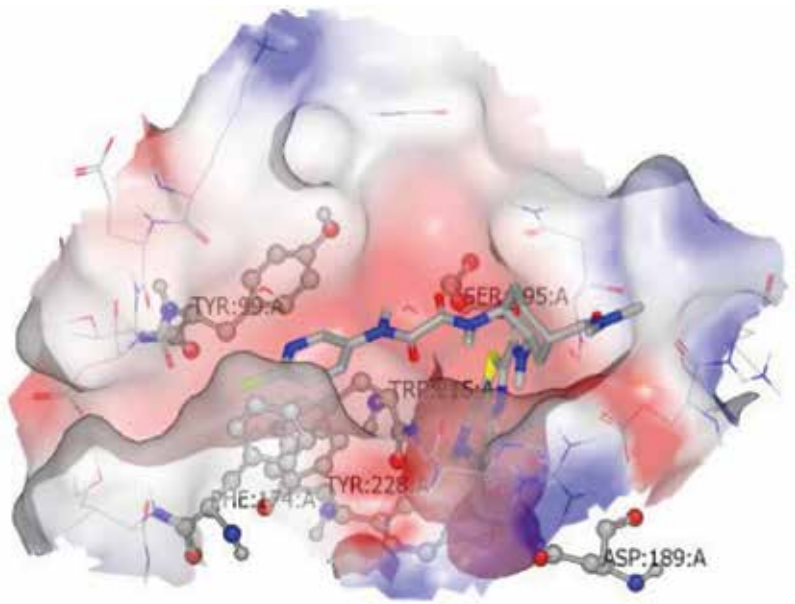


**Figure 18.** Edoxaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

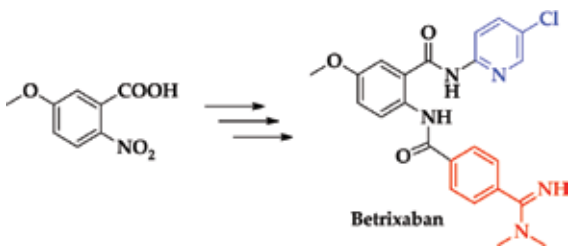


**Figure 19.** Chemical structures: commercial starting material and Betrixaban. The moiety that interacts with S1 is shown in blue, and the portion involved in the S4 interaction is shown in red.

This new DOAC is a competitive and reversible FXa inhibitor and it has a Ki 0.117 pM and $IC_{50}$ 1.5 nM [119]. Betrixaban may therefore have several potential advantages over the other FXa inhibitors (**Figure 20**).

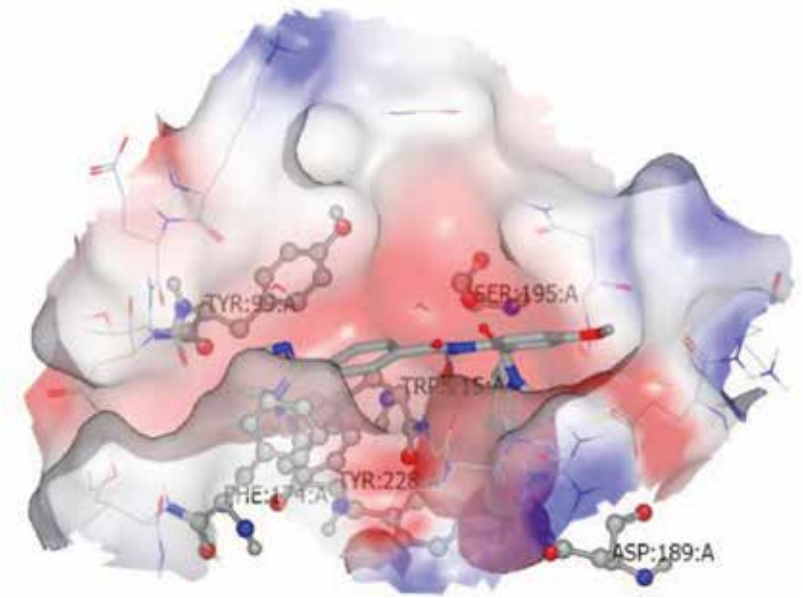


**Figure 20.** Betrixaban bound to FXa (PDB ID 2w26). The binding site is shown in surface mode. Important residues are labeled.

## 5. Conclusions

A perfect anticoagulant would prevent thrombosis without inducing systemic hypocoagulation, and would prevent undesired bleeding complications. Thus, an FXa inhibitor could potentially have the properties of a desirable anticoagulant. In the search for new anticoagulant drugs, the serine protease FXa is a particularly promising target and has attracted a strong interest over the last 15 years.

Development of DOACs such as direct FXa inhibitors is an important innovation in the anticoagulation drug discovery field. In spite of widespread knowledge about the clotting mechanism, its complexity generates significant challenge for the investigation and production of innovative anticoagulants that are both efficient and safe. DOACs availability embraces a vast field of the clinical health system for prevention of diverse pathologies. Currently, with the development and approval of DOACs such as FXa inhibitors, clinical health professionals can use these novel therapeutics approaches.

The continuous and future development of an innovative oral anticoagulant drug that is designed to prevent several thrombotic disorders and related pathologies would be of remarkable wide-reaching health value.

## Acknowledgements

The author would like to thanks Centro de Investigación en Nanotecnología y Materiales Avanzados, CIEN-UC, Pontificia Universidad Católica de Chile and the Institute for Biological

and Medical Engineering. This work was financially supported to F.C.Z. by the CONICYT/FONDECYT Fondecyt Iniciación N° 11130595 and Fondecyt Regular N° 1181408 project.

## Conflict of interest

The author declares no conflict of interest.

## Thanks

The author would like to express a sincere gratitude to Nicolás E. Núñez-Navarro for his valuable assistance with the bibliographical search and to Fabián M. Santana for the images improvement.

## Author details

Flavia C. Zacconi

Address all correspondence to: fzacconi@uc.cl

Pontificia Universidad Católica de Chile, Santiago de Chile, Chile

## References

[1] Austin S. Haemostasis. Medicine. 2013;**41**:208-211

[2] Pérez-Gómez F, Bover R. La nueva cascada de la coagulación y su posible influencia en el difícil equilibrio entre trombosis y hemorragia. Revista Española de Cardiología 2007;**60**:1217-1219

[3] Riddel J, Aouizerat B, Miaskowski C, Lillicrap D. Theories of blood coagulation. Journal of Pediatric Oncology Nursing. 2007;**24**:123-131

[4] Smith S. The cell-based model of coagulation. Journal of Veterinary Emergency and Critical Care. 2009;**19**:3-10

[5] Hoffman M, Monroe D III. A cell-based model of hemostasis. Thrombosis and Haemostasis. 2001;**85**:958-965

[6] Hoffman M, Monroe D. Rethinking the coagulation cascade. Current Hematology Reports. 2005;**4**:391-396

[7] Palta S, Saroa R. Overview of the coagulation system. Indian Journal of Anaesthesia. 2014;**58**:515-523

[8] Vojacek J. Should we replace the terms intrinsic and extrinsic coagulation pathways with tissue factor pathway? Clinical and Applied Thrombosis/Hemostasis. 2016;**1**:1-6

[9] Principales causas de muerte en Chile por regiones 1997-2003. Instituto Nacional de Estadísticas; 2006

[10] Feigin V, Lawes C, Bennett D, Anderson C. Stroke epidemiology: a review of population based studies of incidence, prevalence and case fatality in the late 20th century. The Lancet Neurology. 2003;**2**:43-53

[11] Estrategia Nacional de Salud. 2011-2020. Ministerio de Salud. Gobierno de Chile

[12] Protocolo prevención enfermedad tromboembólica en pacientes quirúrgicos. Dirección Servicio de Salud Metropolitano Oriente. Ministerio de Salud de Chile; 2010

[13] Raskob G, Angchaisuksiri P, Blanco A, Buller H, Gallus A, Hunt B, Hylek E, Kakkar A, Konstantinides S, McCumber M, Ozaki Y, Wendelboe A, Weitz J. A major contributor to global disease burden. ISTH steering Committee for World Thrombosis day. Journal of Thrombosis and Haemostasis. 2014;**12**:1580-1590

[14] Gustafsson D, Bylund R, Antonsson T, Nilsson I, Nyström J-E, Eriksson U, Bredberg U, Teger-Nilsson A-C. A new oral anticoagulant: the 50-year challenge. Nature Reviews Drug Discovery. 2004;**3**:549-659

[15] Wolf P, Abbott R, Kannel W. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. Stroke. 1991;**22**:983-988

[16] Flisfisch H, Aguiló J, Lillo Cuevas D. Trombosis venosa profunda. Revista Medicina y Humanidades. 2014;**VI**:46-50

[17] Bombin F, Kotlik A, Díaz G, Vera O, Contreras T, Vásquez Z. Secuelas de la trombosis venosa profunda de las extremidades inferiores luego de un tratamiento anticoagulante controlado. Revista Chilena de Cirugía. 2005;**57**:311-319

[18] Palomo G, Pereira G, Alarcón L, Pinochet P, Vélez S, Hidalgo P, Skagerberg K, Poblete CF. Factor V Leiden y mutación de la protrombina G20210A en pacientes con trombosis venosa y arterial. Revista Médica De Chile. 2005;**133**:1425-1433

[19] Wolf P, Mitchell J, Baker C, Kannel W, D'Agostino R. Impact of atrial fibrillation on mortality, stroke, and medical costs. Archives of Internal Medicine. 1998;**158**:229-234

[20] Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Harrison's Principles of Internal Medicine, 1 and 2. 18th ed. New York: McGraw-Hill Professional; 2011

[21] Abraham D, editor. Burger's Medicinal Chemistry and Drug Discovery. Cardiovascular Agents and Endocrines. 6th ed. Vol. Vol. 3. New Jersey: Wiley–Interscience; 2003

[22] Lee M, Smith S, Galor A, Hoffman G. Antiplatelet and anticoagulant therapy in patients with giant cell arteritis. Arthritis and Rheumatism. 2006;**54**:3306-3309

[23] Rao P, Burkart T. Advances in oral anticoagulation therapy – What's in the pipeline? Blood Reviews. 2017;**31**:205-211

[24] Oh J, Smiddy W, Kim S. Antiplatelet and anticoagulation therapy in vitroretinal surgery. The American Journal of Ophthalmology. 2011;**151**:934-939

[25] Cohen A, Imfeld S, Markham J, Granziera S. The use of aspirin for primary and secondary prevention in venous thromboembolism and other cardiovascular disorders Thrombosis Research. 2015;**135**:217-225

[26] Koenig-Oberhuber V, Filipovic M. New antiplatelet drugs and new oral anticoagulants. British Journal of Anaesthesia. 2016;**117**:ii74-ii84

[27] Aizman A, Abbott E, Rojas L. Profilaxis de enfermedad tromboembólica en pacientes hospitalizados con patología médica, estrechando la brecha entre las guías y la práctica clínica. Revista Médica de Chile. 2011;**139**:1210-1217

[28] Sobieraj-Teague M, O'Donnell M, Eikelboom J. New anticoagulants for atrial fibrillation. Seminars in Thrombosis and Hemostasis. 2009;**35**:515-524

[29] Gómez-Outes A, Suárez-Gea M, Lecumberri R, Rocha E, Pozo-Hernández C, Vargas-Castrillón E. Nuevos anticoagulantes parenterales en desarrollo clínico. Actualidad en Farmacología y Terapéutica. 2011;**9**:167-181

[30] Weits JI. Emerging anticoagulants for the treatment of venous thromboembolism. Thrombosis and Haemostasis. 2006;**96**:274-284

[31] Side effects of commercial antithrombotic agents. https://www.drugs.com/sfx/ [Accessed: July 12, 2017]

[32] Dimitropoulos G, Rahim S, Moss A, Lip G. New anticoagulants for venous thromboembolism and atrial fibrillation: what the future holds. 2018;**27**:71-86

[33] Perzborn E, Roehrig S, Straub A, Kubitza D, Misselwitz F. The discovery and development or rivaroxaban, an oral, direct factor Xa inhibitor. Nature Reviews Drug Discovery. 2011;**10**:61-75

[34] Eriksson BI, Dahl OE, Lassen MR, Ward DP, Rothlein R, Davis G, Turpie AGG. Partial factor IXa inhibition with TTP889 for prevention of venous thromboembolism: An exploratory study. Journal of Thrombosis and Haemostasis. 2008;**6**:457-463

[35] Goto S. Factor XIa as a possible new target of antithrombotic therapy. Journal of Thrombosis and Haemostasis. 2006;**4**:1494-1495

[36] Schumacher WE. Antithrombotic and hemostatic effects of a small molecule factor XIa inhibitor in rats. European Journal of Pharmacology. 2007;**570**:167-174

[37] Kearon C. Dose-response study of recombinant human soluble thrombomodulin (ART-123) in the prevention of venous thromboembolism after total hip replacement. Journal of Thrombosis and Haemostasis. 2005;**3**:962-968

[38] Koller F. History of factor X. Thrombosis Et Diathesis Haemorrhagica. 1960;**4**:58-65

[39] Brown DL, Kouides PA. Diagnosis and treatment of inherited factor X deficiency. Haemophilia. 2008;**14**:1176-1182

[40] Telfer TP, Denson KW, Wright DR. A "new" coagulation defect. British Journal of Haematology. 1956;**2**:308-316

[41] Butenas S, van't Veer C, Mann KG. "Normal" thrombin generation. Blood. 1999;**94**: 2169-2178

[42] Mann G. Thrombin formation. Chest. 2003;**124**:S4-S10

[43] Di Cera E. Thrombin interactions. Chest. 2003;**124**:S11-S17

[44] Mann KG, Brummel K, Butenas S. What is all that thrombin for? Journal of Thrombosis and Haemostasis. 2003;**1**:1504-1514

[45] Komoriya S, Kobayashi S, Osanai K, Yoshino T, Nagata T, Haginoya N, Nakamoto Y, Mochizuki A, Nagahara T, Suzuki M, Shimada T, Watanabe K, Isobe Y, Furugoori T. Bioorganic & Medicinal Chemistry. 2006;**14**:1309-1330

[46] Medscape. Factor X deficiency. https://emedicine.medscape.com/article/209867-overview#a4 [Accessed: Janauary 25, 2018]

[47] Ansell J. Factor Xa or thrombin: Is factor Xa a better target? Journal of Thrombosis and Haemostasis. 2007;**5**:60-64

[48] Perzborn E. Factor Xa inhibitors-new anticoagulants for secondary haemostasis. Hämostaseologie. 2009;**29**:260-267

[49] Beynon R, Bond J, editors. Proteolytic Enzymes. 2nd ed. New York: Oxford University Press; 2001

[50] Quan M, Lam P, Han Q, Pinto D, He M, Li R, Ellis C, Clark C, Teleha C, Sun J, Alexander R, Bai S, Luettgen J, Knabb R, Wong P, Wexler R. Discovery of 1-(3′-aminobenzisoxazol-5′-yl)-3-trifluoromethyl-N-[2-fluoro-4- [(2′-dimethylaminomethyl)imidazol-1-yl]phenyl]-1H-pyrazole-5-carboxyamide hydrochloride (razaxaban), a highly potent, selective, and orally bioavailable factor Xa inhibitor. Journal of Medicinal Chemistry. 2005;**48**:1729-1744

[51] Nagar S, Argikar U, Tweedie D, editors. Enzyme Kinetics in Drug Metabolism. Fundamentals and Applications. New York: Humana Press/Springer; 2014

[52] Hanna M, Mohan P, Knabb R, Gupta E, Frost C, Lawrence J. Development of Apixaban: A novel anticoagulant for prevention of stroke in patients with atrial fibrillation. Annals of the New York Academy of Sciences. 2014;**1329**:93-106

[53] Jiang J, Ji Y. Alternate synthesis of Apixaban (BMS-562247), an inhibitor of blood coagulation factor Xa. Synthetic Communications. 2013;**43**:72-79

[54] Meyer S, Vanhoorelbeke K, Ulrichts H, Staelens S, Feys H, Salles I, Fontayne A, Deckmyn H. Development of Monoclonal Antibodies that Inhibit Platelet Adhesion or Aggregation as Potential Anti-Thrombotic Drugs Cardiovascular & Hematological Disorders - Drug Targets. 2006;**6**:191-207

[55] Vanhoorelbeke K, Ulrichts H, Van de Walle G, Fontayne A, Deckmyn H. Inhibition of Platelet Glycoprotein Ib and Its Antithrombotic Potential. Current Pharmaceutical Design. 2007;**13**:2684-2697

[56] Rezaie R. Prothrombin protects factor Xa in the prothrombinase complex from inhibition by the heparineantithrombin complex. Blood. 2001;**97**:2308-2313

[57] Desai U. New antithrombin-based anticoagulants. Medicinal Research Reviews. 2004; **24**:151-181

[58] Undas A, Brummel-Ziedins K, Mann K. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. Blood. 2007;**109**:2285-2292

[59] Assessing and treating disorders of primary hemostasis. Clinical Advisor. https://www.clinicaladvisor.com/features/treating-disorders-primary-hemostasis/article/706749/ [Accessed March 07, 2018]

[60] Berg J, Tymoczko J, Stryer L. Biochemistry. 5th Edition: International Version. New York: W. H. Freeman; 2002

[61] Núñez-Navarro N, Santana F, Parra L, Zacconi F. Surfing the blood coagulation Cascade: Insight into the vital factor Xa. Current Medicinal Chemistry. DOI: 10.2174/0929867325666180125165340

[62] Lip G, Pan X, Kamble S, Kawabata H, Mardekian J, Masseria C, Bruno A, Phatak H. Major bleeding risk among non-valvular atrial fibrillation patients initiated on apixaban, dabigatran, rivaroxaban or warfarin: a "real- world" observational study in the United States. International Journal of Clinical Practice. 2016;**70**:752-763

[63] Vene N, Mavri A, Gubensek M, Tratar G, Cuderman T, Perme M, Blinc A. Risk of Thromboembolic Events in Patients with Non-Valvular Atrial Fibrillation After Dabigatran or Rivaroxaban Discontinuation - Data from the Ljubljana Registry. PLoS One. 2016;**11**:e0156943

[64] Acanfora D, Acanfora C, Scicchitano P, Longobardi M, Furgi G, Casucci G, Lanzillo B, Dentamaro I, Zito A, Incalzi R, Ciccone M. Evidence Gaps in the Era of Non–Vitamin K Oral Anticoagulants. Clinical Drug Investigation. 2016;**36**:857-862

[65] Pruitt J, Pinto D, Galemmo R, Alexander R, Rossi K, Wells B, Drummond S, Bostrom L, Burdick D, Bruckner R, Chen H, Smallwood A, Wong P, Wright M, Bai S, Luettgen J, Knabb R, Lam P, Wexler R. Discovery of 1-(2-aminomethylphenyl)-3-trifluoromethyl-N-[3-fluoro-2′-(aminosulfonyl)[1,1′-biphenyl)]-4-yl]-1H-pyrazole-5-carboxyamide (DPC602), a potent, selective, and orally bioavailable factor Xa inhibitor(1). Journal of Medicinal Chemistry. 2003;**46**:5298-5315

[66] Mederski W, Cezanne B, van Amsterdam C, Buhring K-U, Dorsch D, Gleitz J, Marz J, Tsaklakidis C. Chlorothiophenecarboxamides as P1 surrogates of inhibitors of blood coagulation factor Xa. Bioorganic & Medicinal Chemistry Letters. 2004;**14**:5817-5822

[67] Wang W, Yuan J, Fu X, Meng F, Zhang S, Xu W, Xu Y, Huang C. Novel Anthranilamide-Based FXa Inhibitors: Drug Design, Synthesis and Biological Evaluation. Molecules. 2016;**21**:491-507

[68] Roehrig S, Straub A, Pohlmann J, Lampe T, Pernerstorfer J, Schlemmer K-H, Reinemer P, Perzborn E. Discovery of the novel antithrombotic agent 5-chloro-*N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide (BAY 59-7939): An oral, direct factor Xa inhibitor. Journal of Medicinal Chemistry. 2005;**48**:5900-5908

[69] Anselm L, Banner D, Benz J, Zbinden K, Himber J, Hilpert H, Huber W, Kuhn B, Mary J-L, Otteneder M, Panday N, Ricklin F, Stahl M, Thomi S, Haap W. Discovery of 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (Apixaban, BMS-562247), a Highly Potent, Selective, Efficacious, and Orally Bioavailable Inhibitor of Blood Coagulation Factor Xa. Bioorganic & Medicinal Chemistry Letters. 2010;**20**:5313-5319

[70] Song H, Cho Y, Lee D, Park H, Baek S, Chae S, Jo S, Kim Y, Lee H, Park J, Park T, Woo S, Kim Y. 2011. US 2011/0112083 A1

[71] Samama MM, Mendell J, Guinet C, Le Flem L, Kunitada S. In vitro study of the anticoagulant effects of edoxaban and its effect on thrombin generation in comparison to fondaparinux. Thrombosis Research. 2012;**129**:E77-E82

[72] Douxfils J, Ageno W, Samama C, Lessire S, Ten Cate H, Verhamme P, Dogné J, Mullier F. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. Journal of Thrombosis and Haemostasis. 2018;**16**:209-219

[73] Myerson J, He L, Lanza G, Tollefsen D, Wickline S. Thrombin-inhibiting perfluorocarbon nanoparticles provide a novel strategy for the treatment and magnetic resonance imaging of acute thrombosis. Thrombosis and Haemostasis. 2011;**9**:1292-1300

[74] Merlini P, Ardissino D, Bauer K, Oltrona L, Pezzano A, Bottasso B, Rosenberg R, Mannucci P. Persistent thrombin generation during heparin therapy in patients with acute coronary syndromes. Arteriosclerosis, Thrombosis, and Vascular Biology. 1977;**17**:1325-1330

[75] Hermans C, Claeys D. Review of the rebound phenomenon in new anticoagulant treatments. Current Medical Research and Opinion. 2006;**22**:471-481

[76] Weitz J, Hudoba M, Massel D, Maraganore J, Hirsh J. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III independent inhibitors. The Journal of Clinical Investigation. 1990;**86**:385-391

[77] Pinto D, Smallheer J, Cheney D, Knabb R, Wexler R. Factor Xa inhibitors: Next-generation antithrombotic agents. Journal of Medicinal Chemistry. 2010;**53**:6243-6274

[78] Young R. The successful quest for oral factor Xa inhibitors; learnings for all of medicinal chemistry? Bioorganic & Medicinal Chemistry Letters. 2011;**21**:6228-6235

[79] Bauer K, Hawkins D, Peters P, Petitou M, Herbert J-M, van Boeckel C, Meuleman D. Fondaparinux, a synthetic Pentasaccharide: The first in a new class of antithrombotic agents—The selective factor Xa inhibitors. Cardiovascular Drug Reviews. 2002;**20**:37-52

[80] Zhang P, Huang W, Wang L, Bao L, Jia Z, Bauer S, Goldman E, Probst G, Song Y, Su T, Fan J, Wu Y, Li W, Woolfrey J, Sinha U, Wong P, Edwards S, Arfsten A, Clizbe L,

Kanter J, Pandey A, Park G, Hutchaleelaha A, Lambing J, Hollenbach S, Scarborough R, Zhu B. Discovery of betrixaban (PRT054021), *N*-(5-chloropyridin-2-yl)-2-(4-(*N*,*N*-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide, a highly potent, selective, and orally efficacious factor Xa inhibitor. Bioorganic & Medicinal Chemistry Letters. 2009;**19**:2179-2185

[81] Cabral K, Ansell J. The role of factor Xa inhibitors in venous thromboembolism treatment. Vascular Health and Risk Management. 2015;**11**:117-123

[82] Samama M. The mechanism of action of rivaroxaban—an oral, direct factor Xa inhibitor—compared with other anticoagulants. Thrombosis Research. 2011;**127**:497-504

[83] Nutt E, Gasic T, Rodkey J, Gasic GJ, Jacobs JW, Friedman PA, Simpson E. The amino acid sequence of antistasin. A potent inhibitor of factor Xa reveals a repeated internal structure. The Journal of Biological Chemistry. 1988;**263**:10162-10167

[84] Tuszynski GP, Gasic TB, Gasic GJ. Isolation and characterization of antistasin. An inhibitor of metastasis and coagulation. The Journal of Biological Chemistry. 1987;**262**:9718-9723

[85] Dunwiddie C, Thornberry NA, Bull HG, Sardana M, Friedman PA, Jacobs JW, Simpson E. Antistasin, a leech-derived inhibitor of factor Xa. Kinetic analysis of enzyme inhibition and identification of the reactive site. The Journal of Biological Chemistry. 1989;**264**:16694-16699

[86] Waxman L, Smith DE, Arcuri KE, Vlasuk GP. Tick anticoagulant peptide (TAP) is a novel inhibitor of blood coagulation factor Xa. Science. 1990;**248**:593-596

[87] Al-Obeidi F, Ostrem J. Factor Xa inhibitors. Expert Opinion on Therapeutic Patents. 1999;**9**:931-953

[88] Nagler M, Haslauer M, Wuillemin W. Fondaparinux—Data on efficacy and safety in special situations. Thrombosis Research. 2012;**129**:407-417

[89] Bauer K. Fondaparinux sodium: A selective inhibitor of factor Xa. American Journal of Health-System Pharmacy. 2001;**58**:14-17

[90] Savi P, Chong B, Greinacher A. Effect of fondaparinux on platelet activation in the presence of heparin-dependent antibodies: A blinded comparative multicenter study with unfractionated heparin. Blood. 2005;**105**:139-144

[91] Warkentin T, Pai M, Sheppard J. Fondaparinux treatment of acute heparin-induced thrombocytopenia confirmed by the serotonin-release assay: A 30-month, 16-patient case series. Journal of Thrombosis and Haemostasis. 2011;**9**:2389-2396

[92] Harenberg J. Development of idraparinux and idrabiotaparinux for anticoagulant therapy. Thrombosis and Haemostasis. 2009;**102**:811-815

[93] Kubitza D, Becka M, Mueck W, Zuehlsdorf M. Rivaroxaban (BAY 59-7939) – An oral, direct factor Xa inhibitor – Has no clinically relevant interaction with naproxen. British Journal of Clinical Pharmacology. 2007;**63**:469-476

[94] Wong P, Crain E, Xin B, Wexler R, Lam P, Pinto D, Luettgen J, Knabb R. Apixaban, an oral, direct and highly selective factor Xa inhibitor: In vitro, antithrombotic and antihemostatic studies. Journal of Thrombosis and Haemostasis. 2008;**6**:820-829

[95] Kubitza D, Becka M, Mueck W, Zuehlsdorf M. Effects of renal impairment on the pharmacokinetics, pharmacodynamics and safety of rivaroxaban – An oral, direct factor Xa inhibitor. British Journal of Clinical Pharmacology. 2010;**70**:703-712

[96] Eriksson B, Quinlan D, Weitz J. Comparative pharmacodynamics and pharmacokinetics of oral direct thrombin and factor Xa inhibitors in development. Clinical Pharmacokinetics. 2009;**48**:1-22

[97] Pinto D, Orwat M, Koch S, Rossi K, Alexander R, Smallwood A, Wong P, Rendina A, Luettgen J, Knabb R, He K, Xin B, Wexler R, Lam P. Discovery of 1-(4-Methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide (Apixaban, BMS-562247), a highly potent, selective, efficacious, and orally bioavailable inhibitor of blood coagulation factor Xa. Journal of Medicinal Chemistry. 2007;**50**:5339-5356

[98] Wong P, Pinto D, Zhang D. Preclinical discovery of apixaban, a direct and orally bioavailable factor Xa inhibitor. Journal of Thrombosis and Thrombolysis. 2011;**31**:478-492

[99] Collins B, Hollidge C. Antithrombotic drug market. Nature Reviews Drug Discovery. 2002;**2**:11-12

[100] De Candia M, Lopopolo G, Altomare C. Novel factor Xa inhibitors: A patent review. Expert Opinion on Therapeutic Patents. 2009;**19**:1535-1580

[101] Masotti L, Campanini M. Pharmacology of new oral anticoagulants: Mechanism of action, pharmacokinetics, pharmacodynamics. The Italian Journal of Medicine. 2013;**7**:1-7

[102] Perzborn E, Roehrig S, Straub A, Kubitza D, Mueck W, Laux V. Rivaroxaban: A new oral factor Xa inhibitor. Arteriosclerosis, Thrombosis, and Vascular Biology. 2010;**30**:376-381

[103] Yuan J, Liu K, Li L, Yuan Y, Liu X, Li Y. A novel synthesis of the oxazolidinone antithrombotic agent rivaroxaban. Molecules. 2014;**19**:14999-15004

[104] Ufer M. Comparative efficacy and safety of the novel oral anticoagulants dabigatran, rivaroxaban and Apixaban in preclinical and clinical development. Thrombosis and Haemostasis. 2010;**103**:572-585

[105] Wong K, Hu D, Oomman A, Tan R, Patel M, Singer D, Breithardt G, Mahaffey K, Becker R, Califf R, Fox K, Berkowitz S, Hacke W, Hankey G. Rivaroxaban for stroke prevention in east Asian patients from the ROCKET AF trial. Stroke. 2014;**45**:1739-1747

[106] Dempfle C. Direct oral anticoagulants-pharmacology, drug interactions, and side effects. Seminars in Hematology. 2014;**51**:89-97

[107] Frost C, Wang J, Nepal S, Schuster A, Barrett Y, Mosqueda-Garcia R, Reeves R, Lacreta F. Apixaban, an oral, direct factor Xa inhibitor: Single dose safety, pharmacokinetics, pharmacodynamics and food effect in healthy subjects. British Journal of Clinical Pharmacology. 2013;**75**:476-487

[108] Pinto DJP, Orwat MJ, Quan ML, Han Q, Galemmo RA, Amparo E, Wells B, Ellis C, He MY, Alexander RS, Rossi KA, Smallwood A, Wong PC, Luettgen JM, Rendina AR, Knabb RM, Mersinger L, Kettner C, Bai S, He K, Wexler RR, Lam PYS. 1-[3-Aminobenzisoxazol-5-yl]-3-Trifluoromethyl-6-[2-(3-(*R*)-Hydroxy-*N*-Pyrrolidinyl)methyl-[1,1]-Biphen-4-yl]-1,4,5,6-Tetrahydropyrazolo-[3,4-C]-Pyridin-7-one (BMS-740808) a highly potent, selective, efficacious, and orally bioavailable inhibitor O. Bioorganic Medicinal Chemistry Letters. 2006;**16**:4141-4147

[109] Cordeanu M, Lambert A, Gaertner S, Nouri S, Mirea C, Alt-Tebacher M, Stephan D. Apixaban-induced hepatotoxicity. The International Journal of Cardiology. 2016;**204**:4-5

[110] Hashimoto T, Suzuki K, Kihara Y, Iwatsubo T, Miyashita A, Heeringa M, Onkels H, Groenendaal D, Verheggen F, Van Marle S, Usui T. Absorption, metabolism and excretion of darexaban (YM150), a new direct factor Xa inhibitor in humans. Xenobiotica. 2013;**43**:534-547

[111] Iwatsuki Y, Sato T, Moritani Y, Shigenaga T, Suzuki M, Kawasaki T, Funatsu T, Kaku S. Biochemical and pharmacological profile of darexaban, an oral direct factor Xa inhibitor. European Journal of Pharmacology. 2011;**673**:49-55

[112] Steg PG, Mehta SR, Jukema JW, Lip GYH, Gibson CM, Kovar F, Kala P, Garcia-Hernandez A, Renfurm RW, Granger CB. RUBY-1: A randomized, double-blind, placebo-controlled trial of the safety and tolerability of the novel oral factor Xa inhibitor darexaban (YM150) following acute coronary syndrome. European Heart Journal. 2011;**32**:2541-2554

[113] Nakamura Y, Mukesh KM, Inamdar MI. Process for the preparation of (1*S*,4*S*,5*S*)-4-bromo-6-oxabicyclo[3.2.1] Octan-7-one, U.S. patent 20150239909, August 27, 2015

[114] Minguet J, Sims HM, Smith KH, Bramlage P. The factor Xa inhibitor edoxaban for the prevention of stroke and systemic embolism in patients with atrial fibrillation. Expert Review of Clinical Pharmacology. 2016;**10**:1-11

[115] Parasrampuria DA, Truitt KE. Pharmacokinetics and pharmacodynamics of edoxaban, a non-vitamin K antagonist oral anticoagulant that inhibits clotting factor Xa. Clinical Pharmacokinetics. 2016;**55**:641-655

[116] Poulakos M, Walker JN, Baig U, David T. Edoxaban: A direct oral anticoagulant. The American Journal of Health-System Pharmacy. 2017;**74**:117-129

[117] Lip GYH, Agnelli G. Edoxaban: A focused review of its clinical pharmacology. European Heart Journal. 2014;**35**:1844-1855

[118] Thoenes M, Minguet J, Bramlage K, Bramlage P, Ferrero C. Betrixaban – The next direct factor Xa inhibitor? Expert Review of Hematology. 2016;**9**:1111-1117

[119] Administration, F. & D. FDA Approved Betrixaban (BEVYXXA, Portola) for the Prophylaxis of Venous Thromboembolism (VTE) in Adult Patients. https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm564422.htm [Accessed September 21, 2017]

Chapter 3

# Anticoagulants from Hematophagous

Ana Marisa Chudzinski-Tavassi, Fernanda Faria and Miryam Paola Alvarez Flores

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78025

**Abstract**

This chapter will focus on anticoagulant molecules described until now from hematophagous animals. The evolutionary scenario for hematophagous animals is convergent and has resulted on a wide diversity of saliva anticoagulants, substances with platelet anti-aggregation action, and also with vase-dilating action. Hematophagous animals such as bloodsuckers (leeches, mosquitoes, and ticks) have developed strategies that specifically target proteinases from the hemostatic system of the animals they feed, thus keeping the blood incoagulable. The saliva of those animals provides a large amount of molecules to modulate the innate immune response of the host and to inhibit blood coagulation in order to facilitate the feeding. Thus, anticoagulants from hematophagous animals represent a very interesting tool for studies ranging from basic research to applications in the therapeutic area, as anticoagulant medication. Several studies have pointed out that anticoagulants from hematophagous can also display non-hemostatic functions as anti-tumor, bringing new perspectives for the study of these molecules. The comprehension of the multi-faced physiological roles of those new anticoagulants from hematophagous opens new perspectives for therapeutic and biotechnological approaches.

**Keywords:** anticoagulant, hematophagous animals, blood coagulation, FXa inhibitor, thrombin inhibitor

## 1. Introduction

### 1.1. Hematophagous animals

On the search for niches to ensure the survival of their populations, some animal species adapted as parasites on other species, retrieving nutrients directly from the inner mediums

of their hosts. In this way, these animals acquired the capacity to mediate the host defenses in order to succeed on their search for food [1, 2].

Among the arthropods, there are more than 14,000 species which are classified in around 400 genders of blood sucking animals with specific need to suck the host's blood, mostly vertebrate. These types of ectoparasites are called hematophagous animals and their saliva feature a rich array of substances capable to keep the ingested blood unclotted in their digestive system [3, 4].

The hematophagous animals feature exquisite and highly specialized mouthparts and a saliva rich in anti-hemostatic components. These substances are able to interfere on different stages of the blood coagulation mechanism and fibrinolysis. There are different groups of hematophagous animals, such as annelids, like leeches [5, 6] and intestinal worms [7], including arthropods, like mosquitoes [8] and ticks (arachnids) [9], and even mammals such as vampire bats [10].

### 1.2. A little about hirudotherapy

The number of compounds that have been discovered with anticoagulant potential in leeches and other bloodsuckers has become increasingly larger with the advent of transcriptomic analysis [11–13]. Nevertheless, the use of the animal itself has being also an alternative. Hirudotherapy, after many time, re-emerged in the 1970s. Leeches have been used with success in some kinds of microsurgeries and surgeries, as for example, in repair of lost limbs or still in plastic surgeries, where the animal helps in the blood fluid reestablishment [14, 15]. Hirudotherapy has also been used to treat soft tissue swelling and hematomas in trauma [16]. In the literature, you can find many reviews that report the use of this animal in different applications [14, 16–18]. On the other hand, a study where the use of leeches as an adjunct for the management of venous congestion after reconstructive surgery, realized with 87 patients, demonstrated that the morbidity associated with it should be considered, particularly the need for a blood transfusion [19].

There are even studies that demonstrate the opinion of the population regarding the use of the animal in surgery, of course that is not very comfortable for the patient. However, in general social cognition, the acceptance of hirudotherapy may not be very welcome at first, but provided with proper information and explanations, overall compliance of patients and caregivers can be improved and consequently result in superior outcomes in flap salvage [20].

Actually, no international protocols on leech therapy instructions have been established; some reported that leech application for a week is sufficient to get good results [21, 22]. However, it already was published in the literature, a guide of procedure for the use of leeches in surgical interventions [23].

### 1.3. Hematophagous animals and role in hemostasis

In all animals, the circulatory system exists in a perfect balance between coagulation (clot formation) and fibrinolysis (clot dissolution); in other words, to maintain blood in a fluid state, minimizing blood loss when the vascular system is injured. In this mechanism, endothelial cells and platelets are extremely important to form the hemostatic plug helping to arrest

bleeding. First, after injury, circulating platelets bind to collagen in the exposed vessel wall and aggregate, and second, the clotting factors are activated in the coagulation cascade, resulting in the formation of a fibrin clot. Thus, hemostasis coagulation reactions and fibrinolysis are crucial for the proper functioning of the hemostatic system [24].

Hematophagous animals such as bloodsuckers (leeches, mosquitoes, and ticks) are rich sources of anticoagulant molecules; among them, clotting inhibitors, fibrinogenolytics, plasminogen activators, and platelet inhibitors, all are present in their fluids and secretions, with roles on physiological processes such as feeding, digestion, self-defense, etc. Bloodsuckers access the blood fluid through the wound made by specialized structures targeting blood coagulation components, specially thrombin, factor Xa and the prothrombinase and tissue factor/FVIIa complexes, as a strategy to maintain blood incoagulable over a relatively long period of time. These new anticoagulants from hematophagous animals have opened new perspectives in the scientific area for basic and applied researches, having applications in the therapeutic area, as anticoagulant medication. Therefore, hematophagous animals have been an excellent target for studies, including pharmacologists [3, 25, 26].

## 2. Anticoagulant from leeches

Leeches are annelids (Annelida, Hirudinea), and feature over 650 known species found on many parts of the planet, including the oceans. Some species do not suck blood, but prey on worms and other small animals. Many sensorial organs are deployed to detect feeding opportunities, such as receptors over the body sensitive to movements and vibrations of water and soil, and also the "ocelli," light-sensitive cells. Some receptors of these animals can detect very small quantities of some substances such as oils and blood [6].

Even before the medicinal principles of leeches were studied in depth, it was known that these animals had powerful anticlotting and antiprotease substances as the blood found in their intestines remained liquid for weeks [27]. The most studied substances come from the salivary glands of hematophagous leeches. As an example, we have the European leech *Hirudo medicinalis*, for over a century, reaching its highest popularity on the XIX century in Europe. These leeches feature three adapted jaws in their mouth system that perforate the host's skin [6].

Other well-studied leech is the *Haementeria ghilianii*, popularly named as Giant Leech, found mainly in the North of Brazil and in the French Guyana, reaching up to a 50 cm length. However, differently from the European ones, leeches of the *Haementeria* genus have a proboscis on the mouth system, which is introduced on the pores of the host animal to find peripheral blood vessels, from which they then feed [6].

The salivary secretions of leeches have different roles, which are more important for the sucking process than for digestion itself. Some of these functions are performed by substances that have not even been isolated and/or studied, such as an anesthetic agent that causes the "bite" of the mandibular leeches to be painless and also a vasodilator of the histamine type that prolongs the bleeding of the host [6]. Many other substances of pharmacological interest have been characterized and described [28].

### 2.1. Molecules with activity in the hemostatic system from leeches

Among different anticlotting molecules from leeches and involved in the coagulation cascade, fibrinolysis, or on the platelet aggregation process, three substances have been the main focus of investigation. They are hirudin (a thrombin inhibitor) [29], antistasin (factor Xa inhibitor) [30], and decorsin (an antagonist of the llb-llla glycoprotein of the platelet membrane) [31]. The amino acid sequences of these substances, together with studies of inhibitory activities from different molecules and designs of the three-dimension structure have been determined, and, then, the structural similarity of these molecules was observed, allowing for the design of a structure motif (L.A.P.: Cys-X6-12-Cys-X-Cys-X3-6-Cys-X3-6-Cys8-14) [32]. However, the mechanisms of action of these inhibitors and important epitopes for the connection to their respective targets are distinct [32], demonstrating the relevance of the many inhibition mechanisms on clotting processes, as well as the evolution of these processes. Many of these substances that come from leeches have been developed by the industry, as targets for different therapies and in different clinical trial stages.

#### *2.1.1. Thrombin inhibitors*

Thrombin is a key enzyme on the pathogenesis of coronary acute thrombosis. Therapies with heparin, an indirect thrombin inhibitor, have been used during the last four decades. Search for new alternatives has demonstrated that the development of direct thrombin inhibitors (DTIs) is a translational success story; an example in which the combination of scientific ingenuity, structure-based design (including leech molecules models), and rigorous clinical trials has created a new class of anticoagulants that has improved patient care [33].

Hirudin was discovered on the salivary glands of the *Hirudo medicinalis* leeches in 1884 [34], and its role as a powerful antithrombotic drug started to be investigated on the 1920s. Markwardt in 1957 started studies with hirudin as a direct agent on the inhibition of thrombin (DTI), and these studies have been progressing significantly [28, 29].

Hirudin is a natural peptide with a simple chain, featuring 65 with three disulfide bridges and one residue of sulfated tyrosine amino acid residues. Part of its N-terminal region is globular and very compact, due to the presence of three disulfide bridges. On the other hand, the C-terminal region is made up of a great number of negatively charged residues [35–38]. More than 100 years after its discovery, the cDNA of hirudin was cloned and the recombinant (rH) obtained in large scale on *Escherichia coli* [39], on *Saccharomyces cerevisiae* [40], and, more recently, on *Acremonium chrysogenum* [41]. Its way of action has been extensively compared to low-molecular-weight heparins. Hirudin is a strict thrombin inhibitor of the "tight binding" type [42], and cofactors are not needed for its activity. Preclinical evaluation and rH clinical selection of analog forms have been improved on the last years [43].

The complex formed between hirudin and thrombin involves the three amino acid residues from the N-terminal region, which link near to the active site, and the C-terminal tail is linked to the fibrinogen-linking site. Crystallographic studies have shown that 10 residues of amino acids of the C-terminal portion (residues 55–65) react with the anion present on the exosite of thrombin, an important region for linking to fibrinogen. The residues 1–48 of the N-terminal portion are also important for the action of hirudin over thrombin; they interact with the enzyme's catalytic site. These types of interaction explain why hirudin links only to thrombin and not to the blood semiproteases [44].

A significant advance was reached with the resolution of the tridimensional structure of hirudin, which allowed for the understanding and development of recombinants equivalent to this protein (rH). The increase of interest on protein inhibitors also was due to studies that demonstrated thrombocytopenia induced by heparin. These new agents produce a direct anticlotting response, having thrombin as target, and they also inhibit the activation of platelets and the increase of thrombin's activity on the coagulation cascade, as thrombin is a multifunctional enzyme responsible for the activation of manly factors, for example, factor V, VIII, and XI [45]. The use of rH has been promising in patients with unstable angina [46].

Lepirudin (Refludan) is an rH, and it was the first direct thrombin inhibitor (DTI) licensed for treatment of thrombosis complicating HIT and associated thromboembolic disease in order to prevent further thromboembolic complications [47]. It is given as an intravenous infusion with or without a bolus, and its dosing is dependent on body weight. It is renally excreted and dose adjustments are required in patients with renal impairment [48]. Significant limitations to its use are its narrow therapeutic window and potential for increased bleeding events [49]. Besides, it is a drug that forms immunogenic complexes and causes a delay in renal excretion causing its accumulation [50, 51]. Therefore, during the treatment, the dose adjustment based on aPTT is recommended. Although not common, anaphylaxis can also occur in patients with hirudin-induced antibodies during the re-exposition to drug [52]. To date, there are no reports of antidotes that reverse these effects of DTIs [53]. There are recent reports that lepirudin has been discontinued from the market [54, 55].

Desirudin (Iprivask) is also an rH, with very similar characteristics as lepirudin. Both rH are structurally identical except for their N-terminus sequences, which are Leu1-Tyr2 in lepirudin and Val1-Val2 in desirudin. It reversibly binds to the active thrombin site of free and clot-associated thrombin. Desirudin is able to inhibit different actions of thrombin as fibrin formation, activation of coagulation factors V, VII, and XIII, and platelet aggregation, resulting in a dose-dependent prolongation of aPTT. It is the only fixed-dose subcutaneously administered DTI approved by FDA for postoperative prevention of VTE in patients undergoing elective hip replacement surgery [56]. Eriksson and collaborators published two clinical studies comparing the efficacy and safety of desirudin (15 mg s.c. twice daily injections) with unfractionated heparin (5000 units s.c. three times daily) and enoxaparin (40 mg s.c. daily), for the prophylaxis of DVT in patients undergoing major orthopedic surgeries. After 8–12 days of treatment, desirudin proved to be superior to both heparin anticoagulants, while showing a similar safety profile [57, 58]. Recently, desirudin was also under investigation as a potential anticoagulant for patients with heparin induced-thrombocytopenia (HIT) with or without thrombosis. Desirudin was also compared with argatroban in PREVENT-HIT study. This is a small, randomized, open-label trial comparing the clinical efficacy, safety, and economic utility of fixed-dose s.c. of drugs. However, just as lepirudin, desirudin is also renally excreted; there is still a risk of accumulation if the renal function is impaired [59].

Bivalirudin, formerly named Hirulog, is not properly a molecule from leech, but is a synthetic peptide (20 amino acids) [60] and bivalent analog of hirudin with a thrombin inhibition activity nearly 800 times weaker than that of hirudin [61]. Unlike the rH, the binding of bivalirudin to thrombin is reversible, and after the binding, the inhibitor is slowly cleaved by thrombin. Then, thrombin activity is only transiently inhibited and its enzymatic activity is restored. This reversible relationship between bivalirudin and thrombin can be seen as a benefit, once may contribute

to its decreased bleeding risk when compared with rHs [62, 63]. Another advantage of bivalirudin was demonstrated in animal studies, where bivalirudin presented a wider therapeutic index than rHs, and an additional advantage of bivalirudin was its lack of immunogenicity [64].

There are many studies with bivalirudin as an alternative to heparin or the combination of heparin and a GpIIb/IIIa inhibitor in patients with acute coronary syndromes and those undergoing a PCI [65–67]. These trials demonstrated that bivalirudin was not significantly different from other tested inhibitors in relation to reduction in major bleeding; on the other hand, bivalirudin, unlike heparin and GpIIb/IIIa inhibitors, does not cause thrombocytopenia. In this study, it also was demonstrated that bivalirudin reduced cardiac mortality and all-cause mortality among patients undergoing primary PCI for ST-elevation-myocardial infarction in the HORIZONS-AMI trial [66]. Accordingly, bivalirudin (Angiomax, The Medicines Company, Parsippany, NJ, USA) has become one of most widely used antithrombotics in the United States for PCI. Bivalirudin has been further studied in other kind of surgeries, but has not been further developed for these indications. Some examples of clinical studies with bivalirudin were as an alternative to heparin in coronary artery bypass [68, 69] in a dose-finding study for VTE prevention in patients after hip or knee surgery [70] and for the treatment of calf vein thrombosis [71]. Finally, the FDA expanded its approval of bivalirudin to include its use as an alternative to heparin in HIT patients with or without thrombosis undergoing PCI [72].

### *2.1.2. Other thrombin inhibitors*

Besides hirudin, other thrombin inhibitors less studied have been isolated from leeches. Among them are a granuline-similar peptide [73], bufrudin [74], theromin [75], and haemadin [76]. Haemadin and theromin are inhibitors and do not present homology in their sequences with the other inhibitors described up to now in all animal kingdom. Haemadin was isolated from the *Haemadipsa sylvestris*, leech, and it is a 5 kDa peptide with a Ki of 100 fM, kinetically less efficient than hirudin (21 fM) [76, 77]. In addition, in literature, we can find only studies about crystal of haemadin and formation of haemadin-thrombin complex, nothing more besides [78, 79].

Theromin is a potent inhibitor (Ki = 12 fM) which was isolated from the intestines of *Theromyzon tessulatum* leeches [75]. It is homodimer 67 amino acid residues, with 16 cysteines that share 8 disulfide bridges. Just like hirudin, the N-terminal sequence of theromin is highly negatively charged and its C-terminal portion is very compact, due to 10 residues of cysteine present on the sequence. Around 24% of the residues of the molecule be cysteins and this approaches it, in sequence similarity, to protease inhibitors of the antistasin family (more detailed below). Hence, considering the low identity on the general sequence between theromin and the peptides of this family, it is difficult to include theromin as a new member of the mentioned family. However, comparisons of sequences have been made between theromin and four different serine-protease inhibitors isolated from *T. tessulatum* leeches: cytin, therin, therostasin, and tessulin [80–83]. These comparisons revealed that in the case of therostasin [82] and tessulin [83], there was a high degree of sequence identity with theromin (70 and 52%, respectively).

It can also be added that among the leeches from the *Theromyzon* genus, three other thrombin inhibitors were also described [84]. In fact, Merck Company, in 1994, deposited patents for different applications observing three thrombin inhibitors with masses of 3, 9, and 14 kDa

[28]. The N-terminal of the 9 kDa inhibitor, EDDNPGPPRACPGE, presented homology with theromin (ECENTECPRACPGE), factor Xa inhibitor (DCENTECPRACPGE) [82], and trypsin inhibitor tessulin (MCENTECPRACPGE) [83]. This 9 kDa inhibitor features a pI of 4.9 and a specific activity at the end of the purification process of 25 IU for inhibition of thrombin and of 0.2 IU for factor Xa inhibition.

### *2.1.3. Factor Xa inhibitors*

While FXa inhibition has emerged as a convenient pathway for management of VTE, currently three FXa inhibitors are available for anticoagulation management—rivaroxaban, apixaban, and edoxaban [85]. New researches about FXa inhibitors of hematophagous animals constantly have been sought.

Antistasin was the first factor Xa inhibitor described that originates from leeches. It is a 15 kDa protein isolated from the salivary glands of the Mexican leech *H. officinalis* [86, 87]. Soon after, a homologous protein, ghilanten, was isolated from the *H. ghiliani* leech [88]. Antistasin features 119 residues of amino acids with the domain I (residues 1–55) being 56% similar to the domain II (residues 56–110). Of the nine residues of the C-terminal (111–119), domain portion four was positively charged [86], and their active site was located on domain I [88–90]. The cDNA of antistasin was cloned [89] and the recombinant protein expressed in system of baculovirus vector in insect cells [90]. Pharmacological studies were carried out, and data showed that the protein remains active after 30 h of injection in animals. Besides this, when tested in different thrombosis models, antistasin proved superior to heparin [91].

Administration of recombinant antistasin in rabbits with atherosclerosis in the femoral artery, as an example, demonstrated reduction of restenosis after balloon angioplasty [91]. Besides this, chimeric peptides corresponding only to domain I were also tested, and it was checked that domains II and III do not feature any intrinsic inhibitory activity over factor Xa, and also do not contribute to activity of domain I [86]. The most powerful synthetic peptide derived from antistasin corresponds to amino acids 27–49, with a disulfide bridge (ATS29–47); this peptide was able to inhibit factor Xa with a Ki of 35 nM. The DRCRVHCP peptide, in micromolar concentrations, featured anticlotting activity and was able to prolong the coagulation time in 50%, when compared with the control [92].

### *2.1.4. Other inhibitors of factor Xa*

Therostasin is a powerful inhibitor for FXa of the "tight binding" type, isolated from *T. tessulatum*, featuring a Ki of 34 pM [82]. The cDNA (825 bp) encodes 82 amino acids polypeptide (with 16 of them being cysteines) preceded by 19 residues representing the signal peptide. Therefore, just as other inhibitors, therostasin is expressed and kept in cells from the salivary glands of leeches [82].

Vizottin is a FXa inhibitor from the salivary complex of the leech *Haementeria vizottoi*. It has shown anticoagulant effects in human plasma, prolonging the recalcification time in a dose-dependent manner (IC50 40 nM). Vizottin was able to induce blood incoagulability in FX-deficient plasma, whereas in normal and reconstituted plasma, vizottin doubled the prothrombin time at 160 nM. At high concentrations, vizottin inhibited the amidolytic activity of factor VIIa/tissue factor (IC50 96.4 nM). It is a compound which is also able to inhibit FXa in

the prothrombinase complex and Gla-domain less FXa. The authors demonstrated that the inhibition of FXa by vizottin is through binding to the active site rather than an exosite. The structure of this molecule still need to be better studied [93].

A FXa inhibitor has been described in leech that are proven not part of the antistasin-family, the Lefaxin. This inhibitor was obtained from the salivary glands of the Brazilian leech, *Haementeria depressa*. It is a competitive inhibitor of FXa with a Ki of 3.6 nM, and is able to inhibit the FXa also in the prothrombinase complex with IC50 of 10 nM. It has a simple chain with 30 kDa and pI of 5.7 [94].

Among the FXa inhibitors from leeches, antistasin was the one that came closest to drug development; however, it did not get there. Even if these natural substances, as antistasin, are not being directly used in the human medical clinic, it was through the study of them that synthetic molecules focused on FXa were and are still being designed. This has provided potent and selective tools for evaluating the potential role of FXa in various diseases. In addition, these advances have been instrumental in defining the biology of FXa and have aided in the discovery of specific receptors and intracellular signaling pathways for FXa that may be important in the progression of, or the response to, various diseases [95].

### *2.1.5. Antiplatelet agents*

#### *2.1.5.1. Collagen-binding proteins*

Leech antiplatelet protein (LAPP) is a specific inhibitor by collagen pathway from *Haementeria officinalis* leech salivary glands. It has around 13 kDa and pI 4.0. Recombinant LAPP (rLAPP) is able to inhibit collagen-mediated platelet aggregation under test-tube stirring conditions (IC50 ~ 60–100 nM) and, also, it is able to block platelet adhesion to soluble collagen under static conditions, a step mediated by integrin $\alpha 2\beta 1$ [96, 97]. There are reports demonstrating that this recombinant prevents integrin $\alpha$-I domain binding to collagen with IC50 ~ 125 nM [98]. The platelet adhesion to collagen type I is inhibited by rLAPP at high shear rate (1600 $s^{-1}$) and this inhibitor is also able to prevent the binding of vWF to collagen type III [99]. In spite of this, rLAPP inhibits platelet deposition to cross sections of human atherosclerotic coronary arteries [99], and studies in baboons proved that rLAPP did not block collagen graft thrombosis, suggesting that inhibition of collagen alone is not enough to prevent thrombosis, possibly because TF exposure plays an important role in the model [100]. The crystal structure of LAPP has been determined and consists of a C-terminal domain which is very compact and a disordered N-terminal region [101].

Calin is isolated from the salivary secretion of the European leech *H. medicinalis*, as well as the rLAPP; it is able to inhibit the vWF-binding and platelet adhesion to collagen both under static and flow conditions [102]. Similarly, Saratin, from *Haementeria ghilianii* leeches, has been described as a platelet aggregation inhibitor that acts on collagen preventing the binding to integrin $\alpha 2\beta 1$ and vWF [103]. The recombinant Saratin was obtained in yeasts (*Hansenula polymorpha*) [104] and it is being commercialized by BioVascular which has developed this product to GMP standards and is evaluating the effects in clinical studies [105]. To date, in the literature, only a few animal studies have been published, where it has been given alone or together with other drugs in glaucoma rabbit models [106, 107]. Saratin, when administrated alone in rat carotid endarterectomy model, significantly decreased platelet adhesion,

intimal hyperplasia, luminal stenosis, and thrombosis. This inhibitor did not increase suture line bleeding or bleeding times, and did not decrease platelet counts. In this study, the authors also have concluded that Saratin may serve as a topical agent to be used for the site-specific inhibition of thrombosis and intimal hyperplasia after vascular manipulation [108].

*2.1.5.2. Disintegrins*

Disintegrins were first discovered in snake venoms where they are very well studied, and were instrumental in our understanding of integrin function and also for the development of antithrombotic drugs [109]. However, this molecule class also has been found in bloodsucker animals. In leeches, there are two more studied molecules with this profile, decorsin and ornatin.

Decorsin is a 39 amino acids protein purified from *Macrobdella decora* leech salivary glands that acts as an antagonist of glycoprotein GPIIb-IIIa. This disintegrin, like snake family of inhibitors, has six cysteines and an RGD motif near its C-terminus. It completely inhibits platelet aggregation ADP induced at high concentrations (1 μM) and is able to inhibit the interaction of GPIIb-IIIa with fibrinogen in ELISA assays (IC50 ~ 1.5 nM). The secretion of decorsin in the salive of this animal probably is one of its strategy to keep host blood flowing or to keep ingested blood from clotting, as leeches store ingested blood for long periods of time [31]. The structure of decorsin was determined by nuclear magnetic resonance (NMR) and it is interestingly similar to that of hirudin from *Hirudo medicinalis* leech [32].

Ornatin is a disintegrin described on *Placobdella ornate* leech that is 40% similar to decorsin. Studies with ornatin demonstrated that it is able to inhibit fibrinogen binding to GPIIb-IIIa (IC50 ~ 5 nM); on the other hand, it inhibits platelet aggregation at higher concentrations (IC50 ~ 300 nM) [110]. Studies with the recombinant protein demonstrated that the native disulfide bonds are required for the optimal GPIIb-IIIa antagonist activity of the ornatin [111].

*2.1.6. Regulators of fibrinogenolysis*

As described in this chapter, various thrombin inhibitors from hematophagous animals together with other kind of anticoagulant as FXa inhibitor and anti-platelets not only maintain anticoagulant potential of the salivary gland secretions but also play a role of blood preservatives in the gut channel of the bloodsuckers. On the other hand, little is known on the degradation of fibrinogen and fibrin by secretions of bloodsuckers. However, we relate here some data obtained about molecules from some leeches of *Haementeria* genus and from specie *Hirudo medicinalis* that act as regulators of fibrinogenolysis and/or fibrinolysis.

*2.1.6.1. FXIIIa inhibitors*

Factor XIIIa promotes the covalent crosslinking of fibrin polymers and incorporation of proteins into the fibrin network and thus the thrombus can be stable and relative resistance to plasmin-mediated degradation. Besides, FXIIIa is involved in other processes such as wound healing and arteriosclerosis. Therefore, selective FXIIIa inhibitors may be a valuable tool for evaluation of the various functions of FXIIIa and their pharmacological control [112]. In this field, a potent FXIIIa inhibitor was found in leeches. Tridegin was discovered in salivary glands of blood-sucking leech, *Haementeria ghilianii*. It is a highly specific inhibitor of factor

XIIIa with about 7 kDa, this inhibitor works with effective concentrations in the nanomolar range [113]. It was also related the presence of transcripts similar to tridegin in some transcriptome analysis of other leeches specie [12], but the obtaining of new molecules from leeches with this function was not yet published. Some tridegin analog peptides have been synthesized and analyzed for their action improvement, but so far, nothing very relevant has been exposed [114]. Although not used in clinical trials on its recombinant form (T087), a derivative of tridegin is being marketed by more than one company for use in laboratory research.

*2.1.6.2. Fibrino(geno)lytics molecules*

Hementin is responsible for proteolysis of blood fibrinogen with formation of products which block conversion of fibrinogen into fibrin catalyzed by thrombin; this molecule was discovered in salivary gland from *Haementeria ghilianii* [115]. Since fibrinogen is involved in the formation of platelet clot, hementin is able to prevent the platelet aggregation induced by ADP and collagen; on the other hand, it can also induce disaggregation of platelet aggregation induced by ADP, but not collagen [116]. Hementin can lyse fibrin clots; but its fibrinolytic activity is less potent than the fibrinogenolytic one. It does not influence the activity of other plasma proteins [117].

It was also demonstrated that plasma clots formed in the presence of tridegin are more sensitive to lyses by hementin (time required for 50% lysis in the presence and absence of hementin was 16 and about 22 h, respectively) [118]. Study of lysis of clots formed from PRP revealed that in the presence of tridegin the effect of fibrinolytic enzymes was the same as in PPP, whereas lysis of platelet-containing clots occurred slower. Thus, the importance of the platelets in the resistance of plasma clots to fibrinolytic enzymes and also the importance of cross-linking in this process [119].

Considering that both molecules are obtained in the same leech species, it was suggested that hementin and tridegin have a synergic action in feeding process of *Haementeria ghilianii*. They may be considered as promising thrombolytic agents.

Hementerin (HT) is a single-chain 80 kDa, Ca++-dependent metalloproteinase, which specifically degrades fibrin(ogen) through a plasminogen-independent pathway. The amino terminal sequence of 8 residues shows 80% similarity with hementin. However, their activities differ somewhat in terms of kinetics and with regard to the structure of the fibrin(ogen) fragments they may produce. Cleavage by HT of fibrinogen A-alpha, gamma, and B-beta chains, in that order, produces fragments differ from those produced by plasmin. HT was also able to degrade cross-linked fibrin although at a lower rate as compared to fibrinogen. HT is a plasminogen-independent fibrino(geno)lytic metalloproteinase that degrades fibrinogen faster than fibrin, prevents the coagulation and destroys fibrin clots *in vitro* [120]. The action of HT was also studied in different platelet assays and the studies have indicated that HT is an effective inhibitor of human platelet aggregation, presumably through activation of the platelet's nitridergic pathway [121].

Destabilase was discovered in salivary glands from *Hirudo medicinalis* and it was able to hydrolyze the epsilon-(gamma-glutamyl)-lysine bonds as a result of fibrin stabilization by FXIIIa in the presence of calcium ions [122]. It was characterized as a polyfunctional molecule

and is a unique representative of invertebrate lysozymes. This molecule combines the properties of endo-s-lysyl-y-glutamyl isopeptidase (D-dimer monomerase), lysozyme, and chitinase and simultaneously is also a non-enzymatic antimicrobial agent. Its ability to hydrolyze endoisopeptide bonds formed by transglutaminases, which are involved in many pathological conditions, including thrombosis, causes this enzyme to become a focus to seek its use in practice [123], on the other hand, none was presented after that.

The substrate of destabilase is the D-D-dimer, a protein of 190 kDa that contains fragments of all three chains of monomer fibrin (alpha, beta, and gamma) and there is a nonlinear dependence of the reaction rate on substrate concentration. The crosslinked fibrin is also a substrate of destabilase, which catalyzes hydrolysis of isopeptide bonds connecting gamma-gamma and alpha-alpha-chains of this protein [124, 125].

Recently, a study demonstrated an optimization procedures related to the expression, isolation, and purification of active destabilase isoforms (mlDL-Ds1, 2, 3) using an *Escherichia coli* expression system, where their muramidase, lytic, isopeptidase and antimicrobial activities were detected and compared. Analyses of the tested activities revealed that all isoforms had almost identical patterns of pH and ionic strength effects. It was determined that three isoforms possessed non-enzymatic antibacterial activity independent of their muramidase activity. It was also demonstrated, for the first time, the fibrinolytic activity of the recombinant destabilase and showed that only intact proteins possessed this activity, suggesting being an enzymatic property [126].

## 3. Anticoagulants from ticks

Most anticoagulants from ticks are produced for the salivary glands and play essential functions during feeding. Ticks inject the saliva into the skin of a wide range of terrestrial vertebrates and absorb it along with the blood of the animal. Faced with an injury inflicted by tick bite, the animal respond by activating blood coagulation, vasoconstriction, inflammation, and tissue remodeling related to wound healing. However, these ectoparasites have a complex and potent pharmacological mechanism to overcome the host defenses, blocking pain and itch and facilitating blood flow to allow the feeding [25, 127, 128].

Differences in the composition of tick saliva are reflected in the co-evolution between ticks and their host, the feeding strategies, the tick developmental stage, the process of penetration of the host skin, and the duration of the feeding. This can be observed between the two major families, Argasidae and Ixodidae. The first family (family Argasidae) is called soft ticks. They feed fast, less than 1 h, for multiple times causing profound damage to the host skin due the deep mouthparts penetration, while hard ticks (family Ixodidae) feed for a prolonged period (days to weeks) in each developmental stage. Hard ticks have strategies to firmly attach to its host, producing large amount of cement or glue to penetrate the host skin and cause a superficial damage (Metastriata ticks, e.g., *Dermacentor* or *Rhipicephalus* genera), or by attaching more deeply to the host skin by physical mechanisms using longer, barbed mouthparts. Females hard tick feed only once and may ingest more blood than 100-times their initial body weight to die later after oviposition (Prostriata, e.g., *Ixodes*, *Metastriata*, and *Amblyomma* genera) [127, 129–131].

### 3.1. Components affecting coagulation

Ticks saliva has other strategies besides inhibiting blood coagulation factors, in order to facilitate the feeding. After injury, subendothelial tissue get exposed, activated platelets bind to exposed von Willebrand factor and collagen through its surface receptors and platelets release soluble vasoconstrictor mediators (ADP, serotonin, and thromboxane $A_2$). Physiologically, there are three major mechanisms that regulate anticoagulation: TFPI, antithrombin III (ATIII), and protein C/thrombomodulin/activated protein C. Until now, there are no description of tick saliva components interfering with or imitating antithrombin, protein S, protein C, heparin, or thrombomodulin [24]. However, many ticks can inhibit thrombin-induced platelet aggregation. On the other hand, anticoagulant molecules from tick saliva also regulate hemostasis by inhibiting blood coagulation factors (FXa or thrombin) or tenase complexes (FVIIa/TF and FIXa) and/or platelet aggregation [132].

Anticoagulants from tick saliva can be classified according with their biochemical characteristics and structure, some of them belonging to the Kunitz-type domain inhibitors and Serpin domain inhibitors [127]. Members of those families can modulate coagulation, inflammation, or vasoconstriction. For example, the Serpin IRS-2 (*I ricinus* Serpin-2) from *I. ricinus* inhibits cathepsin G and chymase, both known as mediators of platelet aggregation and inflammation [133], as well as to mediate vascular permeability [25]. Besides, Kunitz domain inhibitors are widely expressed and characterized as anticoagulants, some of them having just one Kunitz domain being able to inhibit factor Xa [134] or thrombin, such as savigin from *Ornithodoros savignyi* [135].

Depending on the mechanism of action, they can include platelet inhibitors, factor Xa inhibitors and thrombin inhibitors, since they are able to prevent blood clotting and maintain blood incoagulable. Those blood coagulation inhibitors from tick are the major focus of this section.

#### *3.1.1. Antiplatelet agents*

The primary response to injury is the activation of circulating platelets, which bind to collagen in the exposed vessel wall and aggregate, arresting bleeding. In addition, thrombin, a multifunctional serine protease, activates platelets by cleaving platelet receptors [24]. Thus, saliva from ticks possess molecules to able to target platelet activation and aggregation in several ways, some of them inhibiting thrombin-induced platelet activation [136], other interfering with the adhesion of platelet to collagen or other ligands [136] or inhibiting the activation of protease-activated receptors (PARs). An example of the first group is the Serpin IRS-2 (*I ricinus* Serpin-2) from *Ixodes ricinus* which inhibits platelet aggregation induced by both thrombin and cathepsin G [133]. Another Serpin, IxscS from *I. scapularis*, was described to inhibit thrombin and to interfere with platelet aggregation induced by thrombin or ADP [137]. Also, in *I. scapularis*, the enzyme apyrase (an adenosine triphosphate (ATP) diphosphohydrolase) degrades active ATP and ADP into non-active AMP [138].

Some molecules can interfere with the adhesion of platelets to collagen, for example, the tick adhesion inhibitor (TAI) from *Ornithodoros moubata* [139, 140]. Other inhibitors act by binding competition through an integrin recognition motif RGD or KGD preventing the binding to

fibrinogen or other ligands to platelet receptors such as savignygrin from *O. savignyi* [141]. Variabilin is another anti-platelet RGD-containing peptide from *Dermacentor variabilis* [142]. Some inhibitors identified in *I. pacificus* and *I. scapularis*, known as ixodegrins, display some differences with variabilin by having cysteines flanking the RGD motif, and with savignygrin, which have a non-canonical RGD peptide inserted into a Kunitz fold [127, 143].

Other anti-platelet molecules from ticks were reported: monogrin from *Argas monolakensis* [144], moubatin, a lipocalin derived from *O. moubata* which inhibits collagen-induced platelet aggregation by scavenging thromboxane A2 [139, 140, 144], longicornin, isolated from the salivary gland of *Haemaphysalis longicornis*, which also inhibits collagen-mediated platelet aggregation [145].

### *3.1.2. Tenase complex inhibitors*

To target blood coagulation, components from tick saliva have inhibitory activities on the extrinsic tenase complex in blood coagulation [132]. From the studies in *I. scapularis* tick (Acari: Ixodidae) [146], two classes of extrinsic tenase complex inhibitors were identified acting similarly, but not identically, to the physiological inhibitor, tissue factor pathway inhibitor (TFPI) [136]. The first group is represented by ixolaris [147], a 15.7 kDa molecule obtained from the cDNA library of the salivary glands of *I. scapularis* consisting of 140 amino acid residues containing 10 cysteine and two-Kunitz tandem domain which does not bind to FXa active site, in contrast TFPI. It was hypothesized that the second Kunitz domain of ixolaris binds first to FX/FXa (on a heparin binding proexosite/exosite) before binding to the FVIIa-TF complex via the first Kunitz domain. The native inhibitor has a molecular mass of 24 kDa, and both forms are equally effective as anticoagulants. Functionally, the Ixolaris is structurally distinct from human tissue factor pathway inhibitor (TFPI) [146]. The second group is represented by penthalaris [148], a five-Kunitz tandem domain which uses FX or FXa as scaffold to inhibit the FVIIa-TF complex.

### *3.1.3. Factor Xa inhibitors*

One of the main classes of FXa inhibitors characterized from soft tick saliva is the atypical, non-canonical Kunitz-type inhibitors including the tick anticoagulant peptide (TAP), obtained from the *Ornithodoros moubata* tick [9] and FXa-inhibitor (FXaI) from *O. savignyi* tick [149] (Acari: Argasidae). Both inhibitors possess a single Kunitz domain, in contrast to the tandem Kunitz type thrombin inhibitors. Kinetically, both are slow, tight-binding, competitive inhibitors of FXa. The recombinant (rTAP) TAP has a single-chain acidic polypeptide composed of 60 amino acids including 6 cysteine residues, and is a competitive FXa inhibitor highly selective and reversible. Its molecular weight is 6.8 kDa, pI 4.5 and Ki of 0.588 for the native form, and Ki of 0.18 nM for the recombinant form, expressed in *Saccharomyces cerevisiae* [150–152].

Amblyomin-X is a FXa inhibitor identified molecule in the transcriptomics profile of the salivary glands by Expressed Sequence Tags (ESTs) from the hard tick *Amblyomma cajennense* (currently *Amblyomma sculptum*) [153], containing an unique structure with a N-terminal Kunitz-type domain of 60 amino acids and a C-terminal with 49 amino acids. Amblyomin-X is able to inhibit factor Xa, prothrombinase and tenase activities. As FXa inhibitor, Amblyomin-X

acts as a noncompetitive inhibitor (Ki = 3.9 μM) of factor Xa. It is a substrate for plasmin and trypsin, but not for factor Xa and thrombin. The prolongation of PT and aPTT is reversible [154]. Interestingly, several studies pointed out Amblyomin-X as an anti-cancer molecule *in vitro* and *in vivo* [154–161].

Other FXa inhibitors were reported in *I. scapularis* belonging to the salivary protein (Salp) family, which specifically inhibits the FXa active site [162]. Other inhibitors act on FXa through binding to prothrombinase complex [163].

### *3.1.4. Thrombin inhibitors*

The main effector blood coagulation factor is thrombin, which is the enzyme involved in the final (common pathway of the blood coagulation, responsible for the conversion of fibrinogen in fibrin and also regulates the activity of other coagulation factor with great specificity. Thrombin is a multifunctional molecule acting in cell signaling, fibrinolysis, and inflammation system [164]. Thrombin has three domains, the active site and two regulator sites, named exosites. Exosite I is the site that links the enzyme with fibrinogen, the platelet receptor and protease activated receptors (PARs), as well as the endothelial receptor, thrombomodulin. Exosite II recognizes glycosaminoglycans such as heparin, platelet receptor GP Ib-IX-V and fibrin (for a recent review on the role of thrombin exosites, see Ref. [165]. Thus, the choice of thrombin as a target for new anticoagulants seems logical, since its inhibition not only attenuates fibrin formation, but also blocks thrombin-mediated feedback amplification of clotting [166].

Kunitz-type thrombin inhibitors from ticks were identified in hard (Ixodidae family) and soft (Argasidae family) ticks, and have differences that place them in two different protein subclasses, based on their sequences, probably as an adaptation of their different blood-feeding behaviors [2]. Avathrin is a recombinant thrombin inhibitor from the salivary glands of the ixodid tick, *Amblyomma variegatum*. It shares 31–34% of identity with variegin. Kinetically, avathrin is a fast, tight binding competitive inhibitor (545 pM) with high affinity for thrombin rather than other serine proteases of the coagulation system. Cristal structure of avathrin and thrombin reveal an interaction through the active site and exosite-I of thrombin. Moreover, cleavage products continue to exert prolonged inhibition in a murine carotid artery thrombosis model [167]. From hard ticks, other thrombin inhibitor was isolated including amblin from *Amblyomma hebraeum* [168], boophilin from the cattle tick *Boophilus microplus* [169], and hemalin from *Haemaphysalis longicornis* [170].

Boophilin has been cloned and overexpressed in *E. coli*, which potently inhibits additional trypsin-like serine proteases, including trypsin and plasmin and displays an apparent molecular mass of ~23 kDa. This inhibitor binds bovine thrombin with tight-binding kinetics, and was determined an apparent Ki of 1.8 nM. The crystal structure of the bovine $\alpha$-thrombin boophilin complex reveals a non-canonical binding mode to the protease. The N-terminal region of the mature inhibitor binds in a parallel manner across the active site of the protease, while the C-terminal Kunitz domain is negatively charged and docks into the basic exosite I of thrombin [169].

Recently, a new thrombin inhibitor from *Amblyomma sculptum* was identified in the transcriptomics analysis of tick's salivary glands [171]. Scupltin was cloned and expressed in

*E. coli* as a 20 kDa protein sharing only few similarities with hirudin and more similarity with serine protease inhibitors of the antistasin family. Sculptin is a novel class of competitive, reversible, and specific inhibitor of thrombin because its mechanism of inhibition is slightly different than hirudin. The Ki is comparable with that of hirudin and lower then hirulogs. Interestingly, sculptin phylogenetically diverges from hirudin. Sculptin has not inhibitory activity on FXa, trypsin and plasmin. However, it is degraded by serine proteases including thrombin, thus would not requires antidotes. The sculptin fragments produced by thrombin have not thrombin inhibitory activity, while sculptin fragments produced by FXa can inhibit thrombin independently. Sculptin increases blood coagulation parameter in concentration dependent manner. Sculptin has been filed for patenting in Brazil [171].

From soft ticks, Kunitz-type thrombin inhibitors include ornithodorin from *Ornithodoros moubata* [172], savignin from *Ornithodoros savignyi* [135, 173] and monobin from *Argas monolakensis* [174]. Kinetically, they are slow, tight-binding, competitive inhibitors of thrombin: savignin (Ki = 4.89 pM) [173], and monobin (Ki = 7 pM) [174].

As mentioned above, most Ixodidae ticks produce a cement or glue to attach to the host skin to facilitate the penetration of mouthparts for feeding. Interestingly, in *Amblyomma americanum*, the compositions of this cement revealed by the presence of glycine-rich proteins, lipids, and certain carbohydrates, besides serine protease inhibitors and metalloproteases. Some molecules from tick cement were considered promising candidates for an anti-tick vaccine because of their antigenic properties [136, 175].

### 3.2. Components affecting fibrinolysis

Fibrinolytic enzyme with metalloprotease activity has been described in the hard tick *I. scapularis* [176]. On the other hand, an activator of plasminogen, called longistatin from *H. longicornis*, was found to cause hydrolysis of fibrinogen and delay formation of the fibrin clot as comparable to that of tissue-type plasminogen activator (t-PA) [177]. The recombinant form of longistatins is able to inhibit inflammation associated to tick feeding [178].

## 4. Conclusions

To conclude, hematophagous animals have evolved effective means of inhibiting thrombosis, thereby facilitating the acquisition and digestion of a blood meal. To date, specific inhibitors of coagulation, platelet function and fibrinolysis regulators have been identified from numerous invertebrate species, mainly leeches, ticks, and mosquitoes, representing an impressive array of convergent functional strategies. These parasites may serve as potentially useful therapeutic agents for the treatment of a variety of conditions associated with activation of thrombosis. A number of anticoagulants and platelet inhibitors from bloodsuckers have been evaluated *in vivo*, with some currently in varying stages of preclinical and clinical development. Because of the unique specificity and potency of anticoagulants from hematophagous, these kinds of products hold great promise for improving the treatment of a variety of human illnesses, as heart disease and stroke.

## Acknowledgements

A.M.C.T. received financial support from São Paulo Research Foundation (CENTD, Grants No. 2015/50040-4; CeTICS 2013/07467-1) and from CNPq, Grant No. 305445/2023-8. F.F. received financial support from CNPq, Grant No. 480703/2013-2.

## Conflict of interest

The authors declare that they have no competing interests.

## Author details

Ana Marisa Chudzinski-Tavassi*, Fernanda Faria and Miryam Paola Alvarez Flores

*Address all correspondence to: ana.chudzinski@butantan.gov.br

Laboratory of Molecular Biology - Division of Innovation and Development – Instituto Butantan, São Paulo, Brazil

## References

[1] Bang NU. Leeches, snakes, ticks, and vampire bats in today's cardiovascular drug development. Circulation. 1991;**84**(1):436-438

[2] Mans BJ, Neitz AW. Adaptation of ticks to a blood-feeding environment: Evolution from a functional perspective. Insect Biochemistry and Molecular Biology. 2004;**34**(1):1-17

[3] Ribeiro JM. Blood-feeding arthropods: Live syringes or invertebrate pharmacologists? Infectious Agents and Disease. 1995;**4**(3):143-152

[4] Ribeiro JM, Schneider M, Isaias T, Jurberg J, Galvao C, Guimaraes JA. Role of salivary antihemostatic components in blood feeding by triatomine bugs (Heteroptera). Journal of Medical Entomology. 1998;**35**(4):599-610

[5] Sawyer RT. Thrombolytics and anti-coagulants from leeches. Biotechnology (N.Y). 1991;**9**(6):513-515, 518

[6] Sawyer RT. Leech Biology and Behaviour: Anatomy, Physiology and Behaviour. 11th ed. Oxford: Oxford University Press; 1986. 464 p

[7] Cappello M, Vlasuk GP, Bergum PW, Huang S, Hotez PJ. Ancylostoma caninum anticoagulant peptide: A hookworm-derived inhibitor of human coagulation factor Xa. Proceedings of the National Academy of Sciences of the United States of America. 1995;**92**(13):6152-6156

[8] Ribeiro JM, Schneider M, Guimaraes JA. Purification and characterization of prolixin S (nitrophorin 2), the salivary anticoagulant of the blood-sucking bug Rhodnius prolixus. The Biochemical Journal. 1995;**308**(Pt 1):243-249

[9] Waxman L, Smith DE, Arcuri KE, Vlasuk GP. Tick anticoagulant peptide (TAP) is a novel inhibitor of blood coagulation factor Xa. Science. 1990;**248**(4955):593-596

[10] Gardell SJ, Ramjit DR, Stabilito II, Fujita T, Lynch JJ, Cuca GC, et al. Effective thrombolysis without marked plasminemia after bolus intravenous administration of vampire bat salivary plasminogen activator in rabbits. Circulation. 1991;**84**(1):244-253

[11] Amorim AM, de Oliveira UC, Faria F, Pasqualoto KF, Junqueira-de-Azevedo Ide L, Chudzinski-Tavassi AM. Transcripts involved in hemostasis: Exploring salivary complexes from *Haementeria vizottoi* leeches through transcriptomics, phylogenetic studies and structural features. Toxicon. 2015;**106**:20-29. DOI: 10.1016/j.toxicon.2015.09.002

[12] Faria F, Junqueira-de-Azevedo Ide L, Ho PL, Sampaio MU, Chudzinski-Tavassi AM. Gene expression in the salivary complexes from *Haementeria depressa* leech through the generation of expressed sequence tags. Gene. 2005;**349**:173-185. DOI: 10.1016/j.gene.2004.12.022

[13] Siddall ME, Brugler MR, Kvist S. Comparative transcriptomic analyses of three species of Placobdella (Rhynchobdellida: Glossiphoniidae) confirms a single origin of blood feeding in leeches. The Journal of Parasitology. 2016;**102**(1):143-150. DOI: 10.1645/15-802

[14] Abdualkader AM, Ghawi AM, Alaama M, Awang M, Merzouk A. Leech therapeutic applications. Indian Journal of Pharmaceutical Sciences. 2013;**75**(2):127-137

[15] Tashiro K, Fujiki M, Arikawa M, Kagaya Y, Miyamoto S. Free flap salvage after recurrent venous thrombosis by means of large-scale treatment with medical leeches. Plastic and Reconstructive Surgery Global Open. 2016;**4**(12):e1157. DOI: 10.1097/GOX.0000000000001157

[16] Porshinsky BS, Saha S, Grossman MD, Beery Ii PR, Stawicki SP. Clinical uses of the medicinal leech: A practical review. Journal of Postgraduate Medicine. 2011;**57**(1):65-71. DOI: 10.4103/0022-3859.74297

[17] Jha K, Garg A, Narang R, Das S. Hirudotherapy in medicine and dentistry. Journal of Clinical and Diagnostic Research. 2015;**9**(12):ZE05-ZE07. DOI: 10.7860/JCDR/2015/16670.6918

[18] Knobloch K, Gohritz A, Busch K, Spies M, Vogt PM. Hirudo medicinalis-leech applications in plastic and reconstructive microsurgery – A literature review. Handchirurgie, Mikrochirurgie, plastische Chirurgie: Organ der Deutschsprachigen Arbeitsgemeinschaft fur Handchirurgie: Organ der Deutschsprachigen Arbeitsgemeinschaft fur Mikrochirurgie der Peripheren Nerven und Gefasse. 2007;**39**(2):103-107. DOI: 10.1055/s-2007-965138

[19] Cornejo A, Shammas RL, Poveromo LP, Lee HJ, Hollenbeck ST. Institutional outcomes of leech therapy for venous congestion in 87 patients. Journal of Reconstructive Microsurgery. 2017;**33**(9):612-618. DOI: 10.1055/s-0037-1604082

[20] Kim KS, Sim HS, Shin JH, Hwang JH, Lee SY. The relationship between explanation and patient compliance in hirudotherapy. Archives of Craniofacial Surgery. 2017;**18**(3):179-185. DOI: 10.7181/acfs.2017.18.3.179

[21] Whitaker IS, Cheung CK, Chahal CA, Karoo RO, Gulati A, Foo IT. By what mechanism do leeches help to salvage ischaemic tissues? A review. The British Journal of Oral & Maxillofacial Surgery. 2005;**43**(2):155-160. DOI: 10.1016/j.bjoms.2004.09.012

[22] Knobloch K. Leeches in microsurgery – An evidence-based approach. In: Kini RM, Clemetson KJ, Markland FS, McLane MA, Morita T, editors. Toxins and Hemostasis. Netherlands: Springer Science; 2011. pp. 735-745

[23] Bennett-Marsden M. Hirudotherapy: A guide to using leeches to drain blood from tissue. Clinical Pharmacist. 2014;**6**(3):69. DOI: 10.1211/CP.2014.11136626

[24] Hoffman M, Monroe DM. Coagulation 2006: A modern view of hemostasis. Hematology/Oncology Clinics of North America. 2007;**21**(1):1-11. DOI: 10.1016/j.hoc.2006.11.004

[25] Chmelar J, Calvo E, Pedra JH, Francischetti IM, Kotsyfakis M. Tick salivary secretion as a source of antihemostatics. Journal of Proteomics. 2012;**75**(13):3842-3854. DOI: 10.1016/j.jprot.2012.04.026

[26] Champagne DE. Antihemostatic strategies of blood-feeding arthropods. Current Drug Targets Cardiovascular & Haematological Disorders. 2004;**4**(4):375-396

[27] Bagdy D, Barabas E, Graf L, Petersen TE, Magnusson S. Hirudin. Methods in Enzymology. 1976;**45**:669-678

[28] Salzet M. Leech thrombin inhibitors. Current Pharmaceutical Design. 2002;**8**(7):493-503

[29] Markwardt F. Isolation and chemical characterization of hirudin. Hoppe-Seyler's Zeitschrift fur physiologische Chemie. 1957;**308**(2-4):147-156

[30] Tuszynski GP, Gasic TB, Gasic GJ. Isolation and characterization of antistasin. An inhibitor of metastasis and coagulation. The Journal of Biological Chemistry. 1987;**262**(20):9718-9723

[31] Seymour JL, Henzel WJ, Nevins B, Stults JT, Lazarus RA. Decorsin. A potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor from the leech Macrobdella decora. The Journal of Biological Chemistry. 1990;**265**(17):10143-10147

[32] Krezel AM, Wagner G, Seymour-Ulmer J, Lazarus RA. Structure of the RGD protein decorsin: Conserved motif and distinct function in leech proteins that affect blood clotting. Science. 1994;**264**(5167):1944-1947

[33] Coppens M, Eikelboom JW, Gustafsson D, Weitz JI, Hirsh J. Translational success stories: Development of direct thrombin inhibitors. Circulation Research. 2012;**111**(7):920-929. DOI: 10.1161/CIRCRESAHA.112.264903

[34] Haycraft JB. Ueber die Einwirkung eines Secretes des officinellen Blutegels auf die Gerinnbarkeit des Blutes. Arch exp Path Pharmak. 1884;**18**:209-217

[35] Markwardt F. The hirudin tolerance test. Klinische Wochenschrift. 1959;**37**:1142-1143

[36] Markwardt F, Kaiser B, Nowak G. Studies on antithrombotic effects of recombinant hirudin. Thrombosis Research. 1989;**54**(5):377-388

[37] Markwardt F, Nowak G, Sturzebecher U, Walsmann P. Studies on the pharmacokinetics of hirudin. Biomedica Biochimica Acta. 1987;**46**(4):237-244

[38] Markwardt F, Schulz E. On the mechanism of the inhibitory effect of tabanine on blood coagulation. Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie. 1960;**238**:320-328

[39] Fortkamp E, Rieger M, Heisterberg-Moutses G, Schweitzer S, Sommer R. Cloning and expression in *Escherichia coli* of a synthetic DNA for hirudin, the blood coagulation inhibitor in the leech. DNA Journal. 1986;**5**(6):511-517

[40] Courtney M, Loison G, Lemoine Y, Riehl-Bellon N, Degryse E, Brown SW, et al. Production and evaluation of recombinant hirudin. Seminars in Thrombosis and Hemostasis. 1989;**15**(3):288-292. DOI: 10.1055/s-2007-1002721

[41] Radzio R, Kuck U. Efficient synthesis of the blood-coagulation inhibitor hirudin in the filamentous fungus Acremonium chrysogenum. Applied Microbiology and Biotechnology. 1997;**48**(1):58-65

[42] Talbot MD, Ambler J, Butler KD, Findlay VS, Mitchell KA, Peters RF, et al. Recombinant desulphatohirudin (CGP 39393) anticoagulant and antithrombotic properties in vivo. Thrombosis and Haemostasis. 1989;**61**(1):77-80

[43] Verstraete M, Zoldhelyi P. Novel antithrombotic drugs in development. Drugs. 1995; **49**(6):856-884

[44] De Filippis V, Vindigni A, Altichieri L, Fontana A. Core domain of hirudin from the leech Hirudinaria manillensis: Chemical synthesis, purification, and characterization of a Trp3 analog of fragment 1-47. Biochemistry. 1995;**34**(29):9552-9564

[45] Narayanan S. Multifunctional roles of thrombin. Annals of Clinical and Laboratory Science. 1999;**29**(4):275-280

[46] Merlini PA, Ardissino D, Rosenberg RD, Colombi E, Agricola P, Oltrona L, et al. In vivo thrombin generation and activity during and after intravenous infusion of heparin or recombinant hirudin in patients with unstable angina pectoris. Arteriosclerosis, Thrombosis, and Vascular Biology. 2000;**20**(9):2162-2166

[47] Gajra A, Husain J, Smith A. Lepirudin in the management of heparin-induced thrombocytopenia. Expert Opinion on Drug Metabolism & Toxicology. 2008;**4**(8):1131-1141. DOI: 10.1517/17425255.4.8.1131

[48] Hirsh J, Bauer KA, Donati MB, Gould M, Samama MM, Weitz JI. Parenteral anticoagulants: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th ed.). Chest. 2008;**133**(Suppl 6):141S-159S. DOI: 10.1378/chest.08-0689

[49] Alban S. Pharmacological strategies for inhibition of thrombin activity. Current Pharmaceutical Design. 2008;**14**(12):1152-1175

[50] Nutescu EA, Shapiro NL, Chevalier A. New anticoagulant agents: Direct thrombin inhibitors. Cardiology Clinics. 2008;**26**(2):169-187, v-vi. DOI: 10.1016/j.ccl.2007.12.005

[51] Greinacher A, Warkentin TE. The direct thrombin inhibitor hirudin. Thrombosis and Haemostasis. 2008;**99**(5):819-829. DOI: 10.1160/TH07-11-0693

[52] Warkentin TE, Greinacher A, Koster A. Bivalirudin. Thrombosis and Haemostasis. 2008;**99**(5):830-839. DOI: 10.1160/TH07-10-0644

[53] Hirsh J, O'Donnell M, Weitz JI. New anticoagulants. Blood. 2005;**105**(2):453-463. DOI: 10.1182/blood-2003-12-4195

[54] Schindewolf M, Steindl J, Beyer-Westendorf J, Schellong S, Dohmen PM, Brachmann J, et al. Use of Fondaparinux off-label or approved anticoagulants for management of heparin-induced thrombocytopenia. Journal of the American College of Cardiology. 2017;**70**(21):2636-2648. DOI: 10.1016/j.jacc.2017.09.1099

[55] Lepirudin [Internet]. 2017. Available from: https://www.drugs.com/pro/refludan.html [Accessed: Dec 19, 2017]

[56] Desirudin [Internet]. 2017. Available from https://www.drugs.com/pro/refludan.html [Accessed: Nov 29, 2017]

[57] Eriksson BI, Wille-Jorgensen P, Kalebo P, Mouret P, Rosencher N, Bosch P, et al. A comparison of recombinant hirudin with a low-molecular-weight heparin to prevent thromboembolic complications after total hip replacement. The New England Journal of Medicine. 1997;**337**(19):1329-1335. DOI: 10.1056/NEJM199711063371901

[58] Eriksson BI, Ekman S, Lindbratt S, Baur M, Bach D, Torholm C, et al. Prevention of thromboembolism with use of recombinant hirudin. Results of a double-blind, multicenter trial comparing the efficacy of desirudin (Revasc) with that of unfractionated heparin in patients having a total hip replacement. The Journal of Bone and Joint Surgery. American Volume. 1997;**79**(3):326-333

[59] Frame JN, Rice L, Bartholomew JR, Whelton A. Rationale and design of the PREVENT-HIT study: A randomized, open-label pilot study to compare desirudin and argatroban in patients with suspected heparin-induced thrombocytopenia with or without thrombosis. Clinical Therapeutics. 2010;**32**(4):626-636. DOI: 10.1016/j.clinthera.2010.04.012

[60] Maraganore JM, Chao B, Joseph ML, Jablonski J, Ramachandran KL. Anticoagulant activity of synthetic hirudin peptides. The Journal of Biological Chemistry. 1989;**264**(15): 8692-8698

[61] Steinmetzer T, Sturzebecher J. Progress in the development of synthetic thrombin inhibitors as new orally active anticoagulants. Current Medicinal Chemistry. 2004;**11**(17):2297-2321

[62] Bates SM, Weitz JI. The mechanism of action of thrombin inhibitors. The Journal of Invasive Cardiology. 2000;**12**(Suppl F):27F-232F

[63] Reed MD, Bell D. Clinical pharmacology of bivalirudin. Pharmacotherapy. 2002;**22**(6 Pt 2): 105S-111S

[64] Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: Antithrombotic therapy and prevention of thrombosis. 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;**141**(Suppl 2):e24S-e43S. DOI: 10.1378/chest.11-2291

[65] Lincoff AM, Bittl JA, Harrington RA, Feit F, Kleiman NS, Jackman JD, et al. Bivalirudin and provisional glycoprotein IIb/IIIa blockade compared with heparin and planned glycoprotein IIb/IIIa blockade during percutaneous coronary intervention: REPLACE-2 randomized trial. Journal of the American Medical Association. 2003;**289**(7):853-863

[66] Stone GW, Witzenbichler B, Guagliumi G, Peruga JZ, Brodie BR, Dudek D, et al. Heparin plus a glycoprotein IIb/IIIa inhibitor versus bivalirudin monotherapy and paclitaxel-eluting stents versus bare-metal stents in acute myocardial infarction (HORIZONS-AMI): Final 3-year results from a multicentre, randomised controlled trial. Lancet. 2011;**377**(9784):2193-2204. DOI: 10.1016/S0140-6736(11)60764-2

[67] Stone GW, McLaurin BT, Cox DA, Bertrand ME, Lincoff AM, Moses JW, et al. Bivalirudin for patients with acute coronary syndromes. The New England Journal of Medicine. 2006;**355**(21):2203-2216. DOI: 10.1056/NEJMoa062437

[68] Dyke CM, Smedira NG, Koster A, Aronson S, McCarthy 2nd HL, Kirshner R, et al. A comparison of bivalirudin to heparin with protamine reversal in patients undergoing cardiac surgery with cardiopulmonary bypass: The EVOLUTION-ON study. The Journal of Thoracic and Cardiovascular Surgery. 2006;**131**(3):533-539. DOI: 10.1016/j.jtcvs.2005.09.057

[69] Smedira NG, Dyke CM, Koster A, Jurmann M, Bhatia DS, Hu T, et al. Anticoagulation with bivalirudin for off-pump coronary artery bypass grafting: The results of the EVOLUTION-OFF study. The Journal of Thoracic and Cardiovascular Surgery. 2006;**131**(3):686-692. DOI: 10.1016/j.jtcvs.2005.10.049

[70] Ginsberg JS, Nurmohamed MT, Gent M, MacKinnon B, Sicurella J, Brill-Edwards P, et al. Use of Hirulog in the prevention of venous thrombosis after major hip or knee surgery. Circulation. 1994;**90**(5):2385-2389

[71] Ginsberg JS, Nurmohamed MT, Gent M, MacKinnon B, Stevens P, Weitz J, et al. Effects on thrombin generation of single injections of Hirulog in patients with calf vein thrombosis. Thrombosis and Haemostasis. 1994;**72**(4):523-525

[72] Lee CJ, Ansell JE. Direct thrombin inhibitors. British Journal of Clinical Pharmacology. 2011;**72**(4):581-592. DOI: 10.1111/j.1365-2125.2011.03916.x

[73] Hong SJ, Kang KW. Purification of granulin-like polypeptide from the blood-sucking leech, Hirudo nipponia. Protein Expression and Purification. 1999;**16**(2):340-346. DOI: 10.1006/prep.1999.1077

[74] Electricwala A, Sawyer RT, Jones CP, Atkinson T. Isolation of thrombin inhibitor from the leech Hirudinaria manillensis. Blood Coagulation & Fibrinolysis. 1991;**2**(1):83-89

[75] Salzet M, Chopin V, Baert J, Matias I, Malecha J. Theromin, a novel leech thrombin inhibitor. The Journal of Biological Chemistry. 2000;**275**(40):30774-30780. DOI: 10.1074/jbc.M000787200

[76] Strube KH, Kroger B, Bialojan S, Otte M, Dodt J. Isolation, sequence analysis, and cloning of haemadin. An anticoagulant peptide from the Indian leech. The Journal of Biological Chemistry. 1993;**268**(12):8590-8595

[77] Strube KH, Bialojan S, Kroeger B, Friedrich T. Thrombin-inhibitory proteins from terrestrial leeches. Google Patents. 1995

[78] Richardson JL, Fuentes-Prior P, Sadler JE, Huber R, Bode W. Characterization of the residues involved in the human alpha-thrombin-haemadin complex: An exosite II-binding inhibitor. Biochemistry. 2002;**41**(8):2535-2542

[79] Richardson JL, Kroger B, Hoeffken W, Sadler JE, Pereira P, Huber R, et al. Crystal structure of the human alpha-thrombin-haemadin complex: An exosite II-binding inhibitor. The EMBO Journal. 2000;**19**(21):5650-5660. DOI: 10.1093/emboj/19.21.5650

[80] Chopin V, Bilfinger TV, Stefano GB, Matias I, Salzet M. Amino-acid-sequence determination and biological activity of cytin, a naturally occurring specific chymotrypsin inhibitor from the leech *Theromyzon tessulatum*. European Journal of Biochemistry. 1997;**249**(3):733-738

[81] Chopin V, Matias I, Stefano GB, Salzet M. Amino acid sequence determination and biological activity of therin, a naturally occuring specific trypsin inhibitor from the leech *Theromyzon tessulatum*. European Journal of Biochemistry. 1998;**254**(3):565-570

[82] Chopin V, Salzet M, Baert J, Vandenbulcke F, Sautiere PE, Kerckaert JP, et al. Therostasin, a novel clotting factor Xa inhibitor from the rhynchobdellid leech, *Theromyzon tessulatum*. The Journal of Biological Chemistry. 2000;**275**(42):32701-32707. DOI: 10.1074/jbc.M90-9217199

[83] Chopin V, Stefano GB, Salzet M. Amino-acid-sequence determination and biological activity of tessulin, a naturally occurring trypsin-chymotrypsin inhibitor isolated from the leech *Theromyzon tessulatum*. European Journal of Biochemistry. 1998;**258**(2):662-668

[84] Hemberger J, Sawyer R, Wolf S, Dodt J. Thrombin-inhibitors. Google Patents. 2003

[85] Rao PSS, Burkart T. Advances in oral anticoagulation therapy – What's in the pipeline? Blood Reviews. 2017;**31**(4):205-211. DOI: 10.1016/j.blre.2017.02.002

[86] Nutt E, Gasic T, Rodkey J, Gasic GJ, Jacobs JW, Friedman PA, et al. The amino acid sequence of antistasin. A potent inhibitor of factor Xa reveals a repeated internal structure. The Journal of Biological Chemistry. 1988;**263**(21):10162-10167

[87] Bott JA. Leech protein having anticoagulant and antimetastatic properties. Google Patents. 1993

[88] Blankenship DT, Brankamp RG, Manley GD, Cardin AD. Amino acid sequence of ghilanten: Anticoagulant-antimetastatic principle of the South American leech, *Haementeria ghilianii*. Biochemical and Biophysical Research Communications. 1990;**166**(3):1384-1389

[89] Theunissen HJ, Dijkema R, Swinkels JC, de Poorter TL, Vink PM, van Dinther TG. Mutational analysis of antistasin, an inhibitor of blood coagulation factor Xa derived from the Mexican leech Haementeria officinalis. Thrombosis Research. 1994;**75**(1):41-50

[90] Han JH, Law SW, Keller PM, Kniskern PJ, Silberklang M, Tung JS, et al. Cloning and expression of cDNA encoding antistasin, a leech-derived protein having anti-coagulant and anti-metastatic properties. Gene. 1989;**75**(1):47-57

[91] Ragosta M, Gimple LW, Gertz SD, Dunwiddie CT, Vlasuk GP, Haber HL, et al. Specific factor Xa inhibition reduces restenosis after balloon angioplasty of atherosclerotic femoral arteries in rabbits. Circulation. 1994;**89**(3):1262-1271

[92] Ohta N, Brush M, Jacobs JW. Interaction of antistasin-related peptides with factor Xa: Identification of a core inhibitory sequence. Thrombosis and Haemostasis. 1994;**72**(6):825-830

[93] Oliveira DG, Alvarez-Flores MP, Lopes AR, Chudzinski-Tavassi AM. Functional characterisation of vizottin, the first factor Xa inhibitor purified from the leech *Haementeria vizottoi*. Thrombosis and Haemostasis. 2012;**108**(3):570-578. DOI: 10.1160/TH12-04-0235

[94] Faria F, Kelen EM, Sampaio CA, Bon C, Duval N, Chudzinski-Tavassi AM. A new factor Xa inhibitor (lefaxin) from the *Haementeria depressa* leech. Thrombosis and Haemostasis. 1999;**82**(5):1469-1473

[95] Chudzinski-Tavassi AM, Carrijo-Carvalho LC, Faria F, Alvarez Flores MP, Simons SM. Exogenous factors affecting hemostasis: New therapeutic perspectives and biotechnological approaches. In: Lima ME, Pimenta AMC, Martin-Eauclaire MF, Zingali RB, Rochat H, editors. Animal Toxins: State of the Art Perspectives in Health and Biotechnology. Belo Horizonte: UFMG; 2009. pp. 495-523

[96] Connolly TM, Jacobs JW, Condra C. An inhibitor of collagen-stimulated platelet activation from the salivary glands of the *Haementeria officinalis* leech. I. Identification, isolation, and characterization. The Journal of Biological Chemistry. 1992;**267**(10):6893-6898

[97] Keller PM, Schultz LD, Condra C, Karczewski J, Connolly TM. An inhibitor of collagen-stimulated platelet activation from the salivary glands of the *Haementeria officinalis* leech. II. Cloning of the cDNA and expression. The Journal of Biological Chemistry. 1992;**267**(10):6899-6904

[98] Depraetere H, Kerekes A, Deckmyn H. The collagen-binding leech products rLAPP and calin prevent both von Willebrand factor and alpha2beta1(GPIa/IIa)-I-domain binding to collagen in a different manner. Thrombosis and Haemostasis. 1999;**82**(3):1160-1163

[99] van Zanten GH, Connolly TM, Schiphorst ME, de Graaf S, Slootweg PJ, Sixma JJ. Recombinant leech antiplatelet protein specifically blocks platelet deposition on collagen surfaces under flow conditions. Arteriosclerosis, Thrombosis, and Vascular Biology. 1995;**15**(9):1424-1431

[100] Schaffer LW, Davidson JT, Vlasuk GP, Dunwiddie CT, Siegl PK. Selective factor Xa inhibition by recombinant antistasin prevents vascular graft thrombosis in baboons. Arteriosclerosis and Thrombosis : A Journal of Vascular Biology. 1992;**12**(8):879-885

[101] Huizinga EG, Schouten A, Connolly TM, Kroon J, Sixma JJ, Gros P. The structure of leech anti-platelet protein, an inhibitor of haemostasis. Acta Crystallographica Section D, Biological Crystallography. 2001;**57**(Pt 8):1071-1078

[102] Harsfalvi J, Stassen JM, Hoylaerts MF, Van Houtte E, Sawyer RT, Vermylen J, et al. Calin from Hirudo medicinalis, an inhibitor of von Willebrand factor binding to collagen under static and flow conditions. Blood. 1995;**85**(3):705-711

[103] White TC, Berny MA, Robinson DK, Yin H, DeGrado WF, Hanson SR, et al. The leech product saratin is a potent inhibitor of platelet integrin alpha2beta1 and von Willebrand factor binding to collagen. The FEBS Journal. 2007;**274**(6):1481-1491

[104] Barnes CS, Krafft B, Frech M, Hofmann UR, Papendieck A, Dahlems U, et al. Production and characterization of saratin, an inhibitor of von Willebrand factor-dependent platelet adhesion to collagen. Seminars in Thrombosis and Hemostasis. 2001;**27**(4):337-348. DOI: 10.1055/s-2001-16887

[105] Saratin [Internet]. 2017. Available from: http://www.biovascularinc.com/saratin.html [Accessed: Dec 17, 2017]

[106] Min J, Lukowski ZL, Levine MA, Meyers CA, Beattie AR, Schultz GS, et al. Prevention of ocular scarring post glaucoma filtration surgery using the inflammatory cell and platelet binding modulator saratin in a rabbit model. PLoS One. 2012;**7**(4):e35627. DOI: 10.1371/journal.pone.0035627

[107] Martorana GM, Schaefer JL, Levine MA, Lukowski ZL, Min J, Meyers CA, et al. Sequential therapy with saratin, bevacizumab and ilomastat to prolong bleb function following glaucoma filtration surgery in a rabbit model. PLoS One. 2015;**10**(9):e0138054. DOI: 10.1371/journal.pone.0138054

[108] Cruz CP, Eidt J, Drouilhet J, Brown AT, Wang Y, Barnes CS, et al. Saratin, an inhibitor of von Willebrand factor-dependent platelet adhesion, decreases platelet aggregation and intimal hyperplasia in a rat carotid endarterectomy model. Journal of Vascular Surgery. 2001;**34**(4):724-729. DOI: 10.1067/mva.2001.116801

[109] Assumpcao TC, Ribeiro JM, Francischetti IM. Disintegrins from hematophagous sources. Toxins. 2012;**4**(5):296-322. DOI: 10.3390/toxins4050296

[110] Mazur P, Henzel WJ, Seymour JL, Lazarus RA. Ornatins: Potent glycoprotein IIb-IIIa antagonists and platelet aggregation inhibitors from the leech *Placobdella ornata*. European Journal of Biochemistry. 1991;**202**(3):1073-1082

[111] Mazur P, Dennis MS, Seymour JL, Lazarus RA. Expression, purification, and characterization of recombinant ornatin E, a potent glycoprotein IIb-IIIa antagonist. Protein Expression and Purification. 1993;**4**(4):282-289. DOI: 10.1006/prep.1993.1036

[112] Prasa D, Sturzebecher J. Inhibitors of factor XIIIa. Hämostaseologie. 2002;**22**(1):43-47

[113] Finney S, Seale L, Sawyer RT, Wallis RB. Tridegin, a new peptidic inhibitor of factor XIIIa, from the blood-sucking leech *Haementeria ghilianii*. The Biochemical Journal. 1997;**324**(Pt 3):797-805

[114] Bohm M, Kuhl T, Hardes K, Coch R, Arkona C, Schlott B, et al. Synthesis and functional characterization of tridegin and its analogues: Inhibitors and substrates of factor XIIIa. ChemMedChem. 2012;**7**(2):326-333. DOI: 10.1002/cmdc.201100405

[115] Budzynski AZ, Olexa SA, Sawyer RT. Composition of salivary gland extracts from the leech *Haementeria ghilianii*. Proceedings of the Society for Experimental Biology and Medicine. 1981;**168**(2):259-265

[116] Munro R, Jones CP, Sawyer RT. Effects of hementin on fibrinogen mediated platelet aggregation/deaggregation in vitro. Platelets. 1991;**2**(1):55-56. DOI: 10.3109/09537109-109005505

[117] Budzynski AZ. Interaction of hementin with fibrinogen and fibrin. Blood Coagulation & Fibrinolysis. 1991;**2**(1):149-152

[118] Wallis RB, Seale L, Finney S, Sawyer RT, Bennett GM, Ross-Murphy SB. Reduction of plasma clot stability by a novel factor XIIIa inhibitor from the Giant Amazon leech, *Haementeria ghilianii*. Blood Coagulation & Fibrinolysis. 1997;**8**(5):291-295

[119] Seale L, Finney S, Sawyer RT, Wallis RB. Tridegin, a novel peptidic inhibitor of factor XIIIa from the leech, *Haementeria ghilianii*, enhances fibrinolysis in vitro. Thrombosis and Haemostasis. 1997;**77**(5):959-963

[120] Chudzinski-Tavassi AM, Kelen EM, de Paula Rosa AP, Loyau S, Sampaio CA, Bon C, et al. Fibrino(geno)lytic properties of purified hementerin, a metalloproteinase from the leech *Haementeria depressa*. Thrombosis and Haemostasis. 1998;**80**(1):155-160

[121] Chudzinski-Tavassi AM, Bermej E, Rosenstein RE, Faria F, Sarmiento MI, Alberto F, et al. Nitridergic platelet pathway activation by hementerin, a metalloprotease from the leech *Haementeria depressa*. Biological Chemistry. 2003;**384**(9):1333-1339. DOI: 10.1515/BC.2003.150

[122] Baskova IP, Nikonov GI. Destabilase: An enzyme of medicinal leech salivary gland secretion hydrolyzes the isopeptide bonds in stabilized fibrin. Biokhimiia. 1985;**50**(3):424-431

[123] Baskova IP, Zavalova LL. Polyfunctionality of destabilase, a lysozyme from a medicinal leech. Bioorganicheskaia Khimiia. 2008;**34**(3):337-343

[124] Zavalova LL, Nikonov GI, Kuzina EV, Popova G, Baskova IP. A dimer of fragment D from stabilized fibrin – A substrate for the destabilase enzyme (gamma-glutamyl-epsilon-lysyl-isopeptidase). Biokhimiia. 1991;**56**(1):115-124

[125] Baskova IP, Zavalova LL, Basanova AV, Grigor'eva OV. The hydrolysis of Glu(gamma-epsilon)Lys-amide bonds in D-dimer fragment of fibrin by destabilase from medicinal leech. Bioorganicheskaia Khimiia. 1999;**25**(6):435-438

[126] Kurdyumov AS, Manuvera VA, Baskova IP, Lazarev VN. A comparison of the enzymatic properties of three recombinant isoforms of thrombolytic and antibacterial

protein – Destabilase-lysozyme from medicinal leech. BMC Biochemistry. 2015;**16**:27. DOI: 10.1186/s12858-015-0056-3

[127] Francischetti IM, Sa-Nunes A, Mans BJ, Santos IM, Ribeiro JM. The role of saliva in tick feeding. Frontiers in Bioscience (Landmark Ed). 2009;**14**:2051-2088

[128] Kazimirova M, Stibraniova I. Tick salivary compounds: Their role in modulation of host defences and pathogen transmission. Frontiers in Cellular and Infection Microbiology. 2013;**3**:43. DOI: 10.3389/fcimb.2013.00043

[129] Binnington KC, Kemp DH. Role of tick salivary glands in feeding and disease transmission. Advances in Parasitology. 1980;**18**:315-339

[130] Bowman AS, Coons LB, Needham GR, Sauer JR. Tick saliva: Recent advances and implications for vector competence. Medical and Veterinary Entomology. 1997;**11**(3):277-285

[131] Sonenshine DE. Biology of Ticks. New York: Oxford University Press; 1991

[132] Koh CY, Kini RM. Anticoagulants from hematophagous animals. Expert Review of Hematology. 2008;**1**(2):135-139. DOI: 10.1586/17474086.1.2.135

[133] Chmelar J, Oliveira CJ, Rezacova P, Francischetti IM, Kovarova Z, Pejler G, et al. A tick salivary protein targets cathepsin G and chymase and inhibits host inflammation and platelet aggregation. Blood. 2011;**117**(2):736-744. DOI: 10.1182/blood-2010-06-293241

[134] Joubert AM, Louw AI, Joubert F, Neitz AW. Cloning, nucleotide sequence and expression of the gene encoding factor Xa inhibitor from the salivary glands of the tick, *Ornithodoros savignyi*. Experimental & Applied Acarology. 1998;**22**(10):603-619

[135] Mans BJ, Louw AI, Neitz AW. Amino acid sequence and structure modeling of savignin, a thrombin inhibitor from the tick, *Ornithodoros savignyi*. Insect Biochemistry and Molecular Biology. 2002;**32**(7):821-828

[136] Simo L, Kazimirova M, Richardson J, Bonnet SI. The essential role of tick salivary glands and saliva in tick feeding and pathogen transmission. Frontiers in Cellular and Infection Microbiology. 2017;**7**:281. DOI: 10.3389/fcimb.2017.00281

[137] Ibelli AM, Kim TK, Hill CC, Lewis LA, Bakshi M, Miller S, et al. A blood meal-induced *Ixodes scapularis* tick saliva serpin inhibits trypsin and thrombin, and interferes with platelet aggregation and blood clotting. International Journal for Parasitology. 2014;**44**(6):369-379. DOI: 10.1016/j.ijpara.2014.01.010

[138] Mans BJ, Coetzee J, Louw AI, Gaspar AR, Neitz AW. Disaggregation of aggregated platelets by apyrase from the tick, Ornithodoros savignyi (Acari: Argasidae). Experimental & Applied Acarology. 2000;**24**(4):271-282

[139] Waxman L, Connolly TM. Isolation of an inhibitor selective for collagen-stimulated platelet aggregation from the soft tick *Ornithodoros moubata*. The Journal of Biological Chemistry. 1993;**268**(8):5445-5449

[140] Karczewski J, Waxman L, Endris RG, Connolly TM. An inhibitor from the argasid tick *Ornithodoros moubata* of cell adhesion to collagen. Biochemical and Biophysical Research Communications. 1995;**208**(2):532-541. DOI: 10.1006/bbrc.1995.1371

[141] Mans BJ, Louw AI, Neitz AW. Disaggregation of aggregated platelets by savignygrin, a alphaIIbeta3 antagonist from *Ornithodoros savignyi*. Experimental & Applied Acarology. 2002;**27**(3):231-239

[142] Wang X, Coons LB, Taylor DB, Stevens SE Jr, Gartner TK. Variabilin, a novel RGD-containing antagonist of glycoprotein IIb-IIIa and platelet aggregation inhibitor from the hard tick *Dermacentor variabilis*. The Journal of Biological Chemistry. 1996;**271**(30):17785-17790

[143] Francischetti IM. Platelet aggregation inhibitors from hematophagous animals. Toxicon. 2010;**56**(7):1130-1144. DOI: 10.1016/j.toxicon.2009.12.003

[144] Mans BJ, Ribeiro JM. Function, mechanism and evolution of the moubatin-clade of soft tick lipocalins. Insect Biochemistry and Molecular Biology. 2008;**38**(9):841-852. DOI: 10.1016/j.ibmb.2008.06.007

[145] Cheng Y, Wu H, Li D. An inhibitor selective for collagen-stimulated platelet aggregation from the salivary glands of hard tick *Haemaphysalis longicornis* and its mechanism of action. Science in China Series C, Life Sciences. 1999;**42**(5):457-464. DOI: 10.1007/BF02881768

[146] Francischetti IM, Valenzuela JG, Andersen JF, Mather TN, Ribeiro JM. Ixolaris, a novel recombinant tissue factor pathway inhibitor (TFPI) from the salivary gland of the tick, *Ixodes scapularis*: Identification of factor X and factor Xa as scaffolds for the inhibition of factor VIIa/tissue factor complex. Blood. 2002;**99**(10):3602-3612. DOI: 10.1182/blood-2001-12-0237

[147] Francischetti IM, Valenzuela JG, Pham VM, Garfield MK, Ribeiro JM. Toward a catalog for the transcripts and proteins (sialome) from the salivary gland of the malaria vector *Anopheles gambiae*. The Journal of Experimental Biology. 2002;**205**(Pt 16):2429-2451

[148] Francischetti IM, Mather TN, Ribeiro JM. Tick saliva is a potent inhibitor of endothelial cell proliferation and angiogenesis. Thrombosis and Haemostasis. 2005;**94**(1):167-174. DOI: 10.1160/TH04-09-0566

[149] Gaspar AR, Joubert AM, Crause JC, Neitz AW. Isolation and characterization of an anticoagulant from the salivary glands of the tick, *Ornithodoros savignyi* (Acari: Argasidae). Experimental & Applied Acarology. 1996;**20**(10):583-598

[150] Neeper MP, Waxman L, Smith DE, Schulman CA, Sardana M, Ellis RW, et al. Characterization of recombinant tick anticoagulant peptide. A highly selective inhibitor of blood coagulation factor Xa. The Journal of Biological Chemistry. 1990;**265**(29):17746-17752

[151] Vlasuk GP, Ramjit D, Fujita T, Dunwiddie CT, Nutt EM, Smith DE, et al. Comparison of the in vivo anticoagulant properties of standard heparin and the highly selective factor Xa inhibitors antistasin and tick anticoagulant peptide (TAP) in a rabbit model of venous thrombosis. Thrombosis and Haemostasis. 1991;**65**(3):257-262

[152] Koh CY, Kini RM. Molecular diversity of anticoagulants from haematophagous animals. Thrombosis and Haemostasis. 2009;**102**(3):437-453. DOI: 10.1160/TH09-04-0221

[153] Batista IF, Chudzinski-Tavassi AM, Faria F, Simons SM, Barros-Batestti DM, Labruna MB, et al. Expressed sequence tags (ESTs) from the salivary glands of the tick *Amblyomma cajennense* (Acari: Ixodidae). Toxicon. 2008;**51**(5):823-834. DOI: 10.1016/j.toxicon.2007.12.011

[154] Branco VG, Iqbal A, Alvarez-Flores MP, Sciani JM, de Andrade SA, Iwai LK, et al. Amblyomin-X having a Kunitz-type homologous domain, is a noncompetitive inhibitor of FXa and induces anticoagulation in vitro and in vivo. Biochimica et Biophysica Acta. 2016;**1864**(10):1428-1435. DOI: 10.1016/j.bbapap.2016.07.011

[155] de Souza JG, Morais KL, Angles-Cano E, Boufleur P, de Mello ES, Maria DA, et al. Promising pharmacological profile of a Kunitz-type inhibitor in murine renal cell carcinoma model. Oncotarget. 2016;**7**(38):62255-62266. DOI: 10.18632/oncotarget.11555

[156] Morais KL, Pacheco MT, Berra CM, Bosch RV, Sciani JM, Chammas R, et al. Amblyomin-X induces ER stress, mitochondrial dysfunction, and caspase activation in human melanoma and pancreatic tumor cell. Molecular and Cellular Biochemistry. 2016;**415**(1-2):119-131. DOI: 10.1007/s11010-016-2683-4

[157] Chudzinski-Tavassi AM, Morais KL, Pacheco MT, Pasqualoto KF, de Souza JG. Tick salivary gland as potential natural source for the discovery of promising antitumor drug candidates. Biomedicine & Pharmacotherapy. 2016;**77**:14-19. DOI: 10.1016/j.biopha.2015.11.003

[158] Pacheco MT, Morais KL, Berra CM, Demasi M, Sciani JM, Branco VG, et al. Specific role of cytoplasmic dynein in the mechanism of action of an antitumor molecule, Amblyomin-X. Experimental Cell Research. 2016;**340**(2):248-258. DOI: 10.1016/j.yexcr.2015.12.016

[159] Pacheco MT, Berra CM, Morais KL, Sciani JM, Branco VG, Bosch RV, et al. Dynein function and protein clearance changes in tumor cells induced by a Kunitz-type molecule, Amblyomin-X. PLoS One. 2014;**9**(12):e111907. DOI: 10.1371/journal.pone.0111907

[160] Ventura JS, Faria F, Batista IF, Simons SM, Oliveira DG, Morais KL, et al. A Kunitz-type FXa inhibitor affects tumor progression, hypercoagulable state and triggers apoptosis. Biomedicine & Pharmacotherapy. 2013;**67**(3):192-196. DOI: 10.1016/j.biopha.2012.11.009

[161] Maria DA, de Souza JG, Morais KL, Berra CM, Zampolli Hde C, Demasi M, et al. A novel proteasome inhibitor acting in mitochondrial dysfunction, ER stress and ROS production. Investigational New Drugs. 2013;**31**(3):493-505. DOI: 10.1007/s10637-012-9871-1

[162] Narasimhan S, Koski RA, Beaulieu B, Anderson JF, Ramamoorthi N, Kantor F, et al. A novel family of anticoagulants from the saliva of *Ixodes scapularis*. Insect Molecular Biology. 2002;**11**(6):641-650

[163] Limo MK, Voigt WP, Tumbo-Oeri AG, Njogu RM, Ole-MoiYoi OK. Purification and characterization of an anticoagulant from the salivary glands of the ixodid tick *Rhipicephalus appendiculatus*. Experimental Parasitology. 1991;**72**(4):418-429

[164] Crawley JT, Zanardelli S, Chion CK, Lane DA. The central role of thrombin in hemostasis. Journal of Thrombosis and Haemostasis. 2007;**5**(Suppl 1):95-101. DOI: 10.1111/j.1538-7836.2007.02500.x

[165] Bock PE, Panizzi P, Verhamme IM. Exosites in the substrate specificity of blood coagulation reactions. Journal of Thrombosis and Haemostasis. 2007;**5**(Suppl 1):81-94. DOI: 10.1111/j.1538-7836.2007.02496.x

[166] Weitz JI. Factor Xa or thrombin: Is thrombin a better target? Journal of Thrombosis and Haemostasis. 2007;**5**(Suppl 1):65-67. DOI: 10.1111/j.1538-7836.2007.02552.x

[167] Iyer JK, Koh CY, Kazimirova M, Roller L, Jobichen C, Swaminathan K, et al. Avathrin: A novel thrombin inhibitor derived from a multicopy precursor in the salivary glands of the ixodid tick, *Amblyomma variegatum*. The FASEB Journal. 2017;**31**(7):2981-2995. DOI: 10.1096/fj.201601216R

[168] Lai R, Takeuchi H, Jonczy J, Rees HH, Turner PC. A thrombin inhibitor from the ixodid tick, *Amblyomma hebraeum*. Gene. 2004;**342**(2):243-249. DOI: 10.1016/j.gene.2004.07.012

[169] Macedo-Ribeiro S, Almeida C, Calisto BM, Friedrich T, Mentele R, Sturzebecher J, et al. Isolation, cloning and structural characterisation of boophilin, a multifunctional Kunitz-type proteinase inhibitor from the cattle tick. PLoS One. 2008;**3**(2):e1624. DOI: 10.1371/journal.pone.0001624

[170] Liao M, Zhou J, Gong H, Boldbaatar D, Shirafuji R, Battur B, et al. Hemalin, a thrombin inhibitor isolated from a midgut cDNA library from the hard tick Haemaphysalis longicornis. Journal of Insect Physiology. 2009;**55**(2):164-173. DOI: 10.1016/j.jinsphys.2008.11.004

[171] Iqbal A, Goldfeder MB, Marques-Porto R, Asif H, Souza JG, Faria F, et al. Revisiting antithrombotic therapeutics; sculptin, a novel specific, competitive, reversible, scissile and tight binding inhibitor of thrombin. Scientific Reports. 2017;**7**(1):1431. DOI: 10.1038/s41598-017-01486-w

[172] van de Locht A, Stubbs MT, Bode W, Friedrich T, Bollschweiler C, Hoffken W, et al. The ornithodorin-thrombin crystal structure, a key to the TAP enigma? The EMBO Journal. 1996;**15**(22):6011-6017

[173] Nienaber J, Gaspar AR, Neitz AW. Savignin, a potent thrombin inhibitor isolated from the salivary glands of the tick *Ornithodoros savignyi* (Acari: Argasidae). Experimental Parasitology. 1999;**93**(2):82-91. DOI: 10.1006/expr.1999.4448

[174] Mans BJ, Andersen JF, Schwan TG, Ribeiro JM. Characterization of anti-hemostatic factors in the argasid, *Argas monolakensis*: Implications for the evolution of blood-feeding in the soft tick family. Insect Biochemistry and Molecular Biology. 2008;**38**(1):22-41. DOI: 10.1016/j.ibmb.2007.09.002

[175] Bullard R, Allen P, Chao CC, Douglas J, Das P, Morgan SE, et al. Structural characterization of tick cement cones collected from in vivo and artificial membrane blood-fed lone

star ticks (*Amblyomma americanum*). Ticks and Tick-borne Diseases. 2016;**7**(5):880-892. DOI: 10.1016/j.ttbdis.2016.04.006

[176] Francischetti IM, Mather TN, Ribeiro JM. Cloning of a salivary gland metalloprotease and characterization of gelatinase and fibrin(ogen)lytic activities in the saliva of the Lyme disease tick vector *Ixodes scapularis*. Biochemical and Biophysical Research Communications. 2003;**305**(4):869-875

[177] Anisuzzaman IMK, Alim MA, Miyoshi T, Hatta T, Yamaji K, et al. Longistatin, a plasminogen activator, is key to the availability of blood-meals for ixodid ticks. PLoS Pathogens. 2011;**7**(3):e1001312. DOI: 10.1371/journal.ppat.1001312

[178] Anisuzzaman HT, Miyoshi T, Matsubayashi M, Islam MK, Alim MA, et al. Longistatin in tick saliva blocks advanced glycation end-product receptor activation. The Journal of Clinical Investigation. 2014;**124**(10):4429-4444

Chapter 4

# Anticoagulation for Atrial Fibrillation in Patients with End-Stage Kidney Disease

Quoc Tran, Bassim Jebeili, Kamal Sud and Bhadran Bose

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78022

**Abstract**

Atrial fibrillation (AF) is common in patients with kidney disease, with prevalence several times greater than in the general population. Anticoagulation agents are used to prevent thromboembolic events as a consequence of AF. Several randomized trials have established the efficacy of antithrombotic drugs for preventing stroke in patients with AF, with both antiplatelet agents and oral anticoagulants showing benefit. End-stage kidney disease (ESKD) patients have known platelet defects/dysfunction and also receive heparin during their dialysis treatment, which contributes to their overall coagulopathy. Warfarin being vitamin-K antagonist can augment calciphylaxis in patients with ESKD. Taken together, formal anticoagulation use in patients with ESKD may confer additional risk that is not appreciated in patients without kidney disease. In particular, patients on new oral anticoagulants show excess morbidity and mortality from bleeding when compared to warfarin.

**Keywords:** anticoagulation, warfarin, end-stage kidney disease, hemodialysis, atrial fibrillation, new oral anticoagulation

## 1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia that can lead to thrombus formation in the atria and atrial appendages. It also causes reduction in cardiac output and affected individuals are at increased risk of mortality. The prevalence of AF in patients with end-stage kidney disease (ESKD) is higher than in general population.

Patients with AF are managed with antiarrhythmic agents to control their heart rate and with anticoagulant agents to prevent thromboembolic events. The benefits of anticoagulation in patients with AF (without kidney disease) are well established; however, the benefits and safety of anticoagulation in patients with AF and ESKD are still not clear.

In this chapter, we discuss the prevalence of AF in ESKD and management of AF in these patients focusing on anticoagulation including the direct oral anticoagulants (DOACs).

## 2. Epidemiology

The 2010 Global Burden of Disease (GBD) study estimated that the prevalence of AF approximated at 33.5 million individuals worldwide [1]. In particular, AF is believed to affect between 2.2 and 5.0 million Americans, 4.5 million Europeans and is estimated to affect 1.4% of Australians [1, 2]. The prevalence of AF is expected to increase globally over the next decade [3]. The prevalence of AF increases with age, occurring in approximately 1% of the population under 60 years of age and 15% of the population over 80 years of age. Furthermore, the age-adjusted prevalence of AF is higher for men than women [1, 4]. In terms of complications of AF, ischemic stroke is the most common cause of cerebrovascular incident with 75% of these strokes directly linked to AF [3]. In addition, proportion of strokes from embolic sources increases with age, and greater than 35% of strokes in patients over 80 years of age are cardiac in origin, predominantly due to AF [3], making AF the commonest cause of stroke in this patients older than 80 years [1, 3, 4].

The health burden of renal disease is high for patients as well as for health services globally. The 2010 GBD study found that chronic kidney disease (CKD), which previously ranked 27th in the list of causes of total number of global deaths in 1990, ranked 18th in 2010 [1, 5]. The incidence and prevalence of ESKD vary significantly across different countries. The incidence of ESKD is increasing, with reports indicating doubling in the number of patients being treated for ESKD in Europe, the Americas, and Australia, with diabetes and hypertension being the most common causes in developed and many developing countries; however, glomerulonephritis and "undetermined causes" were more common in Asia and sub-Saharan Africa [5].

Cardiovascular disease and its sequelae occur more frequently in patients with CKD, compared to the general population, and it is often more severe [6]. Patients with impaired renal function (estimated glomerular filtration rate (eGFR) ≤80 mL/min) are deemed to be at higher risk for all cardiovascular events. Current literature examining the prevalence of AF in hemodialysis (HD) patients varies widely, describing a range from 7 to 27% [4]. Furthermore, paroxysmal AF was present in 3.5%, persistent AF in 9.6% of patients and permanent AF in 13.9% of patients [4]. In a large cohort study conducted by Cheng-Huang et al., the prevalence of AF in patients receiving peritoneal dialysis and HD was examined [7]. The incidence rate ratios for AF were 2.07 and 1.78 in HD and PD groups, respectively. Additionally, after adjusting for age, gender and comorbidities, the hazard ratios for the AF risk were 1.46 and 1.32 in HD and PD groups, respectively.

In particular, in a study reported by Hohnloser et al., the risk of stroke in patients with CKD increased with decreasing eGFRs, the annual stroke rate was 1.05% in patients with an eGFR of >80 mL/min, 1.46% in patients with an eGFR of 50–80 mL/min and 2.39% in patients with an eGFR of ≤50 mL/min [8].

http://dx.doi.org/10.5772/intechopen.78022

## 3. Goals of therapy for AF

The mechanisms initiating and maintaining AF may be multifactorial in individual patients, including electrophysiological and structural abnormalities. The primary goals of therapy for AF are to control symptomatic effects of the disease and to prevent any disease-related complications such as thromboembolism and tachycardia-induced cardiomyopathy [9]. The management of AF therefore revolves around strategies for rate control, rhythm control and prevention of thromboembolic strokes. In relation to the former two strategies, multiple international guidelines, including the American College of Cardiology (ACC), the American Heart Association (AHA), European Society of Cardiology (ESC) and the Heart Rhythm Society (HRS) recommend that patients with no structural heart disease should be initiated with dofetilide, dronedarone, flecainide, propafenone, or sotalol, as these agents are found to have the lowest level of cardiac toxicity [9]. If first line therapy is contraindicated or shown to be ineffective, second-line therapy is considered and includes either amiodarone or catheter-directed ablation [9]. Interestingly, amiodarone is considered as first line therapy in patients with substantial left ventricular (LV) hypertrophy as these patients are seen to be at increased proarrhythmic risk with most other first line antiarrhythmic drugs.

The prevention of thromboembolism including stroke prevention has been widely proven with the use of anticoagulants such as warfarin and DOACs. Stroke is seen to be the most common clinical thromboembolic event in patients with AF, with AF attributing to 36% of all strokes in individuals aged 80–89 years [10]. Furthermore, stroke occurring in patients who have AF tend to have a higher degree of severity as compared to those without AF [11]. Clinical markers predicting increased risk of stroke in patients with AF include previous history of transient ischemic attacks (TIA) or prior strokes, coronary artery disease, mitral stenosis, left ventricular dysfunction, heart failure (HF), hypertension, diabetes mellitus, female gender and age more than 75 years [9].

Thrombus formation within the left atrial appendage occurs secondary to reduced blood flow velocities due to the loss of organized mechanical contraction in this anatomical area [12, 13]. Along with reduced flow velocity, other factors have also been attributed to the enhanced thrombogenicity in patients with AF. This includes reduced nitric oxide (NO) production in the left atrial endocardium, increased levels of the prothrombotic protein plasminogen activator inhibitor 1 (PAI-1), as well as elevated levels of β-thromboglobulin and platelet factor 4, von Willebrand factor (vWF), soluble thrombomodulin and fibrinogen [14].

## 4. Evaluation of embolic risk

All individuals who have AF are not at equally high risk for thromboembolic events, and several predisposing clinical factors can identify those patients at relatively higher or lower risk. Risk stratification for embolic events assumes added importance, since the individual's risk of embolic events needs to be carefully balanced against the risk of bleeding which is associated with anticoagulation. In patients without CKD, AF in association with any form of valvular heart disease (VHD) is considered for anticoagulation commencement as the stroke risk in this population subset is high [3, 15, 16]. Patients with nonvalvular heart disease (NVHD),

however, do not necessarily require anticoagulation, and the decision to anticoagulate for stroke prevention depends on their individual risk of stroke [15–17].

There are several risk scores that can be used to evaluate stroke and bleeding risk in the NVHD sub-group including the HAS-BLED score, the $CHADS_2$ and $CHA_2DS_2$-VASc score and the ATRIA stroke risk score [18–21]. The $CHADS_2$ stroke risk scoring system was developed based on the analysis of 1773 patients in the National Registry for Atrial Fibrillation and in 2006 and was used in the ACC/AHA/ESC guidelines to tailor therapy for stroke prevention in AF [15]. The scoring system includes points for congestive heart failure, hypertension, age, diabetes and stroke [15, 20]. Previous stroke or TIA is the strongest predictor of stroke and equates for two points, whereas the other risk factors carry one point each. The final score measures the adjusted stroke rate per 100 patient-years [15, 18, 20]. The $CHA_2DS_2$-VASc score is an updated version of the $CHADS_2$ score as not all patients with a $CHADS_2$ score of 0 were found to be at low risk and was also noted that other risk factors that had been identified were not encompassed by this tool [18, 20, 22]. With the improvement to the $CHA_2DS_2$-VASc score, the 2012 ESC guidelines and 2014 ACC/AHA/HRS guidelines changed their recommendations to support the use of CHA2DS2-VASc score over the $CHADS_2$ scoring system [18, 20, 22]. In addition to the $CHADS_2$, the $CHA_2DS_2$-VASc acknowledges that stroke risk in patients with AF is related to age as a continuous variable, the higher risk of stroke in women, and incorporates risk associated with vascular disease, prior MI, complex aortic plaque, and peripheral arterial disease [18, 20, 22]. The $CHA_2DS_2$-VASc score states that antithrombotic therapy may be omitted for a score of 0, either oral anticoagulants, aspirin, or no antithrombotic therapy can be considered for a score of 1, and oral anticoagulation is recommended for patients with a prior stroke, TIA, or a score of 2 or more [3, 9, 15, 16, 18, 20, 22–26]. Although $CHADS_2$ and $CHA_2DS_2$-VASc scores were useful tools in the past in assisting to quantify risk of stroke in patients with NVAF, recent studies have shown that the $CHA_2DS_2$-VASc score is only able to correctly predict strokes in approximately 68% of cases [3, 18, 22]. The HAS-BLED scoring system was developed in 2010 as a result of the Euro Heart Survey and aims to assess the 1-year risk of major bleeding in patients with AF [18, 21]. The scoring system includes points for hypertension (Systolic >160 mmHg), abnormal renal function, liver function, stroke in past, bleeding, labile international normalized ratio (INR), age ≥65, consuming drugs and consuming alcohol [19, 21]. The scoring system is based on a maximum of nine points with each risk factor worth one point each [18, 21]. A score of 3 or more indicating an increased 1-year bleed risk on anticoagulation is sufficient to justify caution or more regular review [19–21].

While there are other more contemporary risk assessment scoring systems available, such as the ABC (age, biomarkers, clinical history) stroke risk score as devised from Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARIS-TOTLE) study, their applicability is somewhat limited, as several key risk factors included are not routinely measured, and have also yet to be widely validated in population studies [27].

Information on how to best predict stroke risk in the ESKD population is limited and precludes the ability to identify patients at high risk for stroke. No stroke risk prediction scores have been specifically developed for patients with ESRD with AF. Existing thromboembolic and bleeding risk prediction scores show good standardization of stroke risk in the general population, but performs poorly in the ESKD population. McAlister et al. conducted a retrospective large cohort study comparing the effectiveness of current thromboembolic and

bleeding risk prediction scores in patient with NVAF and CKD [28]. The seven risk prediction models examined included $CHADS_2$, $CHA2DS_2$-VASc, $R_2CHADS2$, ATRIA stroke, HAS-BLED, $HEMORR_2HAGES$ and ATRIA bleed. The study showed that the thromboembolic risk scores did not perform differently from each other, where the negative predictive value was not seen to be significantly different from each other. In terms of bleeding risk score, $HEMORR_2HAGES$ was the observed to be the most accurate with the highest c-statistic of 0.66 [28]. Furthermore, the study also showed that each of the seven risk prediction scores performed significantly better for patients with normal kidney function than in patients with CKD with performance significantly worsened as severity of kidney disease increased [28]. Therefore, the study suggests that current thromboembolic and bleeding risk prediction scores are inadequate for use in patients with CKD.

There is no difference in the indications for anticoagulation therapy between paroxysmal, persistent, or permanent AF. Clinical risk assessment tools such as the $CHA_2DS_2$-VASc score do not fully account for thromboembolic risk, and stroke can occur even after a sinus rhythm is restored by either pharmacological or electrical cardioversion.

## 5. Anticoagulation therapy in atrial fibrillation

As stipulated previously, no stroke risk prediction scores have been specifically developed for patients with CKD and AF. It has been shown, however, that patients with CKD and nonvalvular AF have a heightened stroke risk regardless of CHADS2DS2-VASc score, where 80% of patients having scores of ≥2 [29]. Current ACC/AHA/ESC guidelines advise that for a $CHADS_2$ score of ≥2 for either men or women, formal anticoagulation is recommended for patients [3, 9, 15, 16, 20, 22–26, 30]. Those with a $CHADS_2$ score of 1, formal anticoagulation or aspirin alone should be considered in conjunction with patient specific comorbidities [3, 9, 15, 16, 20, 22–26]. Finally, the guidelines state for patients with a $CHADS_2$ score of 0, no anticoagulation, neither formal nor antiplatelets, is recommended [3, 9, 15, 16, 20, 22–26]. In comparison with the American guidelines, the European guidelines recommend that males with a $CHA_2DS_2$-VASc score of ≥2 then anticoagulation should be used for stroke prevention, whereas those with a score of 1 should only be considered for anticoagulation, depending also on patient comorbidities and other risk factors [15, 20, 22–24]. Furthermore, for females, as female gender has been shown to be a weak risk factor for stroke in AF, guidelines advise that a $CHA_2DS_2$-VASc score of ≥3, then anticoagulation is recommended; however if the score is 2, then anticoagulation be considered [15, 20, 22–24]. If the CHA2DS2-VASc score is 0 in men and women or is 1 in women, neither formal anticoagulation nor antiplatelet therapy is advised or required [15, 20, 22–24].

### 5.1. Antiplatelets

There are few studies available that directly compare antiplatelet therapy, either single or dual agent, directly with formal anticoagulation. In a study conducted by Connely et al., it was investigated whether the addition of clopidogrel to aspirin in patients would reduce the risk of vascular events in patients with atrial fibrillation [11]. The primary end points examined included stroke, myocardial infarction, noncentral nervous system systemic embolism

and death from vascular causes. The study showed that in patients with AF where vitamin-K antagonists were deemed unsuitable, the addition of clopidogrel to aspirin reduced the risk of major vascular events, in particular stroke, by 28%; however, the combination increased the risk of major hemorrhage from 1.3 to 2.0% per year [10, 11].

The Stroke Prevention in Atrial Fibrillation (SPAF) II study was the only major study to show a positive outcome for use of aspirin in AF for stroke prevention [31]. The study showed that patients treated with aspirin had a statistically significant reduction of 42% in stroke rate over the placebo group [31]. In the more recent Apixaban Versus Acetylsalicylic Acid to Prevent Stroke in Atrial Fibrillation Patients Who Have Failed or Are Unsuitable for Vitamin-K Antagonist Treatment (AVERROES) study, 5599 patients with atrial fibrillation who were at increased risk for stroke and for whom vitamin-K antagonist therapy was unsuitable were assessed and divided into groups whom received apixaban or aspirin [10]. The primary outcome assessed in the study was the occurrence of stroke or systemic embolism. The study was halted at 18 months as a significant benefit from apixaban over aspirin was observed with a 55% risk reduction in ischemic stroke [10]. Furthermore, it was also found that bleeding was comparable between aspirin and apixaban, 44 major bleeding events (a rate of 1.4% per year) among patients taking apixaban and 39 (1.2% per year) among those taking aspirin (hazard ratio with apixaban, 1.13; 95% CI, 0.74–1.75; P = 0.57) [10].

Olesen et al. examined aspirin's use for stroke prevention in patients with AF and CKD. The retrospective cohort study found that aspirin was associated with an increased risk of stroke or systemic thromboembolism among patients who had any form of renal disease, (hazard ratio, 1.17; 95% CI, 1.01–1.35; P = 0.04) [9]. Furthermore, the risk of stroke or systemic thromboembolism in association with CKD was of the same magnitude when adjusted for all baseline characteristics [9].

Aspirin, however, is still frequently used to reduce stroke risk in many patients with high CHA2DS2-VASc scores who would benefit from anticoagulation.

### 5.2. Vitamin-K antagonist

Vitamin-K antagonist, for example, warfarin, was first used as an anticoagulant in the 1960s when it was validated through multiple randomized, controlled clinical trials comparing it versus placebo or no therapy, that it had superior efficacy in reducing strokes in patients with NVAF [31, 32]. Warfarin's effectiveness was confirmed in the pivotal 1992 Veterans Affairs Stroke Prevention in Nonrheumatic Atrial Fibrillation (SPINAF) trial [33]. This trial definitively proved that warfarin reduced stroke rates in patients with NVAF by approximately 70% and mortality by approximately 30% [33]. Furthermore, when investigated with regard to intention to treat, it was found that there was a 68% risk reduction in stroke for patients taking warfarin when compared to the control groups who were not anticoagulated [34, 35].

Although warfarin is extremely effective in reducing stroke and mortality, it is an incredibly difficult drug to use in clinical practice. Warfarin has a slow onset and offset of action and has multiple drug and food interactions. Warfarin requires constant monitoring to ensure the INR remains within the therapeutic range of 2–3. Studies have shown that an INR <2 carries

an increased risk of stroke, whereas an INR of >3 confers an increased risk of bleeding [31, 32, 36, 37]. From an Australian perspective, the difficulties of warfarin's clinical usage were seen in multicenter trials showing the time in therapeutic range (TTR) is near 70% at best, but more often found to be around 50–60% [38]. Interestingly there seems to be an increased risk of bleeding and intracranial hemorrhage (ICH) with warfarin in Asian populations, even in patients with an INR within the therapeutic range. This has seen some major centers in Asia adopt a lower therapeutic range of 1.5–2 [31, 32].

Formal anticoagulation using Warfarin has been shown to significantly reduce the incidence of stroke in CKD patients with AF. The Stroke Prevention in Atrial Fibrillation [SPAF-III] Study analyzed 516 AF participants with CKD and showed that warfarin was able to reduce ischemic stroke or systemic embolism by 76% (95% CI 42–90, $P < 0.001$) [39]. In a population-based retrospective cohort study conducted by Mitesh et al., it was found that CKD patients requiring dialysis with AF, warfarin use, in comparison with no-warfarin use, did not reduce the risk for stroke however it was associated with a 44% higher risk for having a bleeding event, whereas warfarin use in nondialysis patients with AF was associated with a 13% lower risk for stroke with a 19% higher risk for bleeding event [32, 40]. Bleeding in this study was grouped and defined as intracerebral bleeding, gastrointestinal bleeding, intraocular bleeding, hematuria, and unspecified location of bleeding. This data should not be surprising though as it is well known that HD patients have both platelet and coagulation abnormalities and also have associated comorbidities such as uncontrolled hypertension and diabetes mellitus, all of which contribute to an increase in the risk for stroke and bleeding [32, 41]. Furthermore, HD patients usually also receive heparin during dialysis, which also adds to their increased risk for bleeding. Warfarin use in HD patients, through the inhibition of matrix Gla protein and Gas-6, thus causing calciphylaxis, can accelerate vascular calcification, which may also increase the risk for ischemic stroke [32, 42, 43]. This is further supported by a 134,410 patient retrospective study cohort by Chen et al. who compared ESRD patients requiring renal replacement therapy with AF receiving either monotherapy with antiplatelets or Warfarin with a control group who were not using either of the medications [44]. They showed that the incidence of ischemic stroke or TIAs was no different between the intervention group and the control group [44]. Furthermore, the results stayed unchanged after propensity match and also showed no beneficial effect of antiplatelet or warfarin therapy in any subgroups, such as age and gender [44].

### 5.3. Direct oral anticoagulation drugs

Currently available oral direct acting anticoagulants are the direct thrombin inhibitor dabigatran, and the factor Xa inhibitors rivaroxaban and apixaban. Their clinical use in the normal population is favored due to their rapid onset and offset of action. Direct oral anticoagulations (DOACs) achieve full anticoagulation within 2 h of dosing, and are mostly excreted within 24 h of taking the last dose. In addition to their appeal, none of the DOACs require routine monitoring to evaluate their extent of anticoagulation performance. There has been minimal evidence in investigating DOACs for stroke prophylaxis in ESKD patients with AF. All major trials, comparing DOACs to warfarin for AF and stroke prophylaxis, excluded patients with a calculated creatinine clearance rate of <25 or 30 mL/min [45, 46].

The RE-LY study was the first open label study to compare dabigatran, a direct thrombin inhibitor, to warfarin in patients with one or more risk factors for stroke [45]. The study concluded that a higher dose (110 and 150 twice daily) of dabigatran was superior to warfarin in reducing stroke and systemic embolism. The study also revealed the effect of creatinine clearance and renal function on dabigatran's action and pharmacokinetics. Dabigatran is highly dependent on renal excretion with 80% being excreted unchanged in the urine. A 20% of patients in the RE-LY study had a CrCl of 30–50 mL/min (Patients with CrCl<30 were excluded). These patients had a higher risk of major bleeding compared to patients with a CrCl of >80 mL/min [46]. The RE-LY study also saw that warfarin-assigned patients with an eCrCl of 30–49 mL/min had a significant rate of major hemorrhage at 5.4% per year compared to other participants at 3.2% per year [45]. Large-scale trials for dabigatran use in CKD patients are not available, and although dabigatran is partially removed by dialysis, it remains not recommended for anticoagulation during HD [47].

The Rivaroxaban—once daily, oral, direct factor Xa inhibition compared with vitamin-K antagonism for prevention of stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF) trial—examined rivaroxaban versus warfarin in patients with two or more risk factors for a stroke. The study showed that rivaroxaban had similar efficacy to warfarin in reducing stroke and embolism but a significant reduction in ICH. The study was also able to show a statistically significant trend toward a decrease in all-cause mortality, as with other DOACs [48]. Patients with CrCl <30 mL/min were excluded from the trial, whereas patients with moderate renal insufficiency (CrCl of 30–50 mL/min) were included but given an adjusted dose of 15 mg daily based on data showing 25–30% higher residual serum concentration of rivaroxaban in these patients compared to patients with normal renal clearance [49, 50]. The ROCKET AF study was unable to demonstrate noninferiority or superiority of rivaroxaban in patients with moderate renal insufficiency in comparison with warfarin therapy. The rates of stroke and systemic embolism were higher in patients with moderate renal impairment compared to patients with better renal function [47]. The ROCKET AF trial also examined the primary outcome of major hemorrhage in comparison with warfarin and was shown to occur in 3.2% of patients per year for those with an eCrCl of >50 mL/min as compared to 4.7% per year in those with an eCrCl of 30–49 mL/min [48]. There are no major trials for rivaroxaban therapy in patients with a creatinine clearance of <30 mL/min or on dialysis. Rivaroxaban has been shown to be able to be completely and immediately reversed with 50 U/kg prothrombin complex concentrate on patients with normal renal function [51].

Apixaban's effectiveness was examined in the ARISTOTLE trial, which was similar to the other DOAC trials, included patients with one or more risk factors for stroke. The trial revealed that apixaban was superior to warfarin in stroke reduction and systemic embolism [52]. Major and clinically relevant nonmajor bleeding was also found to be significantly less with apixaban when compared to warfarin with ICH being also significantly reduced [3, 52]. Of the three DOACs available, only apixaban has proven to be statistically significant for reduction in total mortality when compared with warfarin, regardless of renal function.

The ARISTOTLE trial involved examining the efficacy of a apixaban in patients with creatinine of 133–221 μmol/L (1.5–2.5 mg/dL) and lower or creatinine clearance of >25 mL/min [45], and noted that the incidence of major bleeding events with apixaban was inversely related to renal functions [8]. Patients with moderate to severe renal insufficiency (creatinine 133–221 μmol/L or CrCl of <25 mL/min) were given 2.5 mg doses twice daily while patients with normal

renal function were given 5 mg twice daily. Bleeding episodes were higher in patients with moderate/severe renal failure when compared to the normal renal function group, however remained lower with apixaban compared to patients with renal impairment on warfarin. In terms of observing patients on warfarin, the ARISTOTLE trial showed that major hemorrhage was at least twice as likely among patients with an eCrCl of 25–50 mL/min compared with others [52]. The ARISTOTLE trial therefore eludes to apixaban being safe for oral anticoagulation in AF patients with creatinine clearance of >25 mL/min, with dose adjustment as decided by the treating physician.

Again, there are no major trials investigating the effectiveness of apixaban on ESKD patients with stroke risks. Further research in this area is awaited. Apixaban like rivaroxaban is not dialyzable and therefore albeit the small renal excretion of 27 and 36%, respectively, they may build up in patients with ESKD [53]. Therefore, it is always advisable to continue monitoring patients with moderate to severe (CrCl 30–80 mL/min) for worsening renal function and possibility of toxicity.

## 6. Conclusions

In conclusion, patients with ESKD have higher prevalence of AF. In the absence of DOACs that can be used in patients with ESRD, vitamin-K antagonists still remain the gold standard for systemic anticoagulation in this group of patients. Anticoagulation with vitamin-K antagonist in patients with ESKD is challenging due to the adverse events such as increased risk of bleeding and augmentation of risk of calciphylaxis.

The risks of administering vitamin-K antagonists in patients with ESRD should be carefully weighed against benefits of these agents in preventing embolic episodes. Since newer DOACs may have a better benefit-risk profile in dialysis patients than vitamin-K antagonists, provided appropriate dose reductions are made, this strategy may yield more on-target anticoagulation, reduce the risk of intracerebral bleeding, and not interfere with vascular calcification biology. Clinical trials with direct oral anticoagulant in dialysis patients are eagerly awaited. In addition, development of a DOAC that has nonrenal mode of excretion and can be safely given in patients on dialysis may also result in lower rates of bleeding complications, thereby shifting the risk-benefit balance toward systemic anticoagulation with this group of agents in the future.

## Author details

Quoc Tran[1], Bassim Jebeili[1], Kamal Sud[2,3] and Bhadran Bose[2,3]*

*Address all correspondence to: bhadran.bose@health.nsw.gov.au

1 Department of Vascular Surgery, Nepean Hospital, Kingswood, NSW, Australia

2 Department of Renal Medicine, Nepean Hospital, Kingswood, NSW, Australia

3 Nepean Clinical School, The University of Sydney, Sydney, NSW, Australia

## References

[1] Chugh SS, Blackshear JL, Shen WK, Hammill SC, Gersh BJ. Epidemiology and natural history of atrial fibrillation: Clinical implications. Journal of the American College of Cardiology. 2001;**37**(2):371-378

[2] Wong CX, Brown A, Tse H-F, Albert CM, Kalman JM, Marwick TH, et al. Epidemiology of atrial fibrillation: The australian and asia-pacific perspective. Heart, Lung & Circulation. 2017;**26**(9):870-879

[3] Amerena J, Ridley D. An update on anticoagulation in atrial fibrillation. Heart, Lung & Circulation. 2017;**26**(9):911-917

[4] Genovesi S, Pogliani D, Faini A, Valsecchi MG, Riva A, Stefani F, et al. Prevalence of atrial fibrillation and associated factors in a population of long-term hemodialysis patients. American Journal of Kidney Diseases. 2005;**46**(5):897-902

[5] Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: Global dimension and perspectives. The Lancet. 2013;**382**(9888):260-272

[6] Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJL, Mann JF, et al. Chronic kidney disease and cardiovascular risk: Epidemiology, mechanisms, and prevention. The Lancet. 2013;**382**(9889):339-352

[7] Shen CH, Zheng CM, Kiu KT, Chen HA, Wu CC, Lu KC, et al. Increased risk of atrial fibrillation in end-stage renal disease patients on dialysis: A nationwide, population-based study in Taiwan. Medicine. 2016;**95**(25):e3933

[8] Hohnloser SH, Hijazi Z, Thomas L, Alexander JH, Amerena J, Hanna M, et al. Efficacy of apixaban when compared with warfarin in relation to renal function in patients with atrial fibrillation: Insights from the ARISTOTLE trial. European Heart Journal. 2012;**33**(22):2821-2830

[9] Olesen JB, Lip GY, Kamper AL, Hommel K, Kober L, Lane DA, et al. Stroke and bleeding in atrial fibrillation with chronic kidney disease. The New England Journal of Medicine. 2012;**367**(7):625-635

[10] Connolly SJ, Eikelboom J, Joyner C, Diener HC, Hart R, Golitsyn S, et al. Apixaban in patients with atrial fibrillation. The New England Journal of Medicine. 2011;**364**(9):806-817

[11] Investigators A, Connolly SJ, Pogue J, Hart RG, Hohnloser SH, Pfeffer M, et al. Effect of clopidogrel added to aspirin in patients with atrial fibrillation. The New England Journal of Medicine. 2009;**360**(20):2066-2078

[12] Grimm RA, Stewart WJ, Maloney JD, Cohen GI, Pearce GL, Salcedo EE, et al. Impact of electrical cardioversion for atrial fibrillation on left atrial appendage function and spontaneous echo contrast: Characterization by simultaneous transesophageal echocardiography. Journal of the American College of Cardiology. 1993;**22**(5):1359-1366

[13] Stoddard MF, Dawkins PR, Prince CR, Ammash NM. Left atrial appendage thrombus is not uncommon in patients with acute atrial fibrillation and a recent embolic event: A

transesophageal echocardiographic study. Journal of the American College of Cardiology. 1995;**25**(2):452-459

[14] Cai H, Li Z, Goette A, Mera F, Honeycutt C, Feterik K, et al. Downregulation of endocardial nitric oxide synthase expression and nitric oxide production in atrial fibrillation: Potential mechanisms for atrial thrombosis and stroke. Circulation. 2002;**106**(22):2854-2858

[15] Gage BF, Waterman AD, Shannon W, Boechler M, Rich MW, Radford MJ. Validation of clinical classification schemes for predicting stroke: Results from the National Registry of atrial fibrillation. Journal of the American Medical Association. 2001;**285**(22):2864-2870

[16] Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. ESC guidelines for the management of atrial fibrillation developed in collaboration with EACTS. European Heart Journal. 2016, 2016;**37**(38):2893-2962

[17] Conway DS, Pearce LA, Chin BS, Hart RG, Lip GY. Plasma von Willebrand factor and soluble p-selectin as indices of endothelial damage and platelet activation in 1321 patients with nonvalvular atrial fibrillation: Relationship to stroke risk factors. Circulation. 2002;**106**(15):1962-1967

[18] Chao TF, Chen SA. Stroke risk predictor scoring systems in atrial fibrillation. Journal of Atrial Fibrillation. 2014;**6**(5):998

[19] Singer DE, Chang Y, Borowsky LH, Fang MC, Pomernacki NK, Udaltsova N, et al. A new risk scheme to predict ischemic stroke and other thromboembolism in atrial fibrillation: The ATRIA study stroke risk score. Journal of the American Heart Association. 2013;**2**(3):e000250

[20] Aspberg S, Chang Y, Atterman A, Bottai M, Go AS, Singer DE. Comparison of the ATRIA, CHADS2, and CHA2DS2-VASc stroke risk scores in predicting ischaemic stroke in a large Swedish cohort of patients with atrial fibrillation. European Heart Journal. 2016;**37**(42):3203-3210

[21] Pisters R, Lane DA, Nieuwlaat R, de Vos CB, Crijns HJ, Lip GY. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: The euro heart survey. Chest. 2010;**138**(5):1093-1100

[22] Lane DA, Lip GY. Use of the CHA(2)DS(2)-VASc and HAS-BLED scores to aid decision making for thromboprophylaxis in nonvalvular atrial fibrillation. Circulation. 2012;**126**(7):860-865

[23] Kalman JM, Sanders P, Brieger DB, Aggarwal A, Zwar NA, Tatoulis J, et al. National Heart Foundation of Australia consensus statement on catheter ablation as a therapy for atrial fibrillation. The Medical Journal of Australia. 2013;**198**(1):27-28

[24] Wang TKM, Sathananthan J, Marshall M, Kerr A, Hood C. Relationships between anticoagulation, risk scores and adverse outcomes in dialysis patients with atrial fibrillation. Heart, Lung & Circulation. 2016;**25**(3):243-249

[25] Risk factors for stroke and efficacy of antithrombotic therapy in atrial fibrillation. Analysis of pooled data from five randomized controlled trials. Archives of Internal Medicine. 1994;**154**(13):1449-1457

[26] Steinberg BA, Piccini JP. State of the art review: Anticoagulation in atrial fibrillation. The BMJ. 2014;**348**:g2116

[27] Hijazi Z, Lindback J, Alexander JH, Hanna M, Held C, Hylek EM, et al. The ABC (age, biomarkers, clinical history) stroke risk score: A biomarker-based risk score for predicting stroke in atrial fibrillation. European Heart Journal. 2016;**37**(20):1582-1590

[28] McAlister FA, Wiebe N, Jun M, Sandhu R, James MT, McMurtry MS, et al. Are existing risk scores for nonvalvular atrial fibrillation useful for prediction or risk adjustment in patients with chronic kidney disease? Canadian Journal of Cardiology. 2017;**33**(2):243-252

[29] Bonde AN, Lip GY, Kamper A-L, Hansen PR, Lamberts M, Hommel K, et al. Net clinical benefit of antithrombotic therapy in patients with atrial fibrillation and chronic kidney disease: A nationwide observational cohort study. Journal of the American College of Cardiology. 2014;**64**(23):2471-2482

[30] Bajpai A, Savelieva I, Camm AJ. Treatment of atrial fibrillation. British Medical Bulletin. 2008;**88**(1):75-94

[31] Investigators SPiAF. Warfarin versus aspirin for prevention of thromboembolism in atrial fibrillation: Stroke prevention in atrial fibrillation II study. The Lancet. 1994;**343**(8899):687-691

[32] Shah M, Tsadok MA, Jackevicius CA, Essebag V, Eisenberg MJ, Rahme E, et al. Warfarin use and the risk for stroke and bleeding in patients with atrial fibrillation undergoing dialysis. Circulation. 2014;**129**:1196-1203. DOI: 10.1161/CIRCULATIONAHA.113.004777

[33] Ezekowitz MD, Bridgers SL, James KE, Carliner NH, Colling CL, Gornick CC, et al. Warfarin in the prevention of stroke associated with nonrheumatic atrial fibrillation. The New England Journal of Medicine. 1992;**327**(20):1406-1412

[34] Investigators AF. Risk factors for stroke and efficacy of antithrombotic therapy in atrial fibrillation: Analysis of pooled data from five randomized clinical trials. Archives of Internal Medicine. 1994;**154**:1949-1957

[35] Albers GW, Sherman DG, Gress DR, Paulseth JE, Petersen P. Stroke prevention in nonvalvular atrial fibrillation: A review of prospective randomized trials. Annals of Neurology. 1991;**30**(4):511-518

[36] Saxena R, Koudstaal PJ. Anticoagulants versus antiplatelet therapy for preventing stroke in patients with nonrheumatic atrial fibrillation and a history of stroke or transient ischemic attack. The Cochrane Library. 2004. Cochrane id: CD000187

[37] Lamberts M, Gislason GH, Olesen JB, Kristensen SL, Olsen A-MS, Mikkelsen A, et al. Oral anticoagulation and antiplatelets in atrial fibrillation patients after myocardial infarction and coronary intervention. Journal of the American College of Cardiology. 2013;**62**(11):981-989

[38] Bubner TK, Laurence CO, Gialamas A, Yelland LN, Ryan P, Willson KJ, et al. Effectiveness of point-of-care testing for therapeutic control of chronic conditions: Results from the PoCT in general practice trial. The Medical Journal of Australia. 2009;**190**(11):624-626

[39] Goldman ME, Pearce LA, Hart RG, Zabalgoitia M, Asinger RW, Safford R, et al. Pathophysiologic correlates of thromboembolism in nonvalvular atrial fibrillation: I. Reduced flow velocity in the left atrial appendage (the stroke prevention in atrial fibrillation [SPAF-III] study). Journal of the American Society of Echocardiography. 1999;**12**(12):1080-1087

[40] Chan KE, Lazarus JM, Thadhani R, Hakim RM. Warfarin use associates with increased risk for stroke in hemodialysis patients with atrial fibrillation. Journal of the American Society of Nephrology. 2009;**20**(10):2223-2233

[41] Marinigh R, Lane DA, Lip GY. Severe renal impairment and stroke prevention in atrial fibrillation: Implications for thromboprophylaxis and bleeding risk. Journal of the American College of Cardiology. 2011;**57**(12):1339-1348

[42] Brandenburg VM, Kramann R, Specht P, Ketteler M. Calciphylaxis in CKD and beyond. Nephrology, Dialysis, Transplantation. 2012;**27**(4):1314-1318

[43] Hafiji J, Deegan P, Brais R, Norris P. Warfarin-induced calciphylaxis successfully treated with sodium thiosulphate. The Australasian Journal of Dermatology. 2013;**54**(2):133-135

[44] Chen J-J, Lin L-Y, Yang Y-H, Hwang J-J, Chen P-C, Lin J-L. Anti-platelet or anti-coagulant agent for the prevention of ischemic stroke in patients with end-stage renal disease and atrial fibrillation—A nation-wide database analyses. International Journal of Cardiology. 2014;**177**(3):1008-1011

[45] Reilly PA, Lehr T, Haertter S, Connolly SJ, Yusuf S, Eikelboom JW, et al. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: The RE-LY trial (randomized evaluation of long-term anticoagulation therapy). Journal of the American College of Cardiology. 2014;**63**(4):321-328

[46] Hijazi Z, Hohnloser SH, Oldgren J, Andersson U, Connolly SJ, Eikelboom JW, et al. Efficacy and safety of dabigatran compared with warfarin in relation to baseline renal function in patients with atrial fibrillation: A RE-LY trial analysis. Circulation. 2013;**129**:961-970. DOI: 10.1161/CIRCULATIONAHA.113.003628

[47] Lutz J, Jurk K, Schinzel H. Direct oral anticoagulants in patients with chronic kidney disease: Patient selection and special considerations. International Journal of Nephrology and Renovascular Disease. 2017;**10**:135

[48] Committee ES, investigators RAs. Rivaroxaban—Once daily, oral, direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and embolism trial in atrial fibrillation: Rationale and design of the ROCKET AF study. American Heart Journal. 2010;**159**(3):340-347. e1

[49] Fox KA, Piccini JP, Wojdyla D, Becker RC, Halperin JL, Nessel CC, et al. Prevention of stroke and systemic embolism with rivaroxaban compared with warfarin in patients with non-valvular atrial fibrillation and moderate renal impairment. European Heart Journal. 2011;**32**(19):2387-2394

[50] Kubitza D, Becka M, Mueck W, Halabi A, Maatouk H, Klause N, et al. Effects of renal impairment on the pharmacokinetics, pharmacodynamics and safety of rivaroxaban, an oral, direct factor Xa inhibitor. British Journal of Clinical Pharmacology. 2010;**70**(5):703-712

[51] Eerenberg ES, Kamphuisen PW, Sijpkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: A randomized, placebo-controlled, crossover study in healthy subjects. Circulation. 2011;**124**:1573-1579. DOI: 10.1161/CIRCULATIONAHA.111.029017

[52] Lopes RD, Alexander JH, Al-Khatib SM, Ansell J, Diaz R, Easton JD, et al. Apixaban for reduction in stroke and other ThromboembоLic events in atrial fibrillation (ARISTOTLE) trial: Design and rationale. American Heart Journal. 2010;**159**(3):331-339

[53] Bansal VK, Herzog CA, Sarnak MJ, Choi MJ, Mehta R, Jaar BG, et al. Oral anticoagulants to prevent stroke in nonvalvular atrial fibrillation in patients with CKD stage 5D: An NKF-KDOQI controversies report. American Journal of Kidney Diseases. 2017;**70**(6):859-868

Chapter 5

# Real-World Safety of Anticoagulants

Niccolò Lombardi, Giada Crescioli and
Alfredo Vannacci

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78023

**Abstract**

The aim of the present chapter is to characterize the management of anticoagulation in the real-world clinical setting, describing all pharmacological aspects of anticoagulant medications, in particular safety aspects. The chapter is structured into three main sections. Within the first two sections, active principles are classified on the basis of their pharmacological properties (pharmacodynamic and pharmacokinetic characteristic) in old and new molecules (or first and second generation class). In the third one, we discuss safety issues of anticoagulants using available data from postmarketing evidences. Furthermore, we also provide some essential information on the use of this kind of medications in special populations (i.e., elderly, coronary artery disease, diabetics, chronic kidney disease, etc.) in clinical practice.

**Keywords:** anticoagulants, safety, comorbidity, observational study

## 1. Introduction

Hemostasis is the physiologic process for the prevention of hemorrhage and bleeding in response to blood vessel damage, while physiologic inhibition of coagulation ensures the fluidity of blood. The ways in which these factors all balance each other can be the difference between hemostasis and thrombosis [1]. Alteration of this balance in favor of coagulation results in thrombosis, a pathological process characterized by the formation of a platelet or fibrin clot, which could occlude both arterial and venous vessels.

This chapter reviews the agents commonly used for controlling blood fluidity, including in particular:

- The coumarin anticoagulants: warfarin, acenocumarol, and phenprocoumon;
- Direct oral anticoagulants (DOACs): dabigatran, rivaroxaban, apixaban, and edoxaban;
- Parenteral anticoagulant heparin and its derivatives.

## 2. Oral anticoagulants

### 2.1. Old agents

*2.1.1. Warfarin*

*2.1.1.1. Mechanism of action*

Warfarin is a racemic mixture of two optically active isomers, the R and S enantiomers, and it produces its anticoagulant effect by interfering with the cyclic interconversion of vitamin K and vitamin K-2,3-epoxide. Warfarin blocks vitamin K epoxide reductase (VKOR), and the consequent conversion of oxidized vitamin K epoxide into its reduced form, vitamin K hydroquinone [2]. However, warfarin also has a simultaneous procoagulant effect caused by blocking the activation of protein C and S, two endogenous anticoagulants. A rapid depletion of these proteins leads to a transient hypercoagulable state in the first 1 or 2 days of therapy.

*2.1.1.2. Indications*

Warfarin is prescribed for the treatment and prophylaxis of various thromboembolic diseases such as atrial fibrillation (AF), deep venous thrombosis (DVT), transient ischemic attacks (TIA), pulmonary embolism, and other thromboembolic disorders that may affect carriers of cardiac valvular prosthesis or patients who underwent electric cardioversion [3, 4]. The dose-response relationship of warfarin is influenced by genetic and environmental factors, including mutations in gene coding for cytochrome P (CYP) 450, the hepatic enzyme responsible for oxidative metabolism of warfarin, mutations in gene coding for VKOR [5], concomitant drugs, diet, and various disease states [6]. Although warfarin and other dicumarol derivatives cross the placenta and contribute to fetal bone and central nervous system abnormalities when mothers are treated with warfarin within the first-trimester of pregnancy, there is no evidence that warfarin directly affects bone metabolism when administered to children or adults [7]. Women who will be managed with therapeutic anticoagulation in pregnancy should be treated preferably with a parenteral agent, such as heparin and low-molecular-weight heparin (LMWH), unfractionated heparin (UFH), and fondaparinux [8].

*2.1.1.3. Pharmacokinetics*

After oral intake, warfarin enantiomers are absorbed rapidly and almost completely from the gastrointestinal tract (100% of bioavailability) and reach their maximal plasma concentration in 90 minutes in healthy people. Racemic warfarin is extensively bound to plasma protein (mainly albumin, 99%) and has a plasma half-life of 36–42 hours [9]. Warfarin is extensively

metabolized in the liver by different CYP enzymes: CYP3A4 and CYP1A2 primarily metabolize the R-enantiomer, whereas the S-enantiomer is mainly metabolized by CYP2C9. The inactive metabolites are excreted with the urine and stool [10].

*2.1.1.4. Interactions*

Drug interactions that alter the pharmacokinetics of warfarin may include alterations in absorption (e.g., cholestyramine), which would decrease the anticoagulant effect. Reduced plasma-binding because of the presence of excessively albumin-bound drugs causes an increase in free drug plasma concentration and therefore an increase in antithrombotic activity. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), and large doses of penicillins inhibit platelet function, prolong bleeding time, and have the potential to increase the risk of warfarin-associated bleeding, especially upper gastrointestinal bleeding due to their gastric erosion effect. Many drug interactions with warfarin are caused by alterations in metabolism either by CYP2C9 enzyme induction [11], which increases warfarin clearance and thereby reducing antithrombotic activity (e.g., phenytoin, rifampin) [12], or stereoselective and nonselective enzyme inhibition (e.g., amiodarone, cimetidine, sulfamethoxazole, metronidazole) [13], which increases its antithrombotic effect (and the INR). Amiodarone is a potent inhibitor of the metabolic clearance of both the S-enantiomer and the R-enantiomer and potentiates warfarin anticoagulation [14]. The anticoagulant effect of warfarin is augmented by second-generation and third-generation cephalosporins, which inhibit the cyclic interconversion of vitamin K, by thyroxine, which increases the metabolism of coagulation factors, by clofibrate and by acetaminophen, by inhibition of VKOR through a toxic metabolite of the drug [2]. The effect of statins or fibrates on the risk of bleeding in patients on VKAs is controversial. The initiation of a fibrate or statin that inhibits CYP3A4 enzymes was reported to increase the risk of gastrointestinal bleeding, whereas statins that are mainly excreted unchanged were not found to be associated with such an increased risk [15, 16]. Furthermore, nutritional supplements and herbal products are particularly problematic in warfarin-treated patients, who often fail to inform physicians and use these products as self-medication. Fluctuating levels of dietary vitamin K derive predominantly from phylloquinones in plant material, i.e., green tea and *natto* [17, 18].

*2.1.1.5. Therapy management*

Patients at low risk of thrombosis (i.e., AF) do not require heparin treatment at the beginning of warfarin therapy and a low initial dose regimen starting with 3 mg warfarin is recommended. The time taken to reach a therapeutic International Normalized Ratio (INR) is not critical; INR values should be monitored weekly on day 1 (baseline), day 8 and day 15, especially in older people who respond more slowly with changes to the INR. All patients who are sensitive to warfarin effects should monitor their INR more frequently (i.e., every 3–4 days). Patients at high risk of thrombosis (i.e., DVT) should be treated with heparin or LMWH when starting warfarin therapy for a minimum of 5 consecutive days. For initiation, a starting dose of 5 mg warfarin with daily INR monitoring for a minimum of 5 days is recommended. After day 4, clinicians should continue regular INR monitoring every 3 to 4 days until stabilized, and if the patient is still on heparin or LMWH, review the ongoing need for these additional anticoagulants. If INR values change of 0.5 over 3 days or 1.0 over 7 days, the INR is considered

unstable. After INR stabilization, clinicians should adopt a maintenance dosing. The weekly dose can be prescribed using a range of dosing regimens (i.e., alternate day dosing or dose regimens with different doses for weekdays compared to the weekend).

Dose modification should be taken into account following the INR monitoring:

- INR < 1.5: weekly dose should be increased by 20%;
- INR between 1.5 and 1.9: weekly dose should be increased by 10% only if the alteration of the values persists for more than a week;
- INR between 2 and 3: no change in dose regimen;
- INR between 3.1 and 3.9: weekly dose should be decreased by 10–20% only if the alteration of the values persists for more than a week;
- INR between 4 and 4.9: the patient has to omit one dose, decrease the weekly dose by 10–20%, and monitor INR in 2–5 days.

For INR values greater than or equal to 5, there is a significantly increased risk of bleeding and vitamin K administration should be evaluated. Patients have to cease warfarin therapy and restart with a reduced dose, when INR is minor than 5 [19].

In case of switch to another anticoagulant agent and in particular to a direct oral anticoagulant agents (DOACs) in patients with atrial fibrillation, INR should be strictly monitored. Apixaban and dabigatran should be started when INR < 2, rivaroxaban when INR < 3, and edoxaban when INR ≤ 2.5 [20].

*2.1.1.6. Real-world safety aspects*

Vitamin K antagonists reduce stroke and systemic embolism by 64% and all-cause mortality by 26%, compared to placebo in patients with atrial fibrillation [21]. However, as already mentioned above, VKAs have many drug and food interactions and require routine INR monitoring, and these limitations result in under-treatment for 30–50% of AF patients [22].

The $CHADS_2$ scoring system [23] is a simple system that can be used to assess the annual risk of stroke in AF. In the $CHADS_2$ scoring system, each point increases the annual risk of stroke by a factor of 1.5. Treatment with warfarin is recommended for a $CHADS_2$ or $CHA_2DS_2VASc$ scores of equal to or greater than 2. While the $CHADS_2$ score is simple, it does not include many common stroke risk factors. The $CHA_2DS_2VASc$ score is inclusive of the most common stroke risk factors in everyday clinical practice and has been validated in multiple cohorts; the accumulated evidence shows that $CHA_2DS_2VASc$ is better at identifying "truly low-risk" patients with AF and is as good as, and possibly better than, scores such as $CHADS_2$ in identifying patients who develop stroke and thromboembolism [24]. Direct comparison between the effects of warfarin and aspirin has been undertaken in several studies, demonstrating that warfarin was significantly superior, with a relative risk (RR) reduction of stroke of 39%.

In clinical practice, the risk of stroke should be weighed against the risk of bleeding to assess appropriateness of anticoagulant therapy. Warfarin causes major bleedings in 1–2% of people treated and intracranial bleeding in 0.1–0.5% of patients each year of treatment [25]. The highest rate of major

bleeding occurs in the first 3 months of treatment [26]. In comparison, aspirin causes major bleeding in 1.3% of patients [26]. Absolute risk increases for intracranial hemorrhage with warfarin compared to aspirin is only 0.2% per year [27]. Risk of bleeding can be assessed using the HAS-BLED scoring system, where a bleeding risk score of equal to or greater than 3 indicates high risk. There are other bleeding risk assessment tools available including HEMORR$_2$HAGES [23]. Assessment may identify reversible risks that can be managed prior to initiation of warfarin. In general, clinicians should be cautious and conduct regular review of the patient if initiating warfarin [24].

#### *2.1.2. Acenocoumarol and phenprocoumon*

Like warfarin, acenocoumarol and phenprocoumon also exist as optical isomers that have different stereochemical characteristics. R-acenocoumarol has an elimination half-life of 9 h; it is primarily metabolized by CYP2C9 and CYP2C19 and is more potent than S-acenocoumarol. In fact, S-acenocoumarol has a faster clearance (elimination half-life of 0.5 h), it is primarily metabolized by CYP2C9 and undergoes extensive first pass metabolism. The treatment dose may vary between patients up to 10-fold, ranging from 1 to 9 mg daily [28, 29].

Phenprocoumon is a much longer acting agent, with both the R- and the S-isomers with elimination half-lives of 5.5 days. Both are metabolized by CYP2C9, and S-phenprocoumon is 1.5–2.5 times more potent than R-phenprocoumon. It is administered in daily maintenance doses of 0.75–9 mg [29, 30].

As for warfarin, allelic variants of CYP2C9, CYP2C9*2, and CYP2C9*3 could lead to bleeding complications, especially if they code for enzymes with approximately 12 and 5% of the enzymatic activity of the wild type genotype CYP2C9*1 [31].

### 2.2. Novel oral anticoagulants

#### *2.2.1. Direct oral anticoagulants*

Four non–vitamin K antagonist oral anticoagulants (NOACs), or direct oral anticoagulants (DOACs), are widely used as alternatives to warfarin: dabigatran etexilate, rivaroxaban, apixaban, and edoxaban. In contrast with warfarin, DOACs have a more predictable therapeutic effect, do not require routine INR monitoring, and have fewer potential drug-drug interactions and no restriction on dietary consumption of vitamin K–containing food [32].

##### *2.2.1.1. Mechanism of action*

DOACs act through direct inhibition of thrombin or inhibition of activated factor X (factor Xa). Dabigatran etexilate mesylate is a competitive direct thrombin inhibitor. Rivaroxaban, apixaban, and edoxaban inhibit factor Xa and prothrombinase activity, thus inhibiting the conversion of prothrombin to thrombin and decreasing thrombus formation.

##### *2.2.1.2. Indications*

DOACs indications comprehend thromboembolic prevention in patients with nonvalvular atrial fibrillation (AF), DVT, and pulmonary embolism. For each one of these conditions, dose regimen has to be adjusted. Creatinine clearance (CrCl) values should be checked for dose management.

*2.2.1.3. Pharmacokinetics*

**Dabigatran** etexilate has a bioavailability of 3–7%, and it is bound to plasma proteins for a total amount of 35%. Its plasma half-life ranges between 12 and 17 hours but increases to 18–28 hours in case of mild to severe renal impairment. Dabigatran is converted into active dabigatran through hepatic and plasma hydrolysis and hepatic glucuronidation. Excretion occurs through the kidney after i.v. administration, while after oral administration, 7% of drug is recovered in urine and 86% is excreted in feces.

**Rivaroxaban** reaches the peak of plasma concentration in 2–4 hours, and its bioavailability ranges between 66% (20 mg) and 80–100% (10 mg); 92–95% of the drug administered is bound to plasma proteins for a total volume of distribution of 50 L. Hepatic oxidation by CYP3A4/5, CYP2J2, and hydrolysis converts rivaroxaban to inactive metabolites excreted through kidney (66%) and feces (28%).

**Apixaban** has a bioavailability of 50% and reaches plasma peak concentration in 3–4 hours. It is bound for 87% to plasmatic proteins, and it is metabolized by CYP3A4/5, 1A2, 2C9, 2C19, 2 J2, O-demethylation, and hydroxylation in the liver. Excretion occurs through the kidney (27%) and intestinal and biliary tract.

**Edoxaban** reaches the peak of plasma concentration in 1–2 hours, has a bioavailability of 62%, and it is bound for 55% to plasma protein. Its plasma half-life ranges from 10 to 14 hours. Edoxaban is converted through CYP3A4, hydrolysis, conjugation, and oxidation. Metabolites are excreted through kidneys (50%) and biliary/intestinal excretion [33].

*2.2.1.4. Interactions*

All DOACs are substrate of P-glycoprotein (P-gp), and apixaban and rivaroxaban are substrates for CYP3A4 metabolism. Thus, concomitant medications that are inducers or inhibitors of these pathways should be evaluated for potential interactions [34]. In particular, concomitant use of rifampin (P-gp inducer), carbamazepine, phenytoin (both P-gp and CYP3A4 inducers) and cyclosporine, ketoconazole, verapamil, and HIV protease inhibitors (both P-gp and CYP3A4 inhibitors) should be avoided or needs an adjustment of DOACs treatment [34, 35].

*2.2.1.5. Therapy management*

The main advantage of DOACs is a more rapid onset and offset of action that eliminates the necessity of routine INR monitoring. In case of acute care or perioperative settings, when there is uncertainty about the timing of last ingestion, renal function, and gastrointestinal absorption, there are several values that can be checked to assess DOACs effect, such as activated partial thromboplastin time (aPPT), prothrombin time (PT), and antifactor Xa activity. During rivaroxaban treatment renal function (CrCl), complete blood count (CBC) tests and hepatic function monitoring are required periodically, at least annually.

**Dabigatran** treatment of nonvalvular AF provides:

- 150 mg twice daily if CrCl>30 mL/min;
- 75 mg twice daily if CrCl is between 15 and 30 mL/min;
- 75 mg twice daily if CrCl is between 30 and 50 mL/min with concomitant P-gp inhibitors.

**Rivaroxaban** is administered with the evening meal on the basis of CrCl values:

- 20 mg daily if CrCl<50 mL/min;
- 15 mg daily if CrCl is between 15 and 50 mL/min.

**Apixaban** is given at the dose of 5 mg twice daily in patients with nonvalvular AF and reduced to 2.5 mg twice daily if there are at least two of the following conditions: body weight ≤ 60 kg, creatinine ≥ 1.5 mg/dL, age ≥ 80 years. Apixaban is not recommended in case of severe hepatic impairment.

**Edoxaban** is administered on the basis of CrCl:

- 60 mg daily if CrCl is between 50 and 95 mL/min;
- 30 mg daily if CrCl is between 15 and 50 mL/min.

*2.2.1.6. Real-world safety aspects*

*2.2.1.6.1. Dabigatran*

A systematic review and meta-analysis was performed with the aim of summarizing all available evidence from high-quality real-world observational studies regarding efficacy and safety of non–vitamin-K oral anticoagulants compared with vitamin-K antagonists in patients with atrial fibrillation [36]. Compared with warfarin, dabigatran is associated with lower risk for intracranial hemorrhage in several studies that included 606.855 patients (hazard ratio (HR), 0.42; 95% confidence interval (CI), 0.37–0.49) and lower risk for death in 319.486 patients (HR, 0.63; 95% CI, 0.52–0.76). There is no statistical difference between dabigatran and warfarin for the outcomes of ischemic strokes (HR, 0.96; 95% CI, 0.80–1.16); ischemic stroke or systemic embolism (HR, 1.17; 95% CI, 0.92–1.50); any stroke or systemic embolism (HR, 0.93; 95% CI, 0.77–1.14); major hemorrhage (HR, 0.83; 95% CI, 0.65–1.05); and myocardial infarction (HR, 0.96; 95% CI, 0.77–1.21). Authors identified 10 studies that included 537.770 patients which assessed the outcome of gastrointestinal hemorrhage and reported higher risk of dabigatran compared with warfarin (HR, 1.20; 95% CI, 1.06–1.36). Authors also reported the presence of a significant heterogeneity in all analyses with the exception of the outcome of intracranial hemorrhage and any stroke or systemic embolism.

*2.2.1.6.2. Rivaroxaban*

As reported in the study authored by Ntaios and Colleagues [36], compared with warfarin, rivaroxaban is associated with lower risk for intracranial hemorrhage in several real-world studies that included 136,221 patients (HR, 0.64; 95% CI, 0.47–0.86). There is no statistical difference between rivaroxaban and warfarin for the outcomes of ischemic stroke (HR, 0.89; 95% CI, 0.76–1.04); ischemic stroke or systemic embolism (HR, 0.73; 95% CI, 0.52–1.04); any stroke or systemic embolism (HR, 0.87; 95% CI, 0.71–1.07); major hemorrhage (HR, 1.00; 95% CI, 0.92–1.08); myocardial infarction (HR, 1.02; 95% CI, 0.54–1.89); and death (HR, 0.67; 95% CI, 0.35–1.30). Authors identified four studies that included 71.368 patients which assessed the

outcome of gastrointestinal hemorrhage and reported higher risk of rivaroxaban compared with warfarin (HR, 1.24; 95% CI, 1.08–1.41). Authors reported a significant heterogeneity for the outcomes of intracranial hemorrhage and death.

*2.2.1.6.3. Apixaban*

Compared with warfarin, apixaban is associated with lower risk for intracranial hemorrhage in several real-world studies that included 66.482 patients (HR, 0.45; 95% CI, 0.31–0.63); lower risk for gastrointestinal hemorrhage in 2 studies that included 33.323 patients (HR, 0.63; 95% CI, 0.42–0.95); and lower risk for major hemorrhage in 4 studies that included 89.036 patients (HR, 0.55; 95% CI, 0.48–0.63) [36]. Authors identified only one study of 41.785 patients which assessed death; it reported lower risk with apixaban compared with warfarin (HR, 0.65; 95% CI, 0.56–0.75). Also, they identified only one study of 15.390 patients which assessed the outcome of any stroke or systemic embolism; it reported lower risk of apixaban compared with warfarin (HR, 0.67; 95% CI, 0.46–0.98). There is neither a statistical difference between apixaban and warfarin for the outcomes of ischemic stroke (HR, 0.95; 95% CI, 0.75–1.19) nor for ischemic stroke or systemic embolism (HR, 1.07; 95% CI, 0.87–1.31). Authors reported a significant degree of heterogeneity in the analysis of the outcomes of gastrointestinal hemorrhage but not for the other outcomes.

*2.2.2. Betrixaban*

Betrixaban is an oral factor Xa inhibitor whose extended duration treatment reduced a composite of asymptomatic DVT, symptomatic DVT, nonfatal PE, and venous thromboembolism (VTE)–related death compared to enoxaparin, without an increase in major bleeding [37]. It acts by competitively binding to the active site of Xa, preventing its ability to convert prothrombin to thrombin. Betrixaban is rapidly absorbed with mean peak concentrations occurring within 3–4 hours after oral administration. Excretion is mostly unchanged through the bile with renal excretion accounting for 5–7% of the orally administered dose. Betrixaban is not a substrate for major cytochrome P450 enzymes, but it is a substrate for P-gp. Potent inhibitors of P-gp (i.e., ketoconazole, amiodarone, and diltiazem) increase betrixaban concentrations around twofold [38]. A recent network meta-analysis aimed to comprehensively analyze the thromboprophylactic drugs that are used to prevent thrombosis and reduce bleeding risk. Results showed that sudoxicam, FXI-ASO (factor XI antisense oligonucleotide), and betrixaban were likely to be associated with the lowest risk of all-cause bleeding after major joint surgery. Furthermore, betrixaban, dalteparin, and warfarin were associated with the lowest risk of major bleeding/nonmajor clinically relevant bleeding events [39].

The FDA (Food and Drug Administration) approved betrixaban on June 2017 for the prophylaxis of VTE in adult patients hospitalized for an acute medical illness, who are at risk for thromboembolic complications due to moderate or severe restricted mobility and other risk factors for VTE. The recommended dose of betrixaban is an initial single dose of 160 mg starting on day 1, followed by 80 mg once daily taken for 35–42 days at the same time each day with food. Dose should be reduced in case of severe renal and hepatic impairment or

concomitant treatment with P-gp inhibitors. The most common adverse reactions (≥5%), observed in the APEX trial considered for approval, were related to bleeding.

#### *2.2.3. Sulodexide*

Sulodexide belongs to a class of substances known as glycosaminoglycans (GAGs), composed of a fast-moving heparin fraction (80%) with affinity for antithrombin III and a dermatan sulfate fraction (20%) with affinity for heparin cofactor II.

##### *2.2.3.1. Mechanism of action*

Sulodexide exerts a strong antithrombotic activity by simultaneously potentiating the anti-protease activities of both antithrombin III and heparin cofactor II. Sulodexide also has a profibrinolytic effect (through activation of tissue plasminogen activator and inhibition of plasminogen activator inhibitor), an antiproliferative effect on smooth muscle cells, and antilipemic and antiatherosclerotic effects [40, 41].

##### *2.2.3.2. Indications*

Several clinical studies have demonstrated the efficacy of sulodexide in the treatment or prevention of vascular diseases associated with increased thrombotic risk, such as peripheral arterial occlusive diseases, post-myocardial infarction, recurrent DVT, and postthrombotic syndrome [42]. Other clinical applications are treatment of venous ulcers, cerebrovascular disorders, diabetic nephropathy, and other diabetic complications (nephropathy and macular edema).

##### *2.2.3.3. Pharmacokinetics*

Sulodexide is available for both intravenous and intramuscular administration, but it can be taken also orally. By the oral route, Sulodexide is absorbed within 1–2 hours and behaves as a mono-compound, reaching the time to peak in about 4 hours with a dose of 50–100 mg. Similar concentrations are maintained at least up to 18 hours. The distribution volume is very large, due to a higher affinity of sulodexide for the extensive surface area of the endothelium rather than for plasma proteins. Metabolism is liver dependent and based on N-desulfation, while excretion is mostly kidney dependent [40, 43].

##### *2.2.3.4. Therapy management*

Nowadays, sulodexide 250 LSU (lipasemic unit) capsules or 600 LSU parenteral preparation is approved only in Europe (Italy) for venous chronic ulcers in adults (250 LSU twice daily, away from meals).

##### *2.2.3.5. Safety*

Oral administration could lead to disorders of the gastrointestinal system with nausea, vomiting, and heartburn.

## 3. Parenteral anticoagulants

### 3.1. Heparins and low-molecular-weight heparins

Heparan sulfate (HS) proteoglycans play vital functions in many biological processes in the animal kingdom, and the GAG moiety is essential for these functions. Heparin is synthesized from UDP-sugar precursors as a polymer of alternating D-glucuronic acid and N-acetyl-D-glucosamine residues [44]. Unfractionated heparin (UFH) is a glycosaminoglycan consisting of heterogeneous mixture of polysaccharide chains with alternating residues of D-glucosamin and uronic acid, glucoronic acid, or iduronic acid, with a molecular weight range of 3000–30,000 Da. Low-molecular-weight heparins (LWMHs) are fragments of UFH produced by controlled enzymatic or chemical depolymerization processes with a mean molecular weight of about 5000 Da [45].

#### *3.1.1. Mechanism of action*

Both UFH and LMWHs exert their anticoagulant activity by inhibiting thrombin-activated conversion of fibrinogen to fibrin [46]: binding of a unique pentasaccharide to antithrombin causes a conformational change in antithrombin that accelerates its interaction with thrombin and factor Xa by about 1000 times. Binding of the pentasaccharide to antithrombin results directly in inhibition of factor Xa, and the pentasaccharide also blocks the activation of factor IX and neutralizes factor Xa by activating factor X inhibitor.

#### *3.1.2. Indications*

Heparins, and in particular LMWHs, indications comprehend: DVT prophylaxis during perioperative or postoperative period of general/orthopedic surgery or in bedridden patients; DVT treatment in patients affected by pulmonary embolism; instable angina and non-ST-elevation myocardial infarction (NSTEMI) prophylaxis with concomitant use of acetylsalicylic acid; coagulation prevention in patients undergoing dialysis; and symptomatic VTE (proximal DVT and/or pulmonary embolism) to reduce the recurrence of VTE in patients with solid tumor cancers.

#### *3.1.3. Pharmacokinetics*

Heparin is not absorbed orally and therefore is administered parenterally. The two preferred routes of administration are continuous intravenous infusion or subcutaneous injection. If administered subcutaneously, the dose of heparin should be higher than the usual intravenous dose because subcutaneous administration is associated with reduced bioavailability [46]. UFH does not have predictable pharmacokinetics, and it has a small volume of distribution and a relatively short half-life of about 0.5–1 hour [47]. After injection heparin rapidly disappears from blood: the rapid, saturable elimination phase is thought to reflect UFH binding to vascular endothelial cells, macrophages, and reticuloendothelial cells, where it is internalized and metabolized into smaller and less sulfated forms [48]. At higher doses, the cellular binding sites are saturated, and heparin is cleared predominantly by renal elimination [49]. UFH binds also to plasma proteins, changing its pharmacokinetic profile and reducing its

anticoagulant activity. Because of the unpredictable anticoagulant response, careful/close monitoring is essential, when UFH is given in therapeutic doses [48].

LMWHs do not bind to endothelial cells, macrophages, or reticuloendothelial cells and have a 2–4 times longer half-life compared to UFH (3–6 hours). Furthermore, LMWHs have much lower affinity for heparin-binding plasma proteins and are mainly removed by nonsaturable renal filtration, and thus, their clearance is independent of dose and plasma concentration [50].

#### *3.1.4. Interactions*

Heparin use should be avoided in case of concomitant treatment with antiplatelet drugs (NSAIDs, diclofenac, piroxicam, ketorolac, nimesulide, and acetylsalicylic acid), anticoagulant agents (warfarin), and glucocorticoids, due to increase in bleeding risk. Furthermore, LMWH should not be used in patients with previous heparin-induced thrombocytopenia/thrombosis (HITT), known hypersensitivity or adverse reaction to LMWH (dalteparin or enoxaparin), severe renal impairment, active bleeding, severe or uncontrolled hypertension, active peptic ulcerations, hemophilia, and severe liver disease.

#### *3.1.5. Therapy management*

Heparin therapy for VTE treatment is typically administered by continuous intravenous infusion, but adjusted dose and fixed dose subcutaneous injections can also be utilized. Obese patients clear LMWHs faster than nonobese patients due to hyperfiltration, and their dose should be adjusted on total body weight or LMWHs should be substituted with UFHs [51]. Therapy monitoring comprehends regular CrCl, platelet count, and antifactor Xa assay measurements.

In case of VTE prophylaxis, dosing and duration of LMWHs follow this pattern:

- dalteparin 5000 units subcutaneously once daily for 5–10 days or enoxaparin 40 mg subcutaneously once daily for 7–10 days in patients undergoing surgery interventions, who had previously VTE or are bedridden;
- dalteparin 2500 units subcutaneously once daily or enoxaparin 20 mg subcutaneously once daily in case of renal impairment.

Dosing recommendations for treatment of NSTEMI with enoxaparin take into account renal functions and concomitant therapies with antiplatelet agents. In particular:

- enoxaparin 1 mg/kg subcutaneously every 12 hours if CrCl is >50 mL/min;
- enoxaparin 1 mg/kg subcutaneously every 12 hours if CrCl is between 30 and 50 mL/min with continue; monitoring of renal function and antifactor Xa levels;
- heparins use is not recommended if CrCl is <30 mL/min.

In case of thrombolysis, anticoagulation is generally given in addition to dual antiplatelet therapy at doses adjusted on patient's age:

- age < 75 years, 30 mg i.v. bolus immediately prior to thrombolysis followed within 15 minutes by 1 mg/kg subcutaneously every 12 hours (each dose should not exceed 100 mg);
- age > 75 years, 0.75 mg/kg subcutaneously every 12 hours (each dose should not exceed 75 mg in the first two administrations);
- UFHs in patients with CrCl is <30 mL/min.

Intravenous anticoagulation with UFHs in addition to antiplatelet therapy is recommended for all patients undergoing primary percutaneous coronary intervention (PCI). Anticoagulant therapy is selected according to patient's ischemic and bleeding risks (70–100 units/kg i.v. bolus or 50–70 units/kg i.v. bolus with GPIIb/IIIa inhibitor). Bivalirudin or intravenous enoxaparin (0.5 mg/kg i.v.) may be used as alternatives to UFH.

*3.1.6. Real-world safety aspects*

Safe and effective use of heparin requires maintaining a delicate balance: dosing low enough to minimize the risk of bleeding, yet high enough to treat or prevent thrombosis. Achieving a therapeutic level of heparin within 24 hours significantly reduces the risk for recurrent VTE [52]. However, non-protocol-driven practice achieves this outcome only 40% of the time [53].

Bleeding is the primary untoward effect of heparin. Major bleeding occurs in 1–5% of patients treated with intravenous heparin for venous thromboembolism [54]. The incidence of bleeding is somewhat less in patients treated with LMWH for this indication, although the risk of bleeding appears to increase with higher total daily doses of heparin.

Heparin-induced thrombocytopenia (platelet count <150,000/mL or a 50% decrease from the pre-treatment value) occurs in ~0.5% of medical patients 5–10 days after initiation of therapy with heparin [55]. Although the incidence may be lower, thrombocytopenia also occurs with LMWHs and fondaparinux and platelet counts should be monitored. Thrombotic complications that can be life-threatening or lead to amputation occur in about one-half of the affected heparin-treated patients and may precede the onset of thrombocytopenia. The incidence of heparin-induced thrombocytopenia and thrombosis is higher in surgical patients than in medical patients. Women are twice as likely as men to develop this condition.

### 3.2. Fondaparinux, idraparinux, and idrabiotaparinux

Fondaparinux is a synthetic heparin-like compound that acts as indirect factor Xa inhibitor [50]. Idraparinux and idrabiotaparinux are second and third generation pentasaccharides derived from fondaparinux.

After subcutaneous administration, fondaparinux is cleared by kidneys and has an elimination half-life of 17 h in healthy volunteers and 29 h in patients with moderate renal insufficiency, and therefore, it is contraindicated in patients with severe renal insufficiency. In this specific condition, laboratory monitoring is performed with anti-Xa assay, aPTT, and PT [50]. Fondaparinux is approved as an alternative for heparin or LMWH in the initial treatment of VTE in conjunction with a vitamin K antagonist (VKA) and, in Europe, for ACS in patients for whom PCI is not indicated [56].

Idraparinux has a longer elimination half-time of 120 h after a single administration and accumulation does occur and after more than 6 months of treatment, the elimination half-time is increased up to 60 days. It is administered once weekly and is thereby suitable for long-term anticoagulation [57]. Idrabiotaparinux was evaluated for treatment of VTE and for stroke prevention in patients with AF, resulting as safe and efficient as idraparinux [58].

### 3.3. Irudin, lepirudin, desirudin, bivalirudin, and argatroban

Hirudin is a direct thrombin inhibitor (DTI) derived from the salivary secretions of leech (*Hirudo medicinalis*). Lepirudin and desirudin are two forms of recombinant hirudin, structurally identical except for minute differences in the amino-terminus sequence [59]. Unlike heparin and others anticoagulants, DTIs do not need antithrombin to perform its anticoagulant action: epirudin and desirudin form a bivalent irreversible complex with thrombin, while hirudin binds to both the active site as well as to exosite I on thrombin.

Lepirudin and desirudin have been evaluated for the prevention and treatment of VTE and in patients with acute coronary syndrome (ACS). These drugs are generally more effective than heparin in prevention of thrombosis but lead to more bleeding complications possibly related to their irreversible binding to thrombin [60]. Their indications of use comprehend: treatment and prevention of suspected or proven HIT; VTE prophylaxis after hip or knee arthroplasty. Lepirudin and desirudin are administered intravenously or subcutaneously and have a half-life of 80 and 60–120 minutes, respectively. The excretion is totally renal. Due to their narrow therapeutic range, therapy monitoring should be done regularly through aPTT, activated clotting time (ACT), and anti-Xa assay evaluation.

Bivalirudin is used in treatment of patients with unstable angina undergoing PCI even if at risk of HIT. This is a synthetic analog of hirudin that forms a reversible, high-affinity complex with thrombin [61]. Consequently, bivalirudin has a shorter half-life (25 minutes) and is a weaker thrombin inhibitor compared to hirudin, with a potentially larger therapeutic window. Its clearance is for 80% enzymatic and for 20% renal. Bivalirudin is now one of the preferred drugs for patients undergoing PCI in American and European guidelines, and it has become one of the most widely used antithrombotics in the USA for PCI [62].

Argatroban is a small, univalent competitive inhibitor of thrombin. It binds selectively and reversibly to the active site of thrombin and has a short elimination half-life of 50 min through hepatobiliary clearance. Since it is metabolized in the liver, it should be used with caution in patients with liver failure. Argatroban has been approved for the prevention and treatment of VTE in patients with HIT [46, 47] and for patients with (a history of) HIT who need to undergo PCI [46, 63].

## Author details

Niccolò Lombardi, Giada Crescioli and Alfredo Vannacci*

*Address all correspondence to: alfredo.vannacci@unifi.it

Department of Neurosciences, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy

## References

[1] Tran R, Myers DR, Ciciliano J, Trybus Hardy EL, Sakurai Y, Ahn B, et al. Biomechanics of haemostasis and thrombosis in health and disease: From the macro- to molecular scale. Journal of Cellular and Molecular Medicine. 2013;**17**:579-596

[2] Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy: Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2012;**141**:e44S-e88S

[3] Di Minno A, Frigerio B, Spadarella G, Ravani A, Sansaro D, Amato M, et al. Old and new oral anticoagulants: Food, herbal medicines and drug interactions. Blood Reviews. 2017;**31**:193-203

[4] Hawkins D. Limitations of traditional anticoagulants. Pharmacotherapy. 2004;**24**:62S-65S

[5] Geisen C, Watzka M, Sittinger K, Steffens M, Daugela L, Seifried E, et al. VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation. Thrombosis and Haemostasis. 2005;**94**:773-779

[6] Hirsh J, Fuster V, Ansell J, Halperin JL, American Heart Association/American College of Cardiology F. American Heart Association/American College of Cardiology Foundation guide to warfarin therapy. Journal of the American College of Cardiology. 2003;**41**: 1633-1652

[7] Hall JG, Pauli RM, Wilson KM. Maternal and fetal sequelae of anticoagulation during pregnancy. The American Journal of Medicine. 1980;**68**:122-140

[8] Fogerty AE. Challenges of anticoagulation therapy in pregnancy. Current Treatment Options in Cardiovascular Medicine. 2017;**19**:76

[9] Hirsh J. Oral anticoagulant drugs. The New England Journal of Medicine. 1991;**324**: 1865-1875

[10] Jacobs LG. Warfarin pharmacology, clinical management, and evaluation of hemorrhagic risk for the elderly. Cardiology Clinics. 2008;**26**:157-167

[11] Cropp JS, Bussey HI. A review of enzyme induction of warfarin metabolism with recommendations for patient management. Pharmacotherapy. 1997;**17**:917-928

[12] Greenblatt DJ, von Moltke LL. Interaction of warfarin with drugs, natural substances, and foods. Journal of Clinical Pharmacology. 2005;**45**:127-132

[13] Breckenridge A, Orme M, Wesseling H, Lewis RJ, Gibbons R. Pharmacokinetics and pharmacodynamics of the enantiomers of warfarin in man. Clinical Pharmacology and Therapeutics. 1974;**15**:424-430

[14] O'Reilly RA, Trager WF, Rettie AE, Goulart DA. Interaction of amiodarone with racemic warfarin and its separated enantiomorphs in humans. Clinical Pharmacology and Therapeutics. 1987;**42**:290-294

[15] Schelleman H, Bilker WB, Brensinger CM, Wan F, Yang YX, Hennessy S. Fibrate/statin initiation in warfarin users and gastrointestinal bleeding risk. The American Journal of Medicine. 2010;**123**:151-157

[16] Douketis JD, Melo M, Bell CM, Mamdani MM. Does statin therapy decrease the risk for bleeding in patients who are receiving warfarin? The American Journal of Medicine. 2007;**120**:369 e9-369 e14

[17] Holbrook AM, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M, et al. Systematic overview of warfarin and its drug and food interactions. Archives of Internal Medicine. 2005;**165**:1095-1106

[18] Schurgers LJ, Teunissen KJ, Hamulyak K, Knapen MH, Vik H, Vermeer C. Vitamin K-containing dietary supplements: Comparison of synthetic vitamin K1 and natto-derived menaquinone-7. Blood. 2007;**109**:3279-3283

[19] Guidelines for warfarin management in the community. Available at: https://www.health.qld.gov.au/clinical%20practice/guidelines-procedures/medicines?a=165945

[20] Kovacs RJ, Flaker GC, Saxonhouse SJ, Doherty JU, Birtcher KK, Cuker A, et al. Practical management of anticoagulation in patients with atrial fibrillation. Journal of the American College of Cardiology. 2015;**65**:1340-1360

[21] Hart RG, Pearce LA, Aguilar MI. Adjusted-dose warfarin versus aspirin for preventing stroke in patients with atrial fibrillation. Annals of Internal Medicine. 2007;**147**:590-592

[22] De Caterina R, Husted S, Wallentin L, Andreotti F, Arnesen H, Bachmann F, et al. Vitamin K antagonists in heart disease: Current status and perspectives (section III). Position paper of the ESC working group on thrombosis—Task force on anticoagulants in heart disease. Thrombosis and Haemostasis. 2013;**110**:1087-1107

[23] Gage BF, Yan Y, Milligan PE, Waterman AD, Culverhouse R, Rich MW, et al. Clinical classification schemes for predicting hemorrhage: Results from the National Registry of Atrial Fibrillation (NRAF). American Heart Journal. 2006;**151**:713-719

[24] European Heart Rhythm A, European Association for Cardio-Thoracic S, Camm AJ, Kirchhof P, Lip GY, Schotten U, et al. Guidelines for the management of atrial fibrillation: The task force for the management of atrial fibrillation of the European Society of Cardiology (ESC). European Heart Journal. 2010;**31**:2369-2429

[25] Gallus AS, Baker RI, Chong BH, Ockelford PA, Street AM. Consensus guidelines for warfarin therapy. Recommendations from the Australasian Society of Thrombosis and Haemostasis. The Medical Journal of Australia. 2000;**172**:600-605

[26] van Walraven C, Hart RG, Singer DE, Laupacis A, Connolly S, Petersen P, et al. Oral anticoagulants vs aspirin in nonvalvular atrial fibrillation: An individual patient meta-analysis. JAMA. 2002;**288**:2441-2448

[27] Hart RG, Pearce LA, Aguilar MI. Meta-analysis: Antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. Annals of Internal Medicine. 2007;**146**:857-867

[28] Godbillon J, Richard J, Gerardin A, Meinertz T, Kasper W, Jahnchen E. Pharmacokinetics of the enantiomers of acenocoumarol in man. British Journal of Clinical Pharmacology. 1981;**12**:621-629

[29] Verhoef TI, Redekop WK, Daly AK, van Schie RM, de Boer A, Maitland-van der Zee AH. Pharmacogenetic-guided dosing of coumarin anticoagulants: Algorithms for warfarin, acenocoumarol and phenprocoumon. British Journal of Clinical Pharmacology. 2014;**77**:626-641

[30] Haustein KO. Pharmacokinetic and pharmacodynamic properties of oral anticoagulants, especially phenprocoumon. Seminars in Thrombosis and Hemostasis. 1999;**25**:5-11

[31] Rettie AE, Wienkers LC, Gonzalez FJ, Trager WF, Korzekwa KR. Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. Pharmacogenetics. 1994;**4**: 39-42

[32] Levy JH, Faraoni D, Spring JL, Douketis JD, Samama CM. Managing new oral anticoagulants in the perioperative and intensive care unit setting. Anesthesiology. 2013;**118**:1466-1474

[33] Raval AN, Cigarroa JE, Chung MK, Diaz-Sandoval LJ, Diercks D, Piccini JP, et al. Management of patients on non-vitamin K antagonist oral anticoagulants in the acute care and periprocedural setting: A scientific statement from the American Heart Association. Circulation. 2017;**135**:e604-e633

[34] Piccini JP, Hellkamp AS, Washam JB, Becker RC, Breithardt G, Berkowitz SD, et al. Polypharmacy and the efficacy and safety of rivaroxaban versus warfarin in the prevention of stroke in patients with nonvalvular atrial fibrillation. Circulation. 2016;**133**:352-360

[35] Ruff CT, Giugliano RP, Braunwald E, Morrow DA, Murphy SA, Kuder JF, et al. Association between edoxaban dose, concentration, anti-factor Xa activity, and outcomes: An analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. Lancet. 2015;**385**:2288-2295

[36] Ntaios G, Papavasileiou V, Makaritsis K, Vemmos K, Michel P, Lip GYH. Real-world setting comparison of nonvitamin-K antagonist oral anticoagulants versus vitamin-K antagonists for stroke prevention in atrial fibrillation: A systematic review and meta-analysis. Stroke; a Journal of Cerebral Circulation. 2017;**48**:2494-2503

[37] Cohen AT, Harrington RA, Goldhaber SZ, Hull RD, Wiens BL, Gold A, et al. Extended thromboprophylaxis with betrixaban in acutely ill medical patients. The New England Journal of Medicine. 2016;**375**:534-544

[38] Cohen AT, Harrington R, Goldhaber SZ, Hull R, Gibson CM, Hernandez AF, et al. The design and rationale for the Acute Medically Ill Venous Thromboembolism Prevention with Extended Duration Betrixaban (APEX) study. American Heart Journal. 2014;**167**:335-341

[39] Wang Z, Zheng J, Zhao Y, Xiang Y, Chen X, Jin Y. Effectiveness and tolerability of anticoagulants for thromboprophylaxis after major joint surgery: A network meta-analysis. Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology. 2017;**42**:1999-2020

[40] Coccheri S, Mannello F. Development and use of sulodexide in vascular diseases: Implications for treatment. Drug Design, Development and Therapy. 2013;**8**:49-65

[41] Borawski J, Dubowski M, Rydzewska-Rosolowska A, Mysliwiec M. Intravenous and oral sulodexide versus coagulation activation markers in humans. Clinical and Applied Thrombosis/Hemostasis: Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis. 2009;**15**:596-598

[42] Elleuch N, Zidi H, Bellamine Z, Hamdane A, Guerchi M, Jellazi N, et al. Sulodexide in patients with chronic venous disease of the lower limbs: Clinical efficacy and impact on quality of life. Advances in Therapy. 2016;**33**:1536-1549

[43] Marchi E, Barbanti M, Milani R, Breccia A, Fini A, Gattavecchia E. Organ glycosaminoglycan distribution after intravenous and oral administration in rats. Seminars in Thrombosis and Hemostasis. 1994;**20**:297-300

[44] Sugahara K, Kitagawa H. Heparin and heparan sulfate biosynthesis. IUBMB Life. 2002;**54**:163-175

[45] Hirsh J, Levine MN. Low molecular weight heparin. Blood. 1992;**79**:1-17

[46] Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: Antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2012;**141**:e24S-e43S

[47] Middeldorp S. Heparin: From animal organ extract to designer drug. Thrombosis Research. 2008;**122**:753-762

[48] Leentjens J, Peters M, Esselink AC, Smulders Y, Kramers C. Initial anticoagulation in patients with pulmonary embolism: Thrombolysis, unfractionated heparin, LMWH, fondaparinux, or DOACs? British Journal of Clinical Pharmacology. 2017;**83**:2356-2366

[49] Boneu B, Caranobe C, Sie P. Pharmacokinetics of heparin and low molecular weight heparin. Baillière's Clinical Haematology. 1990;**3**:531-544

[50] Donat F, Duret JP, Santoni A, Cariou R, Necciari J, Magnani H, et al. The pharmacokinetics of fondaparinux sodium in healthy volunteers. Clinical Pharmacokinetics. 2002;**41**(Suppl 2):1-9

[51] Bazinet A, Almanric K, Brunet C, Turcotte I, Martineau J, Caron S, et al. Dosage of enoxaparin among obese and renal impairment patients. Thrombosis Research. 2005;**116**:41-50

[52] Anand SS, Bates S, Ginsberg JS, Levine M, Buller H, Prins M, et al. Recurrent venous thrombosis and heparin therapy: An evaluation of the importance of early activated partial thromboplastin times. Archives of Internal Medicine. 1999;**159**:2029-2032

[53] Wheeler AP, Jaquiss RD, Newman JH. Physician practices in the treatment of pulmonary embolism and deep venous thrombosis. Archives of Internal Medicine. 1988;**148**: 1321-1325

[54] Hirsh J, Anand SS, Halperin JL, Fuster V, American Heart Association. Guide to anticoagulant therapy: Heparin: A statement for healthcare professionals from the American Heart Association. Circulation. 2001;**103**:2994-3018

[55] Warkentin TE. Heparin-induced thrombocytopenia. Hematology/Oncology Clinics of North America. 2007;**21**:589-607

[56] Greinacher A. Heparin-induced thrombocytopenia. The New England Journal of Medicine. 2015;**373**:1883-1884

[57] Harenberg J, Jorg I, Vukojevic Y, Mikus G, Weiss C. Anticoagulant effects of idraparinux after termination of therapy for prevention of recurrent venous thromboembolism: Observations from the van Gogh trials. European Journal of Clinical Pharmacology. 2008;**64**:555-563

[58] Equinox I. Efficacy and safety of once weekly subcutaneous idrabiotaparinux in the treatment of patients with symptomatic deep venous thrombosis. Journal of Thrombosis and Haemostasis. 2011;**9**:92-99

[59] Di Nisio M, Middeldorp S, Buller HR. Direct thrombin inhibitors. The New England Journal of Medicine. 2005;**353**:1028-1040

[60] Effects of recombinant hirudin (lepirudin) compared with heparin on death, myocardial infarction, refractory angina, and revascularisation procedures in patients with acute myocardial ischaemia without ST elevation: A randomised trial. Organisation to Assess Strategies for Ischemic Syndromes (OASIS-2) Investigators. Lancet. 1999;**353**:429-438

[61] Maraganore JM, Bourdon P, Jablonski J, Ramachandran KL, Fenton JW 2nd. Design and characterization of hirulogs: A novel class of bivalent peptide inhibitors of thrombin. Biochemistry. 1990;**29**:7095-7101

[62] Task Force on the management of STseamiotESoC, Steg PG, James SK, Atar D, Badano LP, Blomstrom-Lundqvist C, et al. ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. European Heart Journal. 2012;**33**:2569-2619

[63] Lewis BE, Wallis DE, Leya F, Hursting MJ, Kelton JG, Argatroban I. Argatroban anticoagulation in patients with heparin-induced thrombocytopenia. Archives of Internal Medicine. 2003;**163**:1849-1856

Chapter 6

# Heparin-Induced Thrombocytopenia (HIT)

Maja Tomicic, Irena Jukic, Ana Hecimovic and Tomislav Vuk

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78024

**Abstract**

Heparin-induced thrombocytopenia (HIT I) is a severe, life-threatening, and immunological drug reaction. According to the clinical-laboratory characteristics, there are two types of HIT: type I (HIT I) and type II (HIT II). HIT I is the result of non-immunologic, direct interaction of heparin with the platelet surface. Contrary, HIT II is immunologically induced (antibody-mediated) and life-threatening side effect of heparin therapy, often associated with thromboembolic complications. All patients receiving heparin are exposed to the development of anti-heparin antibodies, irrespective of the heparin dosage, type, and method of administration. HIT most commonly develops in intensive care patients, dialyzed patients, and cardiosurgical and orthopedic patients. It commonly develops after 5–10 days of heparin therapy. Platelet count decreases by more than 50% from the baseline and ranges from 20 × $10^9$/L to 100 × $10^9$/L. In HIT II, thromboembolic complications usually include deep-vein thrombosis and pulmonary embolism, but they also include arterial occlusion of the extremities, myocardial infarction, stroke, and necrosis and organ damage. Clinical assessment of the HIT probability using 4T´s score system, systematic monitoring of platelet number in heparin-receiving patients, and specific laboratory diagnosis of anti-heparin antibodies substantially contribute to the final confirmation of the diagnosis, enable timely administration of direct non-heparin thrombin antagonists, and reduce mortality from thromboembolic complications.

**Keywords:** heparin, heparin-induced thrombocytopenia (HIT), HIT type I, HIT type II, anti-heparin-PF4 antibodies, thromboembolism, direct thrombin inhibitors, argatroban, lepirudin, anti FXa, fondaparinux, direct oral anticoagulants (DOACs), dabigatran, rivaroxaban, apixaban, danaparoid, IVIg

## 1. Introduction

Heparin-induced thrombocytopenia type II (HIT II) is a severe, life-threatening, immunological drug reaction. HIT II is an important side effect of heparin, the most commonly used anticoagulant agent. As opposed to bleeding caused by heparin overdose, some patients develop a paradoxical complication of heparin treatment – thromboembolism. According to the clinical-laboratory characteristics, there are two types of HIT: type I (HIT I) and type II (HIT II). HIT I is the result of non-immunologic, direct interaction of heparin with the platelet surface. It occurs in approximately 10% of the patients in the first several days of heparin treatment. Thrombocytopenia is mild and resolves within several days with the continuation of heparin therapy. Thromboembolic complications usually do not occur; therefore, it is of minor clinical significance. Contrary, HIT II is immunologically induced (antibody-mediated) and a life-threatening side effect of heparin therapy, often associated with thromboembolic complications [1]. The HIT may occur during the treatment with unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH). All patients receiving heparin are exposed to the development of anti-heparin antibodies, irrespective of heparin dosage (prophylactic or therapeutic), type (UFH or LMWH), and method of administration (subcutaneous or intravenous). Since heparin is also used for flushing intravenous lines, it may lead to the development of anti-heparin antibodies in patients who do not receive subcutaneous or intravenous heparin [1, 2]. HIT most often develops in intensive care patients, hemodialyzed patients, and cardiosurgical and orthopedic patients, who usually receive heparin. Heparin-induced thrombocytopenia (HIT) is a clinical-pathologic syndrome diagnosed based on clinical findings and laboratory evidence of antibodies directed to the heparin and platelet factor 4 complex (H-PF4). HIT II may also be defined as a transitory, autoimmune, and heparin-induced thrombocytopenia. Reaching the diagnosis of HIT is a complex process, because thrombocytopenia in patients receiving heparin may be caused by numerous other factors. Although clinical assessment is very important in suspecting HIT, laboratory diagnosis plays the key role in providing evidence for the diagnosis of HIT II [3–5]. HIT II occurs in 0.1–5.0% heparin-treated patients, predominantly in those receiving UFH. It commonly develops after 5–10 days of therapy, but it may also occur earlier during the treatment course if the patient has been exposed to heparin within the previous 100 days (early form). HIT II rarely develops after 20 or more days from the start of the therapy (late form). In HIT II, platelet count decreases by more than 50% from the baseline and ranges from $20 \times 10^9$/L to $100 \times 10^9$/L. HIT II patients are at high-risk of thromboembolic complications, venous and/or arterial, and allergic reactions. In HIT II, thromboembolic complications usually include deep-vein thrombosis and pulmonary embolism, but they also include arterial occlusion of the extremities, myocardial infarction, stroke, and necrosis and organ damage. Vein thrombosis may be found by duplex ultrasound in more than 50% of the patients with no clinical signs or symptoms of thrombosis [1–4]. In one-fourth of the HIT II patients, an allergic reaction may develop within 5–30 min after intravenous heparin administration (fever, chills, and respiratory distress). Rarely, an erythematous plaque or necrosis with pronouncedly painful skin may be observed. Complications related mortality rate was high (20–30%), but it has been significantly reduced in recent years due to the early diagnosis and treatment of HIT with heparin alternative [5, 6].

http://dx.doi.org/10.5772/intechopen.78024

### 1.1. Heparin

Heparin is a polymer of varying chain size. Two forms of heparin are used as paharmaceuticals: unfractionated heparin (UFH) that has not been fractionated to sequester the fraction of molecules with low molecular weight, and low molecular weight heparin (LMWH), which undergone fractionation. Either UFH or LMWH can be used in the prevention of thromboembolic events LMWH is preferable. Heparin binds to the enzyme inhibitor antithrombin III (AT) via a specific pentasaccharide sulfation sequence contained within the heparin polymer. The formation of a ternary complex between AT, thrombin, and heparin results in the inactivation of thrombin, factor Xa, and other proteases. In contrast, anti-factor Xa activity requires only the pentasaccharide binding site. The highly negative charge density of heparin contributes to its very strong electrostatic interaction with thrombin. For this reason, heparin's activity against thrombin is size-dependent, with the ternary complex requiring at least 18 saccharide units for efficient formation. The rate of inactivation of these proteases by AT can increase by up to 1000-fold due to the binding of heparin. The size difference of heparin has led to the development of low-molecular-weight heparins (LMWHs) and, more recently, to fondaparinux as pharmaceutical anticoagulants. Fondaparinux is a synthetic pentasaccharide, whose chemical structure is almost identical to the AT binding pentasaccharide sequence that can be found within polymeric heparin and heparan sulfate. LMWHs and fondaparinux target anti-factor Xa activity rather than AT activity, with the aim of facilitating a more suitable regulation of coagulation and an improved therapeutic index [6].

## 2. Pathophysiology

The pathophysiologic mechanism of HIT II is mediated by the formation of heparin-platelet factor 4 (PF4) complexes. The PF4 is a positively charged heterodimer found in platelet alpha granules, and heparin is a negatively charged molecule. The formation of heparin-PF4 complex results in the change in the tertiary structure of the PF4 and exposure of neo-peptide, which elicits the formation of antibodies, usually IgG isotype. The immune heparin-PF4-IgG complexes activate platelets via Fc y IIa receptors. The antibodies may bind to monocytes, which then release tissue factor, the most potent blood clotting factor. Activated platelets release procoagulant microparticles and PF4. Antibodies recognize the complexes, bind to the endothelial cells, and activate the coagulation cascade, which leads to the formation of thrombin and eventually thrombosis. The ability of HIT antibodies to strongly activate platelets even in the absence of heparin may cause heparin-independent HIT II [7–9].

HIT II is most often caused by IgG antibodies targeting heparin-PF4 complex. In patients with HIT antibodies present in the blood, re-administration of heparin causes a rapid decrease in the platelet counts (within hours) to extremely low values. In heparin-treated patients, platelet count should be monitored before and during therapy. Before the specific laboratory evidence of anti-heparin antibodies, the probability of HIT should be determined using clinical laboratory indicators.

## 3. Clinical diagnosis

Clinicians should assess whether the platelet count decrease is the result of anti-heparin antibodies or underlying disease. They should also be cautious not to over diagnose HIT, because some HIT antibodies are not pathogenic and will not necessarily lead to the clinical HIT syndrome. Before the specific laboratory evidence of anti-heparin antibodies, the probability of HIT should be determined using clinical-laboratory indicators. In heparin-treated patients, platelet count should be monitored before and during the course of therapy. According to the 2006 British Haematology Standards, platelet count should be determined in all patients on the day of the start of heparin therapy. In patients who received heparin within the previous 100 days, platelet count should be determined on the day of the start of heparin therapy and 24 h later. In patients receiving UFH, platelet count should be measured daily from day 4 to day 14, and every 2–4 days between day 4 and day 14 in patients receiving LWMH [10–13].

The clinical scoring system used for determining the probability of a HIT is the so-called "*4 T score*" - thrombocytopenia, timing of onset, thrombosis, and absence of other causes of thrombocytopenia (**Table 1**) [6]. Each of these symptoms is scored from 0 to 2 points. The total score of 0–3 indicates a low probability of HIT II, 4–5 indicates moderate, and 6–8 indicates a high

| **4Ts** | | **Score** |
|---|---|---|
| T1<br>Thrombo-cytopenia | (a) Platelet count decline by >50%, with lowest value of 20 × 10/9/L | 2 |
| | (b) Platelet count by 30–50%, with lowest value of 10–19 × 10/9/L | 1 |
| | (c) Platelet count decline by <30%, with lowest value below 10 × 10/9/L | 0 |
| T2<br>Timing od platelet count decline | (a) Occurrence of thrombocytopenia 5–10 days of initial heparin administration or <1 day (with previous exposure within 30 days) | 2 |
| | (b) Occurrence of thrombocytopenia >10 days of initial heparin administration or unknown or <1 day (with previous exposure within 30–100 days) | 1 |
| | (c) Occurrence of thrombocytopenia at <4 days (without previous recent heparin exposure) | 0 |
| T3<br>Thrombosis or other sequelae | (a) New thrombosis, skin necrosis, acute systemic reaction following bolus heparin | 2 |
| | (b) Progressive recurrent thrombosis, erythematous skin lesion, unconfirmed suspicion of thrombosis | 1 |
| | (c) No other causes of thrombocytopenia | 0 |
| T4<br>Other cause of thrombocytopenia | (a) No other cause of thrombocytopenia | 2 |
| | (b) Presence of other possible causes of thrombocytopenia | 1 |
| | (c) Definitive other cause of thrombocytopenia is present | 0 |

Low, 0–3; Moderate, 4–5; High, 6–8.

**Table 1.** Clinical assesement of Heparine induced thrombocytopenia (HIT) by use of modified 4T scoring system according to Lo and Warkentin [6].

probability of HIT II. To confirm the diagnosis of HIT II, laboratory evidence of anti-heparin antibodies is needed. Anti-heparin antibodies may be confirmed in about half of the patients with clinically suspected HIT requiring laboratory investigation. The frequency of positive results depends on the clinical 4 T score and sensitivity and specificity of the test used [6–9].

## 4. Laboratory diagnostics

Laboratory investigation of HIT includes two categories of tests: immunologic assays for detecting circulating anti-PF4/heparin antibodies usually of IgG class and functional assays which detect antiplatelet antibodies capable to induce heparin-dependent platelet activation and thrombogenic potential (**Table 2**) [10–12].

In laboratory investigations of HIT II, anti-heparin-PF4 antibody tests are most commonly used in immunologic assays and serotonin-release assay (SRA) to determine anti-heparin antibody-induced platelet activation. In addition to, SRA heparin-induced platelet activation/aggregation assay (HIPA) is used when the thrombogenic potential of the present antibodies should be determined or *in vitro* effectiveness of heparin, alternative should be estimated. Enzyme-immunologic (EIA) method and gel method are most commonly used for immunologic assays. EIA is performed on a microtiter plate, and heparin-PF4 antigen complex is applied to the plate wells. Gel test is performed on gel-filled microcolonies, and the heparin-PF4 complex is added into a microparticle suspension. These tests have a similar sensitivity (80–90%) and specificity (89–97%). The most important advantage of gel test (quick screening test) is a high negative predictive value (>95%) for the exclusion of HIT II. In the other group

| Method | Sensitivity | Specificity | NPV | PPV |
|---|---|---|---|---|
| Anti H-PF4 assays (antigen-antygody assays): | | | | |
| (a) Gel-columns-mycro-particle assay | High | Moderate | High | Low |
| (b) Lateral flow immunodiffudion assay-IgG | High | Moderate | High | Moderate |
| (c) EIA-IgG | High | Moderate | High | Moderate |
| Funtional assays: | Low/moderate | High | | High |
| (a) SRA-cr | | | | |
| (b) SRA-HPLC | | | | |
| (c)SRA-EIA | | | | |
| (d) HIPA | | | | |
| (e) HIMA | | | | |

H-PF4, heparin-platelet factor 4 complex; EIA, enzyme immuno assay; HPLC, high presure liquid cromatography; SRA, serotonin release assay; HIPA, heparin-induced platelet activation/aggregation; HIMA, heparin-induced platelet activation/multilate aggregation; NPV, negative predictive value; PPV, positive predictive value; H-PF4, heparin-platelet factor 4 complex.

**Table 2.** Methods for anti-heparin antibodies detection.

of tests, heparin-induced platelet activation (HIPA), and serotonin-release assay (SRA) are the most commonly used tests. They have lower sensitivity, but higher specificity than the first group of tests. In addition to, EIA test result, OD value is also obtained. OD <1.000 indicates the presence of clinically significant antibodies and a high-risk of thromboembolic complications. OD ranging from 0.400 to 0.999 and low clinical 4T indicate low thrombogenic activity of the antibodies and subclinical HIT. Monitoring antibody titres using OD values is used in the preoperative preparation of patients with previously confirmed HIT II; the surgery in which heparin is given is performed after the antibody titter decreases or the test results become negative [14, 15].

To exclude other causes of thrombocytopenia, differential diagnosis should include pseudo thrombocytopenia (artifact), massive pulmonary embolism, disseminated intravascular coagulation (DIK), sepsis, other drug-induced thrombocytopenia (e.g. by GP IIb/IIIa inhibitors), autoimmune or alloimmune thrombocytopenia, post-transfusion purpura, diabetic ketoacidosis, and antiphospholipid syndrome with thrombocytopenia [16].

## 5. Treatment

Treatment of HIT patients is complex. In severely ill, heparin-treated patients who develop HIT II, there is often a misbalance of antithrombotic molecules including protein C, antithrombine III, thrombomodulin, and others. If there is a lack of these regulators, their substitution may increase the anticoagulant effect of heparin alternatives [16]. Clinical assessment plays a key role not only in discerning the platelet count decrease caused by anti-heparin antibodies from the platelet count decrease caused by the underlying disease but also in the selection of anticoagulant agent [17].

According to the clinical practice guidelines on antithrombotic therapy and prevention of thrombosis, if there is laboratory evidence of anti-heparin antibodies, heparin should be discontinued immediately, and replaced by some other non-heparin anticoagulant. Most commonly used alternative anticoagulants are: direct thrombin inhibitors, heparinoids, and factor Xa inhibitors (**Table 3**) [17].

Hirudin is a direct inhibitor of thrombin and acts independently of cofactors such as antithrombin. (19) Therefore, hirudin may be more effective in the presence of platelet-rich thrombi. Hirudin can also inhibit thrombin bound to fibrin or fibrin degradation products.

| | |
|---|---|
| Direct thrombin inhibitors (DTI) | Hirudin, lepirudin, bivaluridin argartoban |
| Heparionoides | Danaparoid |
| Factor Xa inhibitors | Fondaparinux |
| Direct oral anticoagulants(DOACs) | Dabigatran, apixaban, rivaroxaban |
| Other | Intravenous gamma globulins (IVIG) |

**Table 3.** Alternative anticoagulants for treatment of heparin- induced thrombocytopenia type II (HIT II).

Lepirudin is a recombinant hirudin and is a highly specific direct and irreversible inhibitor of thrombin. One molecule of lepirudin binds with one molecule of thrombin. Lepirudin is almost exclusively excreted by the kidneys and hence systemic clearance of lepirudin is dependent on the glomerular filtration rate. The drug should be avoided in hemodialysis patients and those with acute renal failure with creatinine clearance <15 ml $min^{-1}$ (normally 120 ml $min^{-1}$) or serum creatinine >528 μmol $liter^{-1}$. The 2006 British Guidelines recommend lepirudin, recombinant protein, and direct thrombin inhibitor to be used as heparin alternatives [12].

Bivalirudin is a direct thrombin inhibitor and an analogue of the peptide fragment hirugen, which is a compound derived from hirudin. Unlike lepirudin, the binding of bivalirudin to thrombin is reversible. Bivalirudin binds specifically to the catalytic site and substrate-binding site of thrombin. The US Food and Drug Administration (FDA) has approved bivalirudin for use in patients undergoing coronary angioplasty with unstable angina who are also on aspirin therapy [18].

Argatroban is a direct competitive synthetic inhibitor of thrombin. It binds reversibly to the thrombin catalytic site and therefore, inhibits reactions that are catalyzed or induced by the presence of soluble and clot-bound thrombin. When given i.v. and is metabolized in the liver by cytochrome P450 enzymes it is 100% bioavailable. Unchanged drug is excreted in the urine (16%) and feces (14%). It is eliminated as its metabolite in the feces (65%), presumably through biliary secretion, and in the urine (22%) [17].

It should be taken into account that all non-heparin anticoagulants may also lead to anaphylactic reaction and bleeding. However, unlike heparin, there is no known antidote for these agents and laboratory monitoring of their effects and antibody cross-reactivity is difficult (anti-Xa activity and ellagic time are not standard laboratory tests for hemostasis), in addition to the high price of the substance.

Danaparoid is a low molecular weight heparinoid. It is a mixture of dermatan sulfate, glycosaminoglycans, and chondroitin sulfate. A favorable outcome in 90% of patients with HIT is associated with the use of danaparoid. The main activity of danaparoid is against factor Xa, with the anti-Xa: the anti-IIa ratio of 22:1 resulting in inhibition of fibrin formation. There is a 10–20% cross-reactivity rate with HIT antibodies in vitro although it is less common in vivo. [18].

Coumarin agents (e.g. warfarin) are contraindicated in acute HIT II, because they increase the risk of microvascular thrombosis, necrosis, and gangrene. Replacing UFH with LWMH is also contraindicated in the treatment of HIT II due to the antibody cross-reactivity [12, 13, 18].

Most guidelines suggest the use of argatroban over other nonheparin anticoagulants in patients with HITT and renal insufficiency, and the use of bivalirudin over other nonheparin anticoagulants or heparin plus antiplatelet agents in patients with acute HIT or subacute HIT who require urgent cardiac surgery (Grade 2C) [12, 18, 19].

Recently, the most commonly used agent has been fondaparinux, a synthetic and selective factor Xa inhibitor, which rarely causes anti-heparin antibody cross-reactivity. Clinical experience shows it has beneficial effects in the prevention of thromboembolic complications. The fondaparinux results are an important step in developing the recommendations for the use of alternative agents to heparin, although randomized studies are needed [20, 21].

The use of oral direct thrombin inhibitors in the treatment of HIT II (dabigatran, rivaroxaban, and apixaban) also holds promise. The effectiveness of rivaroxaban in HIT patients was assessed in several studies showing no cross-reactivity with anti-heparin antibodies. Moreover, there was no PF4 release from platelets, as opposed to enoxaparin-LWMH [22, 23]. HIT can be managed with danaparoid in post-cardiac surgery patients. However, in absence of any increase of platelet count after 3–5 days of danaparoid therapy and/or occurrence of a new thrombotic event, danaparoid cross-reactivity with heparin should be suspected and replaced with direct thrombin inhibitor [24].

There is a dozen of evidence that in some patients with severe HIT II refractory to standard treatment immediate and sustained respond could be achieved by the admission of intravenous gamma globulin (IVIg), most probably mediated by inhibition of platelet activation [25].

According to the literature data, prophylactic platelet transfusions in thrombocytopenic HIT patients are contraindicated. Since spontaneous bleeding in HIT is rare, and platelet transfusions may potentially increase the risk of thrombosis, their use is recommended in case of life-threatening bleeding. Contrary, there is not clear evidence suggested that platelet transfusions should be avoided in a critically ill bleeding patient with HIT. [26].

In a couple of studies, platelet transfusion was administered prophylactically to prevent bleeding in post-surgery patients. These patients experienced no new thrombotic complication but expected post-transfusion platelet count increment was not achieved. Furthermore, previous deep venous thrombosis progressed after platelet transfusion and subsequently led to death in one patient [27].

## 6. Conclusion

HIT II is immunologically-induced, a life-threatening side effect of heparin therapy associated with thromboembolic complications. All patients receiving heparin are exposed to the development of anti-heparin antibodies, irrespective of the heparin dosage, type, and method of administration. HIT most commonly develops in intensive care patients, dialyzed patients, and cardiosurgical and orthopedic patients. Clinical assessment of the HIT probability using 4T scoring system, systematic monitoring of platelet number in heparin-receiving patients, and specific laboratory diagnosis of anti-heparin antibodies substantially contribute to the final confirmation of the diagnosis, enable timely administration of direct non-heparin thrombin antagonists, and reduce mortality from thromboembolic complications.

## Author details

Maja Tomicic*, Irena Jukic, Ana Hecimovic and Tomislav Vuk

*Address all correspondence to: maja.tomicic@hztm.hr

Croatian Institute of Transfusion Medicine, Zagreb, Croatia

## References

[1] Warkentin TE. New approaches to the diagnosis of heparin-induced thrombocytopenia. Chest. 2005;**127**:35-45

[2] Chong BH. Heparin-induced thrombocytopenia. Journal of Thrombosis and Haemostasis. 2003;**1**(7):1471-1479

[3] Kelton JG, Warkentin TE. Heparin-induced thrombocytopenia: A historical perspective. Blood. 2008;**112**:2607-2616

[4] Warkentin TE, Crowther MA. When is HIT really HIT? The Annals of Thoracic Surgery. 2007;**83**:21-23

[5] Warkentin TE, Levine MN, Hirsh J, et al. Heparin-induced thrombocytopenia low-molecular weight heparin or unfractionated heparin. The New England Journal of Medicine. 2007;**356**:891-893

[6] Lo GK, Juhl D, Warkentin TE, Sigouin, Eichler P, Greinacher A. Evaluation of pretest clinical 4T score (4T's) for the diagnosis of heparin-induced thrombocytopenia in two clinical settings. Journal of Thrombosis and Haemostasis. 2006;**4**:759-765

[7] Schallmoser K, Drexler C, Rohde E, Groselj-Strele A, Panzer S, Lanzer G. Detection of specific IgG antibodies in heparin-induced thrombocytopenia type II. Haematologica. 2007;(suppl 2):282, Abstract 0757

[8] Warkentin TE, Kelton JG. Delayed-onset heparin-induced thrombocytopenia and thrombosis. Annals of Internal Medicine. 2001;**135**(7):502-506

[9] Fabris F, Ahmad S, Cella G, Jeske WP, Walenga JM, Fareed J. Pathophysiology of heparin-induced thrombocytopenia. Clinical and diagnostic implications – A review. Archives of Pathology & Laboratory Medicine. 2000;**124**(11):1657-1666

[10] Greinacher A, Juhl D, Strobel U, Wessel A, Lubenow N, Selleng K, Eichler P, Warkentin TE. Heparin-induced thrombocytopenia: A prospective study on the incidence, platelet-activating capacity and clinical significance of antiplatelet factor 4/heparin antibodies of the IgG, IgM, and IgA classes. Journal of Thrombosis and Haemostasis. 2007;**5**(8):1666-1673

[11] Smythe MA, Prizoila J, Dobesh PP, Wirth D, Cuker A, Wittkowsky AK. Guidance for the practical management of the heparin anticoagulants in the treatment of venous thromboembolism. Journal of Thrombosis and Thrombolysis. 2016;**41**:16-186

[12] Keeling D, Davidson S, Watson H. Haemostasis and thrombosis task force of the British Committee for Standards in Haematology. The management of heparin-induced thrombocytopenia. British Journal of Haematology. 2006;**133**(3):259-269

[13] Croatian society for haematology and transfusion medicine guidelines on the diagnosis and management of heparin-induced thrombocytopenia (HIT). Liječnički Vjesnik. 2012;**134**(9-10):253-258

[14] Whithlatch NL, Perry SL, Ortel TL. Anti-heparin/platelet factor 4 antibody optical density values and confirmatory procedure in the diagnosis of heparin induced thrombocytopenia. Journal of Thrombosis and Haemostasis. 2008;**100**(4):678-684

[15] Fouassier M, Bourgerette E, Libert F, Pouplard C, Marques-Verdier A. Determination of serotonin release from platelets by HPLC and ELISA in the diagnosis of heparin-induced thrombocytopenia: Comparison with reference method by [$^{14}$C]-serotonin release assay. Journal of Thrombosis and Haemostasis. 2006;**4**:1136-1139

[16] Chan CM, Woods CJ, Warkentin TE, Sheppard JA, Shorr AF. The role of optical density in heparin-induced thrombocytopenia: A cohort study. Chest. 2015;**148**(1):55-61

[17] Brayant A, Low J, Austin S, Joseph JE. Timely diagnosis and management of heparin-induced thrombocytopenia in a frequent request, low incidence single Centre using clinical 4T'score and particle gel immunoassay. British Journal of Haematology. 2008;**143**(5):721-726

[18] Bates MS, Geer IA, Pabinger I, Sofaer S, Hirsh J. Venous thromboembolism, thrombophilia, antithrombotic therapy, and pregnancy: American College of Chest Physicians Evidence-Based Clinical Practise Guidelines (8th edition). Chest. 2008;**133**:844-886

[19] Linkins LA, Dans AL, Moores LK, Bona R, Davidson BL, Schulman Sand Crowther M. Treatment and prevention of heparin-induced thrombocytopenia. Chest. 2012;**141** (Suppl 2):495-530

[20] Grouzi E, Kyriaoku E, Panagou I, Spiliotopoulou I. Fondaparinux for the treatment of acute heparin-induced thrombocytopenia: A single Centre experience. Clinical and Applied Thrombosis/Hemostasis. 2010;**16**(6):663-667

[21] Sun J, Teoh KH, Sheth T, Landry D, Jung H, Warkentin TE, Yusuf S, Lamy A, Eikelboom JW. Randomized trial of fondaparinux versus heparin to prevent graft failure after coronary artery bypass grafting: The Fonda CABG study. Journal of Thrombosis and Thrombolysis. 2011;**32**(3):378-385

[22] Cronin RE, Reilly R. Unfractionated heparin for hemodialysis: Still the best option. Seminars in Dialysis. 2011;**23**(5):510-515

[23] Warkentin TE, Pai M, Linkins LA. Direct oral anticoagulants for treatment of HIT: Update of Hamilton study. Blood. 2017;**130**(9):1104-1113

[24] Weitz JI, Eikelboom JW, Salama MM. Antithrombotic therapy and prevention of thrombosis, 9$^{th}$ ed: American college of chest physicians evidence -based clinical practise guidelines. Chest. 2012;**141**(Suppl 2):120-151

[25] Padmanabhan A, Jones CG, Pechauer SM, Curtis BR, Bougie DW, Irani MS, Brayant BJ, Alperin JB, Deloughery TG, Mulvey KP, Dhakal B, Wen R, Wang D, Aster RH. IVIg for treatment of severe refractory heparin-induced thrombocytopenia. Chest. 2017;**152**(3):478-485

[26] Hopkins CK, Goldfinger D. Platelet transfusions in heparin-induced thrombocytopenia: A report of four cases and review of the literature. Transfusion. 2008;**48**(10):2128-2132

[27] Foroughina F, Farsad F, Gholmani K, Ahmadi S. Usefulness of Dnaparoid sodium in patients with heparin- induced thrombocytopenia after cardiac surgery. Journal of Research in Pharmacy Practice. 2015;**4**(2):73-78

*Edited by Mojca Božič-Mijovski*

Anticoagulant drugs are among the most frequently prescribed drugs in everyday clinical practice. In the past decades, several new direct oral anticoagulants were developed that changed the anticoagulant therapy landscape considerably. This book provides an extensive overview of all the known anticoagulants that can be useful for studying different aspects of the haemostatic system or as a starting point for new drug development. It is also a valuable tool for clinicians providing a description of the mode of action and management of therapy for anticoagulant drugs used in everyday clinical practice in different clinical settings, including direct oral anticoagulants dabigatran, rivaroxaban, apixaban and edoxaban.

Published in London, UK


IntechOpen

ISBN 978-1-83881-498-4