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Oral Direct Thrombin Inhibition: an Effective and Novel Approach for Venous Thromboembolism

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Abstract

Venous thromboembolism is a serious illness that affects patient morbidity and mortality and presents a significant management challenge to healthcare providers world-wide. Despite major achievements in the significant reduction of thromboembolic complications, the most common therapies currently used for prevention and treatment of venous thromboembolism – heparins and vitamin K antagonists such as warfarin – have several limitations. In particular, unfractionated heparin and warfarin show significant inter-patient variability in pharmacokinetics and pharmacodynamics, which makes regular coagulation monitoring necessary. Furthermore, only warfarin is suitable for long-term use, as it is administered orally. A new class of anticoagulants has been developed that directly target thrombin, a key enzyme in the blood coagulation cascade. Unlike warfarin and heparin, these direct thrombin inhibitors are able to inhibit fibrin-bound thrombin and so produce more effective inhibition of coagulation. Importantly, some members of this class of drugs have been developed for oral administration. Ximelagatran, which is converted to its active form melagatran, has predictable pharmacokinetics and pharmacodynamics. Therefore, ximelagatran can be administered in fixed doses with no need for coagulation monitoring. Its efficacy and safety profile have been demonstrated in preclinical and clinical studies. As the first oral agent in the new class, direct thrombin inhibitors, ximelagatran has significant potential for improving the prevention and treatment of venous thromboembolism.

1. Introduction

Venous thromboembolism, including deep vein thrombosis and pulmonary embolism, is a serious and potentially fatal condition that places a significant economic burden on healthcare providers. Its annual incidence is approximately 1 in 1000 individuals per year,[1] but this is likely to be an underestimate, because the condition is often asymptomatic, with the first clinical sign being pulmonary embolism, which can be fatal. The risk of developing venous thromboembolism increases with age, prolonged immobility and major surgery.[2,3] Therefore, venous thromboembolism is a particular problem in elderly patients undergoing major orthopaedic surgery. In patients with unprovoked venous thromboembolism, recurrence is common if anticoagulation therapy is stopped, with a cumulative incidence of 30% observed after 10 years after an initial event;[4] in 20% of cases, recurrence is in the form of pulmonary embolism.[5] Therefore, it is important to identify patients at risk of venous thromboembolism, and to administer appropriate thromboprophylactic treatment.

This review discusses current treatments for venous thromboembolism, the need for new treatment alternatives and the development of oral direct thrombin inhibitors, new agents that have the potential to simplify the prevention and treatment of venous thromboembolism.

Venous thrombi occur as a result of inappropriate plasma coagulation in patients with hypercoagulability or with venous trauma or stasis. Coagulation is dependent on a highly regulated cascade of proteolytic enzymes and co-factors that ultimately leads to the production of an insoluble fibrin network (figure 1). Two coagulation pathways exist: the intrinsic pathway, which is activated when blood comes into contact with negatively charged surfaces, and the extrinsic pathway, which

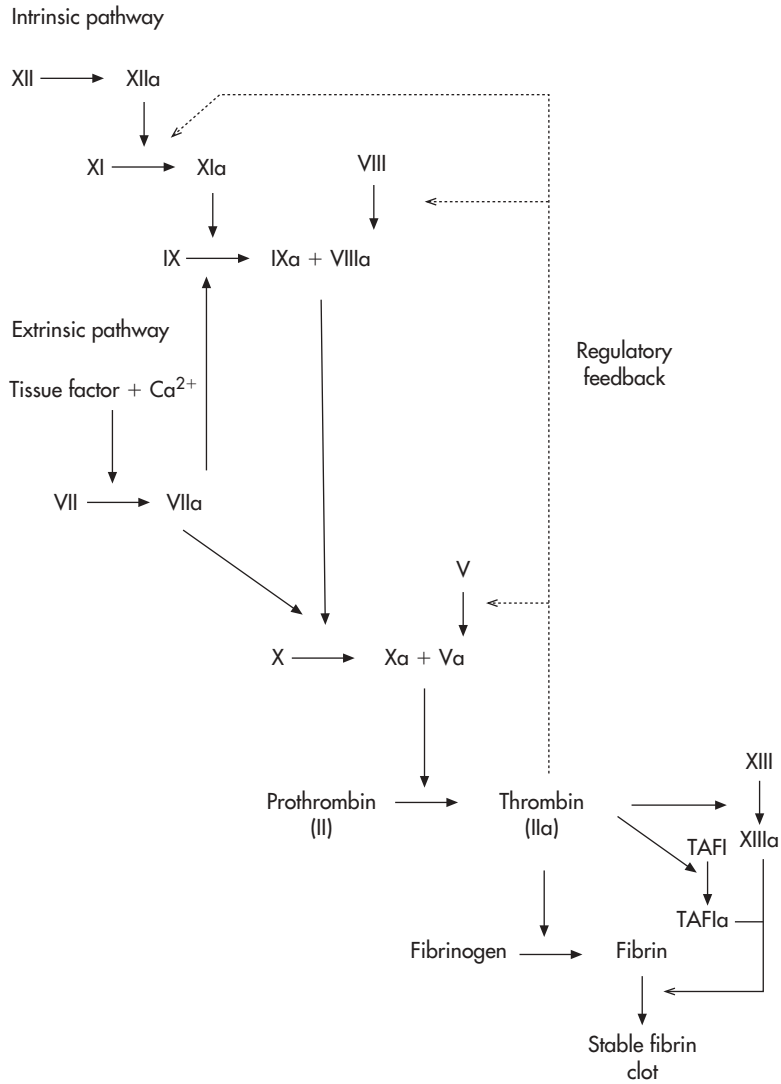

Fig. 1. The coagulation cascade, showing the intrinsic and extrinsic pathways. The suffix 'a' indicates the activated form of the relevant coagulation factor. **V**, **VII**–**XIII** = coagulation factors V, VII–XIII; **TAFI** = thrombin activatable fibrinolysis inhibitor.

is activated by the exposure of tissue factor at sites of vascular injury. Both these pathways are linked and converge after the activation of factor X, which then cleaves prothrombin (factor II) to produce thrombin, the final protease in the coagulation cascade. Thrombin cleaves fibrinopeptides A and B from fibrinogen to yield soluble fibrin, which is cross-linked to form insoluble fibrin and a more stable coagulum.

Thrombin has a central role in controlling thrombus growth. At early stages in the coagulation process, it amplifies the coagulation response by activation of factors V, VIII and XI, and stabilises fibrin by activating factor XIII, the fibrin-stabilising factor. Thrombin acts to promote an anticoagulant pathway after binding to thrombomodulin. Thrombomodulin-bound thrombin activates protein C, which, together with its co-factor (protein S), inactivates factors Va and VIIIa, thus inhibiting the generation of thrombin. The thrombin–thrombomodulin complex also activates thrombin-activatable fibrinolysis inhibitor (TAFI), which reduces the rate of degradation of fibrin.[6] In addition to its role in blood coagulation, thrombin activates platelets. As would be expected for such a pivotal enzyme, thrombin is tightly regulated, and it is inhibited by antithrombin and heparin cofactor II. The crystal structure of thrombin has been determined and a number of domains have been identified that are involved in its proteolytic and regulatory roles, in addition to its interaction with various anticoagulants (figure 2). In addition to the active site, the molecule possesses two positively charged domains that are involved in intermolecular interactions with fibrinogen, in addition to other substrates, and heparin, respectively (figure 2a).

2. Current Treatments for Venous Thromboembolism

The most commonly used treatments for venous thromboembolism are heparins and warfarin. Heparins consist of heterogeneous, highly sulphated polysaccharide chains, some of which include a pentasaccharide sequence that binds to and activates antithrombin.[7] Unfractionated heparin has a mean molecular weight of 15 000, whereas low-molecular-weight heparin (LMWH) has a mean molecular weight of about 5000. Heparin-activated antithrombin inhibits the activity of several proteases in the coagulation cascade, including thrombin and factor Xa (figure 2b).[8] Only pentasaccharide-containing heparin chains consisting of 18 saccharide units are able to catalyse thrombin inhibition by antithrombin (figure 2c),[9] because these chains are long enough to form a ternary heparin–antithrombin–thrombin complex.

Fondaparinux is a synthetic pentasaccharide analogue. This agent is too short to bridge antithrombin to thrombin. Consequently, fondaparinux only catalyses the inhibition of factor Xa by antithrombin, and has no effect on thrombin inhibition.[10]

Although effective, unfractionated heparin has several limitations in the treatment of venous thromboembolism. It produces an unpredictable anticoagulant response, mainly as a result of binding to plasma proteins, endothelial cells and macrophages.[7] Therefore, routine coagulation monitoring is necessary to ensure that a therapeutic anticoagulant response is achieved. LMWH produces a more predictable anticoagulant response than unfractionated heparin and, therefore, coagulation monitoring is not generally required. However, both LMWH and unfractionated heparin require parenteral administration and so are less suitable than warfarin for long-term use. A particular concern with unfractionated heparin is heparin-induced thrombocytopenia, a process triggered by antibodies directed against the complex formed between heparin and platelet factor 4, a protein secreted by activated platelets. When the antibodies bind to the heparin–platelet factor 4 complex, they induce further platelet activation and the generation of platelet microparticles that can trigger thrombosis by promoting coagulation.[11]

Warfarin is an orally active vitamin K antagonist that acts by preventing vitamin K-dependent γ-glutamyl carboxylation of the coagulation factors II, VII, IX and X.[12] γ-Glutamyl carboxylation of

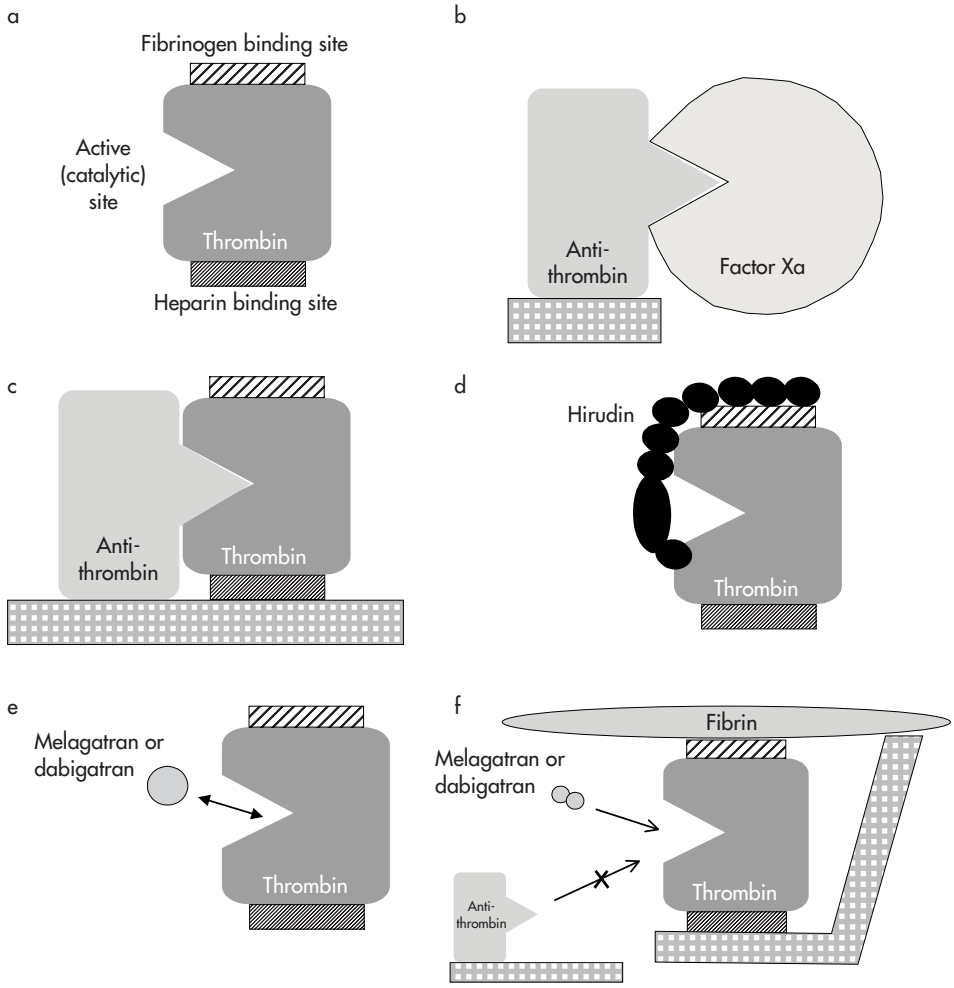

Fig. 2. Schematic diagrams showing **(a)** the interaction sites present on the thrombin molecule, **(b)** the interaction of heparins of chain length <18 saccharide units with antithrombin and factor Xa, **(c)** the interaction of heparins of chain length ⩾18 saccharide units with antithrombin and thrombin, **(d)** the interaction of hirudin with thrombin, **(e)** the interaction of melagatran and dabigatran with thrombin, and **(f)** the interaction of anticoagulants with fibrin-bound thrombin.

these factors is critical for their activity and in its absence they are non-functional.

Although warfarin is a highly effective anticoagulant, like the heparins it has several drawbacks. Warfarin has a narrow therapeutic window, and produces an unpredictable anticoagulant activity.[12] Various genetic factors, disease states, several drugs, dietary vitamin K intake and alcohol can affect the absorption or metabolism of warfarin, thereby leading to over- or under-anticoagulation.[12] Consequently, routine coagulation monitoring and dose adjustment are necessary to ensure that a therapeutic anticoagulant response is obtained. Warfarin also has a slow onset of action, taking up to 5 days to reach its full antithrombotic effect. This means that heparins have to be administered simultaneously with warfarin in the first few days of treatment. Importantly, because warfarin is metabolised by cytochrome P450 enzymes in the liver, it has significant interactions with other

drugs, both prescription and over-the-counter.[12] The regular monitoring that is usually required when using unfractionated heparin or warfarin is inconvenient and resource-intensive in terms of patient and physician time and technical analysis. The limitations of current treatments, in addition to failure to identify patients at risk, mean that many patients who would benefit do not receive adequate anticoagulation therapy.[13]

3. The Ideal Anticoagulant

As discussed earlier, there are several drawbacks to the clinical use of current anticoagulant treatments. In particular, the unpredictable pharmacokinetics of warfarin and heparin and the associated risk of bleeding or undertreatment necessitate routine coagulation monitoring. There is, therefore, a clinical need for alternative anticoagulation therapies. Among other properties, the ideal anticoagulant should have a predictable pharmacokinetic profile such that coagulation monitoring is not required (table I). In addition, oral availability is important if new drugs are to be useful as alternatives to warfarin for long-term, outpatient treatment. Several new therapies are under investigation; these target either the initiation or propagation of coagulation, or fibrin formation.[14]

4. Direct Thrombin Inhibitors

Thrombin is a particularly attractive target for anticoagulation therapies because of the central part that it plays in the coagulation cascade. Molecules that directly inhibit thrombin have some theoretical advantages over current anticoagulants. For example, neither heparins nor other indirect coagulation inhibitors are active against fibrin-bound thrombin.[15] This is important, because fibrin-bound thrombin remains active and promotes continued thrombus growth. As shown in figure 2f, heparin is able to link thrombin to fibrin and so blocks the heparin-binding site on thrombin that is necessary for its interaction with heparin-activated antithrombin. Direct thrombin inhibitors, however, have the potential to inhibit fibrin-bound thrombin, as no steric hindrance is present. In addition, direct thrombin inhibitors do not bind to platelet factor 4 (as do heparins) and so do not cause heparin-induced thrombocytopenia.

The first direct inhibitor of thrombin to be discovered was hirudin, a 65-amino acid residue polypeptide that was isolated from the medicinal leech (*Hirudo medicinalis*).[16] This molecule binds essentially irreversibly to thrombin,[17] with the amino-terminus binding to the active site of thrombin and the carboxy-terminus binding to the fibrinogen-binding site of thrombin (figure 2d).[18] The irreversible binding of hirudin to thrombin means that rapid normalisation of coagulation cannot be achieved, if this is required. The technical difficulties of isolating sufficient quantities of hirudin for clinical use led to its production by recombinant technology, for example as lepirudin and desirudin.

Bivalirudin is a small synthetic peptide with high potency and specificity for thrombin inhibition. Some features that distinguish bivalirudin from hirudin are the reversibility of the bivalirudin–thrombin complex and its shorter half-life.

Table I. The properties of the 'ideal' anticoagulant

- Reproducible pharmacokinetics and pharmacodynamics.
- Rapid onset and offset of action.
- Minimal or no adverse effects.
- Inhibition of free and clot-bound thrombin.
- Minimal interactions (with food and other drugs).
- Wide therapeutic window (good separation of antithrombotic effect and bleeding).
- No coagulation monitoring required.
- Targeted action.
- Oral and parenteral formulations.
- Minimal number of administrations per day.

After intravenous administration, bivalirudin shows reversible anticoagulant effects, with coagulation time returning to baseline in approximately 60 min.

Argatroban is an arginine derivative that binds reversibly to the active site of thrombin.[19] Its half-life in humans is 40–50 min,[20] and the molecule is extensively metabolised by the liver to form four inactive metabolites.[19] As with hirudin and bivalirudin, argatroban is administered parenterally and so is not suitable for long-term use.

Two more recently developed direct thrombin inhibitors are melagatran and dabigatran, both of which bind reversibly to the active site of thrombin (figure 2e). These drugs have low oral bioavailability: that of melagatran is approximately 4–8%[21,22] and that of dabigatran 5%.[23] Ximelagatran was developed to overcome the limited oral bioavailability of melagatran; a prodrug of melagatran, it has oral bioavailability of 20% in both the fed and fasting state.

4.1 Ximelagatran

Ximelagatran is the first new oral anticoagulant to be developed in nearly 60 years and represents a major advance in anticoagulation therapy. The active form of ximelagatran, melagatran, is a dipeptide that binds reversibly to the active site of thrombin and inhibits this protease with an inhibition constant of 2 nmol/L.[24] The pharmacokinetic properties of ximelagatran highlight its potential to achieve great improvement in anticoagulation therapy in patients with venous thromboembolism. In healthy volunteers, melagatran (as ximelagatran) has a bioavailability of 18–24%.[21,25,26] After rapid absorption, ximelagatran is converted to melagatran with a maximum plasma concentration (C_{max}) of melagatran being reached 1.5–2h after administration.[22] The conversion of ximelagatran to melagatran occurs throughout the body in various sites and is neither dependent on the cytochrome P450 enzyme system nor localised to the liver.[27] Co-administration of food or alcohol has no effect on the bioavailability, C_{max} or time to reach C_{max} of ximelagatran in either elderly volunteers or young healthy volunteers.[28] The volume of distribution of ximelagatran is 2.0 L/kg, compared with 0.2 L/kg for melagatran.[29] Elimination of melagatran occurs predominantly via the renal route, with a plasma elimination half-life of 2.5–3.5h in young healthy volunteers and 4–5h in patients.[22,28] The pharmacokinetic properties of ximelagatran are consistent with twice-daily administration, based on total exposure to the drug (area under the plasma concentration versus time curve [AUC]) rather than peak and trough plasma concentration. The utility of twice-daily dosing has been confirmed in clinical studies.

Exposure to melagatran is independent of age,[28] ethnicity,[26] sex[28] and body weight,[30] and appears to depend only on renal function. Therefore, in a typical 70-year-old patient, the plasma elimination half-life is extended to 4–5h as a result of the natural decline of renal function with age.[29] Mild-to-moderate hepatic impairment has no effect on the pharmacokinetics of ximelagatran.[31] In healthy volunteers, the coefficient of variation of melagatran plasma concentrations is approximately 15% after subcutaneous administration[28] and approximately 20% after oral administration as ximelagatran.[21] Furthermore, the activated partial thromboplastin time has been shown not to be affected by age, sex, body weight or creatinine clearance.[32] Melagatran is not metabolised[22] and does not inhibit cytochrome P450 isoenzymes *in vitro* or *in vivo*,[33] therefore it has a low potential for interactions with other drugs.[28-31] Concomitant administration of aspirin does not affect the pharmacokinetic or pharmacodynamic parameters of intravenous melagatran in healthy volunteers.[34] The reproducible pharmacokinetics of ximelagatran mean that fixed-dose regimens can be used without the requirement for coagulation monitoring.[35]

The proposed pharmacodynamic effects of direct thrombin inhibitors have been confirmed for melagatran in both preclinical and clinical settings. In a rat model, the prevention of thrombus formation was investigated after chemical damage to the carotid artery of anaesthetised animals given

warfarin or melagatran.[36] The results obtained, in conjunction with bleeding-time experiments, showed that melagatran has a wider therapeutic window than warfarin (figure 3),[36] and therefore its anticoagulant effects are well separated from the bleeding effects observed at higher doses. The steep dose–response curve of warfarin highlights the potential difficulties of maintaining the correct dose of this compound, especially when it has such great inter-patient variability in pharmacokinetics. In contrast, melagatran has a relatively shallow dose–response curve and this, coupled with its reproducible pharmacokinetics, ensures that coagulation monitoring is not required.

The effectiveness versus bleeding profile of melagatran has also been compared with that of hirudin in a rabbit model of thrombosis prevention and ear bleeding.[37] In this model, thrombosis was measured in the mechanically injured aorta of anaesthetised rabbits. Patency of the aorta was defined as the percentage of time during which blood flow exceeded 0.5 mL/min over a 90-min period of observation. Cumulative blood loss over 30 min was also measured after five cuts had been made in the ear, 15 min after the initiation of treatment. The effectiveness versus bleeding profiles of hirudin and melagatran were assessed by plotting blood loss as a function of the percentage time that vessels remained patent over the 90-min period. The results shown in figure 4[37] demonstrate the reduced bleeding observed with melagatran, compared with hirudin, at concentrations that maintain high levels of patency.

The ability of melagatran to inhibit fibrin-bound thrombin has been demonstrated in an *in-vitro* assay. In this assay, the ability of hirudin to inhibit fibrin-bound thrombin was significantly less than its ability to inhibit fluid-phase thrombin.[37] In contrast, melagatran inhibited fibrin-bound thrombin and fluid-phase thrombin to a similar degree. It also inhibited TAFI activation in a dog model of coronary artery thrombosis, probably as an indirect result of thrombin inhibition.[38]

Clinical trials have confirmed the efficacy and safety of ximelagatran using a twice-daily fixed dosing regimen in both the treatment and prevention of venous thromboembolism. These trials are discussed further in other articles in this Supplement.

4.2 Dabigatran Etexilate

Dabigatran etexilate (BIBR 1048) is an oral form of dabigatran (BIBR 953), which inhibits thrombin with an inhibition constant of 5 nmol/L.[39] Its oral bioavailability is likely to be low and has been estimated at 5%.[23] After oral adminis-

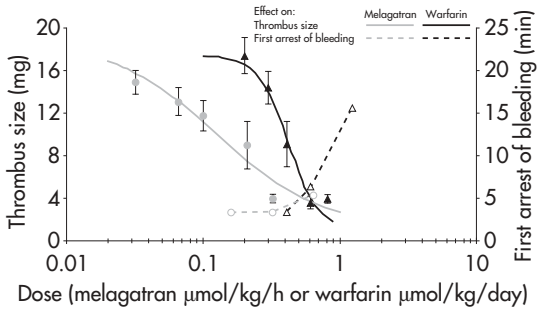

Fig. 3. Effects of melagatran and warfarin on thrombus size and first arrest of bleeding time in a rat model. (With permission from Gustafsson D, Elg M, The pharmacokinetics of the oral direct thrombin inhibitor ximelagatran and its active metabolite melagatran: a mini-review. Thromb Res 2003; 109 (1): S9–15[36])

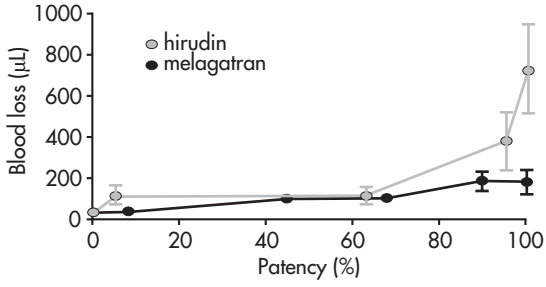

Fig. 4. Effects of melagatran and hirudin on blood loss as a function of the percentage time that vessels remained patent over a 90-min period in a rabbit model of arterial thrombosis prevention. (With permission from Klement P, Carlsson S, Rak J, et al. The benefit-to-risk profile of melagatran is superior to that of hirudin in rabbit arterial thrombosis prevention and bleeding models. Thromb Haemost 2003; 1 (Pt 3): 587-94[37])

tration of dabigatran etexilate, the C_{max} of dabigatran is reached after 1.25–1.75h in healthy volunteers.[40] Dabigatran is excreted mainly by the renal route and has an elimination half-life of 8.2–10.4h after a single dose.[23] Both dabigatran etexilate and dabigatran have been demonstrated to be effective in animal models of venous thrombosis.[41,42] Data from two related phase II trials of dabigatran etexilate were reported recently.[43,44] In one study phase IIa trial (BISTRO I), 289 patients received dabigatran etexilate 25–600 mg/day (as two doses) or single daily doses of 150mg or 300mg for 6–10 days, after hip replacement surgery.[43] Steady-state plasma concentrations of dabigatran were reached after 2–3 days of treatment and declined with an elimination half-life of 15h. There was a trend towards lower incidence of deep vein thrombosis with increasing AUC values of the drugs. A close correlation was observed between the plasma concentration of dabigatran and the activated partial thromboplastin time and ecarin clotting time.[44] The inter-individual variability of the pharmacodynamic response was reported to be low (8–20%). In the BISTRO Ib study, 59 patients received a single 150-mg dose of dabigatran etexilate after hip replacement surgery.[43] Peak plasma concentrations, which were reached after approximately 6h, were lower than those observed at steady state after several doses. In this study, four patients showed markedly lower concentrations, although in two patients, this was likely to have been a result of vomiting after drug intake. Additional studies are needed to establish the efficacy and safety of dabigatran etexilate.

5. Conclusion

Venous thromboembolism is a serious condition that requires effective and safe anticoagulation therapy. The current modalities for its prevention and treatment have many limitations, particularly relating to unpredictable pharmacokinetics and pharmacodynamics. At present, two different types of anticoagulant are needed for treatment of venous thromboembolism: heparins, followed by an oral compound requiring routine coagulation testing. The most commonly used oral anticoagulant, warfarin, has many interactions with food and other drugs. Therefore, although warfarin is used for long-term treatment, the necessity of routine coagulation monitoring and individual dosing is resource-intensive and inconvenient.

Thrombin is a key enzyme in the coagulation cascade and as such is an attractive target for novel anticoagulant therapies. A new class of drugs has been developed that produces direct inhibition of thrombin. Ximelagatran, the first oral direct thrombin inhibitor, is at the most advanced stage of development and its efficacy and safety in venous thromboembolism have been demonstrated in clinical trials. Ximelagatran is the first new oral anticoagulant to be developed in nearly 60 years and its profile shows many advantages over current treatment options. It has a reproducible pharmacokinetic and pharmacodynamic profile, and so fixed doses can be administered without the need for coagulation monitoring. The characteristics of ximelagatran have led to its investigation in the prevention of venous thromboembolism in patients undergoing major elective orthopaedic surgery, the treatment and long-term secondary prevention of venous thromboembolism, prevention of stroke in patients with atrial fibrillation and prevention of major cardiovascular events after acute myocardial infarction. The new class of direct thrombin inhibitors, particularly those members of this class that are orally available, has the potential to achieve considerable improvement in the prevention and treatment of venous thromboembolism.

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