Direct inhibitors of coagulation proteins – the end of the heparin and low-molecular-weight heparin era for anticoagulant therapy?

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Summary
Heparins, either unfractionated or low-molecular-weight (UFH and LMWHs), and vitamin K antagonists (VKAs) are currently the anticoagulants of choice for the prevention of post-operative venous thromboembolism (VTE) and for the treatment of acute venous and arterial thromboembolism. While VKAs are widely used in the US, LMWHs are the standard of care in the EU. Although efficacious, these agents are associated with a number of drawbacks, such as the risk of heparin-induced thrombocytopenia, the need for frequent coagulation monitoring in the case of UFH and VKAs, and the parenteral mode of administration in the case of heparins, which can lead to problems associated with patient compliance. There is a need for new anticoagulants that overcome these limitations. Direct, small-molecule inhibitors of coagulation proteins targeting a single enzyme in the coagulation cascade – particularly thrombin or Factor Xa – have been developed in recent years. Two agents, the direct thrombin inhibitor dabigatran and the direct Factor Xa inhibitor rivaroxaban, have recently been approved in the EU and several other countries for the prevention of VTE after total hip or knee replacement surgery. Here we will review data that suggest that the antithrombin-independent mechanism of action of these agents, particularly that of direct Factor Xa inhibitors, leads to increased efficacy with similar safety profiles compared with the antithrombin-dependent heparins. Although the end of the heparins era is not to be expected, the new anticoagulants presented in this review potentially represent the future of anticoagulation.

Keywords
Factor Xa, direct thrombin inhibitors, antithrombin-dependent inhibitors, rivaroxaban, heparin


Introduction
Anticoagulation therapy is used in many medical specialties, with indications including primary and secondary prevention, as well as the treatment of both venous and arterial thromboembolic events (1). The parenterally administered heparins, comprising unfractionated heparin (UFH) and its derivatives the low-molecular-weight heparins (LMWHs), and the orally administered vitamin K antagonists (VKAs) have long been the anticoagulants of choice, in some cases for decades (2, 3). Although these agents are efficacious, their use in clinical practice is associated with a number of drawbacks and potentially fatal risks (2, 3).

There is a need for new anticoagulants that can overcome the limitations of heparins and VKAs. Direct, small-molecule inhibitors of coagulation proteins targeting a single enzyme in the coagulation cascade (Fig. 1) have been developed in recent years, with two such agents having recently been approved for clinical use in the EU. Although VKAs are widely used in the US, their use remains limited in the EU, where LMWHs are the standard of care (2–4). This review will introduce some of these new agents and compare them with the heparins, with regards to their synthesis and production, mechanism of action, and efficacy/safety profiles as observed in both animal models and clinical trials.

Synthesis and production of heparins
UFH, the antithrombotic properties of which were discovered by McLean approximately 90 years ago, was the first available anti-
Today, the starting point of heparin production is still the raw heparin isolated from animal intestines. The biosynthesis of UFH is a complex process, involving chain initiation, chain polymerisation, and chain modification steps, which depends on various enzymes. Additionally, UFH is a very heterogeneous product, with only approximately 30% of its ingredients having anticoagulant effects. Analyses for quality control can, therefore, be challenging. In January 2008, several batches of UFH had to be withdrawn from the US market due to reports of adverse reactions and one possible death that may have been associated with the product. It was later discovered that the raw heparin, derived from pig intestines, that had been used to produce these batches had been contaminated with oversulfated chondroitin sulfate at a processing plant in China, and batches of UFH were also called back for the same reasons in other countries, including Germany, Japan, Canada, France, Italy, and Denmark.

LMWHs, which were first developed in the 1980s, are produced by chemical or enzymatic depolymerisation of heparin. Depolymerisation is achieved by treatment with nitrous acid or with the enzyme heparinase, by hydrolytic cleavage with hydrogen peroxide, or by beta-elimination. The production of a LMWH from raw heparin is complex and, because the starting material is raw heparin, the same problems of contamination may occur. Because LMWHs are prepared by different methods of depolymerisation and differ to some extent in their pharmacokinetic properties and anticoagulant profile, they may not be clinically interchangeable.

**Mechanism of action: direct versus indirect inhibition of coagulation proteins**

**Antithrombin-dependent versus antithrombin-independent inhibition of coagulation**

UFH is an indirect anticoagulant that requires a plasma cofactor, antithrombin (AT), to exhibit anticoagulant activity. UFH is a heterogeneous mixture of negatively charged glycosaminoglycans. Only one-third of the glycosaminoglycan chains bind to AT, with the remaining two-thirds only having minimal anticoagulant activity at therapeutic concentrations. UFH binds to AT via a unique pentasaccharide sequence; through subsequent binding to clotting factors such as thrombin and Factor Xa, it catalyses their inactivation by AT. Following irreversible inhibition of thrombin and other coagulation enzymes, UFH dissociates from AT and is reutilised by the heparin–AT interaction. Following irreversible inhibition of thrombin and other coagulation enzymes, UFH dissociates from AT and is reutilised. UFH also binds to cells and other plasma proteins, which leads to unpredictable pharmacokinetic and pharmacodynamic properties. This, in turn, necessitates regular monitoring of the anticoagulant effects of UFH in patients.

Similar to UFH, LMWHs produce their major anticoagulant effect by activating AT. Only pentasaccharide-containing heparin chains composed of at least 18 saccharide units are of sufficient length to bridge AT to thrombin. Almost all molecules of UFH contain at least 18 saccharide units, resulting in an antithrombin to AT ratio of 1:1. This ratio is between 2:1 and 4:1 for the LMWHs, depending on their molecular size distribution, showing a higher selectivity of LMWHs for Factor Xa.
tion, LMWHs exhibit less binding to cells and proteins than UFH. Therefore, LMWHs have more predictable pharmacokinetic and pharmacodynamic properties, and a lower risk of side-effects, such as heparin-induced thrombocytopenia (HIT), than UFH (2). HIT is caused by heparin-dependent antibodies that bind to a conformationally modified epitope on platelet factor 4 (PF4) (11). The use of LMWHs is also associated with a risk of HIT, but to a lesser extent than with UFH because the level of binding to PF4 is reduced (2). In addition, LMWHs have a longer half-life, making them more convenient for use in the outpatient setting.

Laux et al. A new era for anticoagulation

<table>
<thead>
<tr>
<th>Agent</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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</table>
| UFH   | – Fast acting  
– Good efficacy | – Potential for severe heparin-induced thrombocytopenia  
– Parenteral administration  
– Unpredictable response (non-specific protein binding)  
– Risk of bleeding complications  
– Variable bioavailability  
– Indirect action via antithrombin  
– No inhibition of clot-bound coagulation factors  
– Laboratory monitoring required at higher doses |
| LMWHs | – Once- or twice-daily dosing  
– No laboratory monitoring  
– Good efficacy | – Parenteral administration  
– Bleeding complications in patients with renal insufficiency  
– Risk of heparin-induced thrombocytopenia  
– Indirect action via antithrombin  
– No inhibition of clot-bound coagulation factors |
| Fondaparinux | – Once-daily dosing  
– No laboratory monitoring  
– Good efficacy | – Parenteral administration  
– Bleeding complications in patients with renal insufficiency  
– Indirect action via antithrombin  
– No inhibition of clot-bound coagulation factors |

Based on the fact that heparin chains of any length that contain the high-affinity pentasaccharide can catalyse Factor Xa inhibition by AT, the AT-binding pentasaccharide was synthesised and developed into a drug called fondaparinux (2). Fondaparinux exclusively potentiates the anti-Factor Xa activity of AT and has no effect on thrombin. Because fondaparinux only has low affinity for PF4, side-effects such as HIT are unlikely to occur (2).

In contrast to the heparins, the new, small-molecule anticoagulants that are currently being developed are AT-independent inhibitors of specific coagulation enzymes. The targeted, direct action of these new agents on coagulation enzymes could potentially increase their predictability and efficacy.

**Direct and indirect inhibition of coagulation enzymes bound to the clot or in the prothrombinase complex**

**Inhibition of clot-bound thrombin**

In the final step of the coagulation cascade, thrombin converts fibrinogen into insoluble fibrin, leading to the formation of a stable clot or thrombus (Fig. 1). During their generation, thrombin incorporate clotting factors, particularly thrombin, which remain in their active form. To inhibit the propagation of thrombosis, thrombin inhibitors should be able to inhibit clot-bound thrombin. It has been shown that clot-bound thrombin is relatively protected from inhibition by heparin, possibly because the heparin-binding site on thrombin is inaccessible when the enzyme is bound to fibrin, and because of the large size of the heparin–AT complex, which prevents it from penetrating the clot (12). Conversely, clot-bound thrombin is susceptible to inactivation by AT-independent inhibitors because their sites of interaction are not masked by thrombin binding to fibrin; this was first shown with the direct thrombin inhibitor (DTI) PPACK (D-phenylalanyl-L-propyl-L-arginyl chloromethyl ketone) (12). Therefore, in some clinical settings, AT-independent inhibitors such as the DTIs may be more effective than heparins.

**Inhibition of prothrombinase Factor Xa activity**

Reducing thrombin generation by inhibiting Factor Xa may have some advantages over directly inhibiting thrombin. Indeed, inhibiting Factor Xa can prevent the amplified burst of thrombin generation that occurs during thrombus formation, with one molecule of Factor Xa leading to the generation of more than 1,000 thrombin molecules (13).

AT-independent inhibitors have the potential to inhibit free Factor Xa, prothrombinase, and clot-bound Factor Xa activity (14–16). Conversely, AT-dependent inhibitors cannot inhibit Factor Xa within the prothrombinase complex. A direct prothrombinase inhibition assay, monitoring thrombin generation under near-physiologic concentrations of prothrombin and AT in the presence of therapeutic doses of LMWHs and fondaparinux, indicated that Factor Xa in the prothrombinase complex was protected from inhibition by AT more than 1,000 times, independent of the molecular size of heparin (17). In a separate study, it was also demonstrated that fondaparinux could not inhibit Factor Xa within the prothrombinase complex at clinically relevant concentrations (18).

**The profile of an ‘ideal’ anticoagulant**

There is a need for new anticoagulants that can overcome the limitations of heparins (Table 1). An ideal anticoagulant would be orally administered, with a rapid onset of action, a good safety profile (particularly in terms of risk of bleeding), be specific,
have predictable pharmacokinetics and pharmacodynamics, no propensity for food or drug interactions, fixed-dose administration, a wide therapeutic window, no need for coagulation monitoring, and be a synthetic drug to reduce the possibility of contamination associated with the use of animal sources as starting material (19, 20). In addition, these small-molecule drugs can be produced through well-defined synthesis (21, 22), which leads to homogeneous and potentially safer compounds, as the risk of contamination is reduced. The direct mechanism of action of these AT-independent anticoagulants also seems to indicate that they could have improved efficacy compared with their AT-dependent counterparts, with similar safety profiles.

In the search for new agents to better fit this ideal anticoagulant profile, a number of different points in the coagulation cascade have been targeted, including direct thrombin inhibition, and inhibition of Factor Xa, Factor IXa, the Factor Vlla–tissue factor complex, and the Factor Va–Factor Vlla complex (protein C pathway). Among these different options, thrombin and Factor Xa are very attractive targets because they are common to both the intrinsic and extrinsic pathways (Fig. 1). Recently, new small-molecule thrombin and Factor Xa inhibitors have reached advanced stages of development, with two such drugs now approved for the prevention of venous thromboembolism (VTE) in adult patients undergoing elective total hip and knee replacement (THR and TKR) surgery.

**Direct thrombin inhibitors**

DTIs are small molecules that inhibit thrombin directly and AT independently by binding to its active catalytic site and blocking its interaction with its substrates. This allows them to inhibit both free and clot-bound thrombin, which constitutes a potential advantage over the indirect thrombin inhibitors (12). Furthermore, they produce a more consistent anticoagulant response, and are not neutralised by PF4, which eliminates the risk of thrombocytopenia. Hirudin (Refludan®; Bayer HealthCare Pharmaceuticals) – the first DTI available for clinical use – irreversibly binds to thrombin. It is more effective than heparin but is associated with increased bleeding. It is administered intravenously and requires individual dosing based on frequent laboratory monitoring (2). Other DTIs include bivalirudin (Angiox®; The Medicines Company) and argatroban (Argatrac®, Mitsubishi Pharma), both of which have a more favorable safety profile than hirudin, possibly because of their reversible binding to thrombin (2). Bivalirudin has received marketing approval as an alternative anticoagulant for percutaneous coronary intervention, and argatroban has been approved for the treatment of patients with HIT (2). However, the clinical use of these DTIs is limited by the need for intravenous administration, which makes them unsuitable for long-term and/or outpatient use. This has led to the development of oral DTIs, such as ximelagatran and dabigatran etexilate.

**Ximelagatran**

Ximelagatran (Exanta®; AstraZeneca), the first orally active thrombin inhibitor, is a prodrug of the active site-directed thrombin inhibitor melagatran (1). Ximelagatran underwent an extensive clinical trial program. It was evaluated for the prevention of VTE after orthopaedic surgery and for the treatment of VTE (1). The efficacy of ximelagatran in stroke prevention in patients with atrial fibrillation (AF) was demonstrated in two phase III trials (1). However, hepatotoxicity and major adverse cardiovascular events were observed in several studies (1), and ximelagatran was withdrawn from all markets and from further development in February 2006.

**Dabigatran**

Dabigatran etexilate (Pradaxa®; Boehringer Ingelheim) is a prodrug of dabigatran, a specific and reversible thrombin inhibitor. After oral administration, dabigatran etexilate is rapidly and completely metabolised to the active form dabigatran, with an oral bioavailability of approximately 6.5% (23). Maximal plasma concentrations are reached within 2 hours of administration. Dabigatran exhibits linear pharmacokinetic characteristics, with dose-proportional increases observed in maximum plasma concentration and area under the plasma concentration–time curve in healthy male subjects. Prolongation of the clotting tests (activated partial thromboplastin time [aPTT], international normalised ratio, thrombin time, and ecarin clotting time) is dose dependent with both single- and multiple-dosing administrations, and correlates with the plasma concentration–time profile of dabigatran. This demonstrates a rapid onset of action without a time delay and illustrates the direct mode of action of the drug on thrombin in plasma (23).

Three phase III studies investigated oral dabigatran etexilate for the prevention of VTE after major orthopaedic surgery (Table 2) (23). These studies compared oral dabigatran etexilate 150 and 220 mg once daily (od) with the subcutaneous LMWH enoxaparin 40 mg od (RE-MODEL and RE-NOVATE) or 30 mg twice daily (bid; RE-MOBILIZE); the initial dabigatran etexilate 180–220 mg od regimen was associated with increased bleeding and thrombocytopenia, whereas the 150–220 mg od dose regimens demonstrated a rapid onset of action with a dose-dependent increase in bleeding and thrombocytopenia.

**Table 2: Dabigatran for the prevention of venous thromboembolism after major orthopaedic surgery: results of phase III studies (1)**

<table>
<thead>
<tr>
<th>DVT, PE, and all-cause mortality (%)</th>
<th>Enoxaparin (150 mg)</th>
<th>Dabigatran (150 mg)</th>
<th>Dabigatran (220 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE-NOVATE (THR)</td>
<td>6.7</td>
<td>8.6</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.0001*</td>
<td></td>
<td>P&lt;0.0001*</td>
</tr>
<tr>
<td>RE-MOBILIZE (TKR)</td>
<td>25.3</td>
<td>33.7</td>
<td>31.1</td>
</tr>
<tr>
<td></td>
<td>P=0.009‡</td>
<td></td>
<td>P&lt;0.021†</td>
</tr>
<tr>
<td>RE-MODEL (TKR)</td>
<td>37.7</td>
<td>40.5</td>
<td>36.4</td>
</tr>
<tr>
<td></td>
<td>P=0.003‡</td>
<td></td>
<td>P=0.017*‡</td>
</tr>
</tbody>
</table>

**Major bleeding (%)**

| RE-NOVATE (THR)                     | 1.6                 | 1.3                 | 2.0                 |
|                                    |                     |                     |                    |
| RE-MOBILIZE (TKR)                   | 1.4                 | 0.6                 | 0.6                 |
|                                    |                     |                     |                    |
| RE-MODEL (TKR)                      | 1.3                 | 1.3                 | 1.5                 |

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ilate dose was given as a half-dose after surgery. In all three studies, the primary efficacy endpoint was the composite of deep vein thrombosis (DVT), pulmonary embolism (PE), and all-cause mortality. In these studies, dabigatran was either non-inferior (RE-MODEL [TKR] and RE-NOVATE [THR]) or inferior to the LMWH enoxaparin (RE-MOBILIZE [TKR]), with a similar safety profile (23).

Dabigatran was approved in the EU in 2008 for VTE prevention in patients undergoing THR and TKR. It is currently undergoing a phase III study (RE-LY) for the prevention of stroke in patients with AF. Dabigatran is also being investigated in three phase III studies for the long-term treatment and secondary prevention of VTE (RE-COVER, RE-COVER 2, RE-MEDY, and RE-SONATE). A multicenter, placebo-controlled, dose-finding, phase II study in patients with acute coronary syndrome (ACS), RE-DEEM, has recently been started.

**Direct Factor Xa inhibitors**

Factor Xa is a different target for coagulation inhibition than thrombin (24). This is due to the amplifying nature of coagulation factor interactions and fibrin formation, with one molecule of Factor Xa resulting in the generation of 1,000 molecules of thrombin (13). This means that smaller concentrations of an anticoagulant are sufficient to block coagulation progression. AT-independent Factor Xa inhibitors can also inhibit prothrombinase and clot-bound Factor Xa activity (14–16, 18).

Compared with thrombin, Factor Xa has limited functions outside the coagulation cascade (25), although it exhibits proinflammatory and proliferative activities (26).

There is evidence for insufficient activation of protein C with DTIs, which could explain the observed phenomenon of hypercoagulability. Activated protein C (APC), together with its cofactor protein S, degrades coagulation Factors Va and VIIIa, thereby limiting further thrombin generation (1). It has recently been shown that low concentrations of DTIs, such as melagatran and dabigatran, might suppress the anticoagulant effects of the thrombin–thrombomodulin/APC system by inhibiting the activation of protein C by the thrombin–thrombomodulin complex (i.e. they suppress the negative feedback reaction by APC), and thereby enhance thrombin generation (27). In the same study, the direct Factor Xa inhibitor rivaroxaban did not increase thrombin generation, suggesting that it does not suppress the negative feedback reaction by APC (27). This study confirms previous studies comparing the direct Factor Xa inhibitors DX-9065a and DU-176b with the thrombin inhibitor melagatran.

**Rivaroxaban**

Rivaroxaban (Xarelto®; Bayer Schering Pharma) is a small-molecule, oral, direct Factor Xa inhibitor that selectively and reversibly inhibits both free and clot-associated Factor Xa activity, as well as prothrombinase activity (14, 15). Rivaroxaban has a favorable efficacy/bleeding ratio in animal models of arterial thrombosis (arteriovenous-shunt and tail-transsection bleeding-time models). In these models, rivaroxaban was compared with the oral vitamin K antagonist warfarin, the AT-independent thrombin inhibitor ximelagatran, the LMWH enoxaparin, and the antiplatelet agent clopidogrel. Rivaroxaban had the most favorable efficacy/bleeding ratio. It was also the only drug to have an effective dose (ED$_{50}$) lower than the concentration required to double bleeding time (5 mg/kg and 5.8 mg/kg, respectively), and, therefore, an efficacy/bleeding ratio of less than 1.0 (28).

Rivaroxaban has a high oral bioavailability and a rapid onset of action, reaching maximum plasma concentrations 2.5–4.0 hours after administration. It has a half-life of up to 9 hours in healthy young subjects and 12–13 hours in healthy elderly subjects (16). Rivaroxaban has a dual mode of elimination, with one-third of the drug eliminated as unchanged drug in the urine and two-thirds undergoing metabolic degradation, half of which is excreted via the kidneys and half via the fecal route (29).

Rivaroxaban shows dose-proportional plasma concentration–time profiles, with no significant accumulation over time. In addition, the plasma concentrations and pharmacodynamic effects of rivaroxaban (inhibition of Factor Xa activity and prolongation of prothrombin time [PT]) correlate closely in healthy subjects and those undergoing major orthopaedic surgery (15, 16).

Rivaroxaban has recently been approved for the prevention of VTE after THR and TKR in the EU and several other countries, based on the results of the phase III RECORD program, which comprised four large studies in more than 12,500 patients in total. In all of the RECORD studies, the primary efficacy endpoint was the composite of DVT, as detected by mandatory, bilateral venography, non-fatal PE, and all-cause mortality (total VTE), and the main secondary efficacy endpoint was the composite of proximal DVT, non-fatal PE, and VTE-related death (major VTE). The primary safety endpoint was major bleeding, defined as clinically overt bleeding that was fatal, occurred in a critical organ (e.g. retroperitoneal, intracranial, intracural, or intrapinal), required re-operation, or extra-surgical-site bleeding that was associated with a fall in haemoglobin of 2 g/dl or more, or required an infusion of 2 or more units of blood (Table 3). RECORD2 investigated the efficacy and safety of extended thromboprophylaxis with rivaroxaban (5 weeks; 31–39 days) compared with short-term enoxaparin (10–14 days followed by placebo) in patients undergoing THR (30). The results of this study demonstrated that extended prophylaxis with rivaroxaban 10 mg od was superior to short-term prophylaxis with enoxaparin 40 mg od for the prevention of VTE, including symptomatic events, after THR (Table 3). Despite rivaroxaban being given for 3 weeks longer than enoxaparin, the incidence of major bleeding at 5 weeks was 0.1% in both groups. This study confirmed the benefits of extended prophylaxis over short-term prophylaxis and the safety of its use (30).

The RECORD1 and 3 studies were designed to compare rivaroxaban 10 mg od (starting 6–8 hours after surgery) with enoxaparin 40 mg od (starting the evening before surgery) given for 31–39 days (extended prophylaxis) after THR (RECORD1) (31) and 10–14 days (short-term prophylaxis) after TKR (RECORD3) (32). In both studies, rivaroxaban was significantly more effective than enoxaparin for the prevention of VTE (Table 3). RECORD 3 also showed a significant reduction in symptomatic VTE, and whereas RECORD1 showed a general trend for reduction in symptomatic VTE, which was not statistically significant. RECORD4 compared the efficacy and safety of oral rivaroxaban 10 mg od (starting 6–8 hours after surgery) with the
North American regimen of enoxaparin 30 mg bid (starting 12–24 hours after surgery), given subcutaneously (10–14 days) in patients undergoing TKR (33). Rivaroxaban was significantly superior to enoxaparin for the primary efficacy endpoint, with no significant difference in the rates of major bleeding between the two groups.

Rivaroxaban is also being investigated in large-scale phase III studies for prophylaxis in hospitalised, medically ill patients (MAGELLAN), VTE treatment (EINSTEIN DVT, EINSTEIN PE, and EINSTEIN EXT) and the prevention of stroke and non-CNS embolism in patients with AF (ROCKET AF and J-ROCKET AF), and phase III studies have also recently been initiated for secondary prevention in patients with ACS (ATLAS ACS TIMI 51).

### Apixaban

Apixaban is a small-molecule, oral, direct Factor Xa inhibitor that selectively and reversibly inhibits both free Factor Xa and prothrombinase activity. Apixaban has high oral bioavailability in large animals, and has a half-life of approximately 12 hours in humans (34).

Apixaban is currently being evaluated in phase III studies for the prevention of VTE after major orthopaedic surgery (the ADVANCE program). ADVANCE-1 investigated the efficacy and safety of oral apixaban 2.5 mg bid (with the first dose started on the morning of the day after surgery), compared with subcutaneous enoxaparin 30 mg bid (started 12–24 hours after surgery), for the prevention of VTE in patients undergoing TKR (both regimens administered for 12 days). This study has been completed recently (35). The results of the ADVANCE-1 trial indicated that apixaban did not meet the prespecified criteria for non-inferiority compared with enoxaparin with respect to the primary efficacy endpoint (a composite of symptomatic or asymptomatic DVT, PE, and all-cause mortality). The rate of the primary efficacy endpoint in the apixaban and enoxaparin groups was 9.0% and 8.9%, respectively (P-value non-significant). Rates of major bleeding (ISTH definition: fatal bleeding, symptomatic bleeding in a critical area or organ, or bleeding causing a fall in haemoglobin of 20 g/l or more, or required an infusion of 2 or more units of blood) with apixaban and enoxaparin were 0.7% and 1.4%, respectively (P = 0.05). ADVANCE-2 (apixaban 2.5 mg bid versus enoxaparin 40 mg od) is currently underway in patients undergoing TKR. A third study, ADVANCE-3, is assessing extended prophylaxis with apixaban 2.5 mg bid compared with enoxaparin 40 mg od (35 days for both regimens) in patients undergoing THR. The estimated study completion date for the two latter studies is early 2009.

Apixaban is currently undergoing two phase III studies for the treatment of VTE (AMPLIFY, AMPLIFY-EXT). The drug is also being investigated in phase III studies for the prevention of stroke or systemic embolism in patients with AF (ARISTOTLE and AVERROES). The results of a phase II study assessing the safety of apixaban in combination with antiplatelet therapy after ACS (APPRAISE-1) were presented at the European Society of Cardiology Congress in 2008. It was reported that the addition of apixaban to antiplatelet therapy resulted in a dose-dependent increase in bleeding and a trend towards a reduction in clinically important ischaemic events. A phase III study is currently being planned.

### Betrixaban

Betrixaban is a small-molecule, oral, direct Factor Xa inhibitor that selectively and reversibly inhibits free Factor Xa in animal models of thrombosis (36). In these animal models, it has a bioavailability of 47% and a half-life of 19 hours (36).

A phase II, randomised, open-label VTE prevention trial (EXPERT) in patients undergoing TKR provided proof of principle for the efficacy and safety of betrixaban (15 or 40 mg bid) (37). The primary efficacy endpoint was the incidence of VTE (symptomatic DVT or PE, or asymptomatic DVT) on days...
10–14. VTE occurred in 20% and 15% of patients receiving betrixaban 15 and 40 mg, respectively, and 10% of those receiving enoxaparin. Major bleeding events were not observed in either of the betrixaban groups, but occurred in 2% of patients in the enoxaparin group. Dose selection will be investigated further in a large dose-ranging study. There are plans to investigate betrixaban for VTE treatment and prevention, stroke prevention in patients with AF, and for the secondary prevention of stroke and myocardial infarction.

Edoxaban

Edoxaban (DU-176b) is an oral, direct and specific Factor Xa inhibitor with an approximate 10,000-fold selectivity for Factor Xa over thrombin (34). In vitro studies have shown that DU-176b dose-dependently prolongs human plasma clotting in the PT and aPTT assays, with concentrations of 0.26 and 0.51 μM doubling the clotting times, respectively. In young, healthy subjects, peak plasma levels of DU-176b were observed at 1.5 hours after a single oral dose, corresponding to the maximum inhibition of Factor Xa activity; the antithrombotic effects were sustained for up to 5 hours (34).

A placebo-controlled phase II study in patients undergoing TKR showed that DU-176b dose-dependently reduced VTE, without increases in major or clinically relevant bleeding (38). The results of a phase Ib study (STARTS) comparing DU-176b with dalteparin in patients undergoing THR have recently been reported (39). In this randomised, double-blind study, DU-176b (15, 30, 60, or 90 mg od) showed a dose-dependent reduction in the incidence of VTE. There were no major or clinically relevant bleeding events associated with dalteparin. The rate of major or clinically relevant bleeding was 1.6%, 1.8%, 2.2%, and 2.3% for the 15, 30, 60, and 90 mg od DU-176b doses, respectively. A phase III study investigating VTE prevention has been planned.

DU-176b was also assessed in a phase II study for the prevention of stroke in patients with AF, compared with standard warfarin therapy for 3 months (40). A significantly higher incidence of major and clinically relevant non-major bleeding events was observed in patients who received DU-176b 30 mg bid or 60 mg bid, compared with those given warfarin, whereas the incidence in the DU-176b 30 mg od and 60 mg od groups were similar to those in warfarin-treated patients (40). A phase III trial will compare DU-176b 30 mg or 60 mg od with warfarin for 24 months in patients with AF (ENGAGE-AF TIMI 48). Studies in patients with ACS are currently being planned.

Conclusions

The efficacy and safety demonstrated by new oral, AT-independent anticoagulants — both DTIs and direct Factor Xa inhibitors — in recent clinical studies potentially heralds a new era for anticoagulation. These first positive results, obtained for the prevention of VTE in patients undergoing elective THR and TKR, have already led to the approval of two agents for this indication, the DTI dabigatran and the direct Factor Xa inhibitor rivaroxaban. These compounds are also currently being investigated in chronic use indications such as the prevention of stroke in patients with AF and the secondary prevention of ACS, as well as for the treatment of VTE. In addition, further compounds are currently at advanced stages of clinical development.

It is very unlikely, however, that these new drugs will completely remove the need for heparins. There is currently no replacement for UFH in the standard intensive care unit setting, and heparins are the only recommended drugs during pregnancy (2). Moreover, the non-anticoagulant actions of heparins and LMWHs indicate a potential for exploring indications beyond anticoagulation (41). The effects of heparin are currently being investigated in a number of diseases, such as interstitial cystitis, asthma, inflammatory bowel disease, allergic rhinitis, cancer, adult respiratory-distress syndrome, arthritis, transplant rejection, delayed-type hypersensitivity reactions, and allergic encephalomyelitis (41).

For many years, LMWHs have been the anticoagulants of choice. Although the end of the heparin and LMWHs era is not to be expected, the new, direct, targeted anticoagulants presented in this review potentially represent the future of anticoagulation.

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