New anticoagulants
From bench to bedside
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Summary
Heparins and vitamin K antagonists have been the cornerstones of anticoagulation therapy for several decades. Although they are very effective at inhibiting the coagulation process, they have several practical limitations. This was a challenge for the development of therapies that will overcome these drawbacks while matching the efficacy of the two classes of anticoagulants. Advances were achieved in the development of safer, convenient, more specific treatments, which should provide more predictable anticoagulant responses and substantially improve the prevention and management of thromboembolic disorders.

Established anticoagulants

Vitamin K antagonists
Warfarin, the most commonly used VKA, exerts its anticoagulant effect by interfering with the metabolism of vitamin K, inhibiting the synthesis of several coagulation proteins such as factors II, VII, IX, X, and proteins C and S. The benefits of warfarin therapy in a wide range of patients with thromboembolic disorders are well established. For example, a metaanalysis of trials involving 2900 patients demonstrated that dose-adjusted warfarin reduced the relative risk of stroke by 62% compared with placebo in patients with atrial fibrillation (14).

However, warfarin’s use is hampered by numerous limitations (Tab. 1), such as its narrow therapeutic window, its need for frequent coagulation monitoring and dose adjustments, dietary restrictions, bleeding risk and its delayed on- and off-set of action (13).

Heparin
UFH and the LMWHs are indirect coagulation inhibitors, too. UFH enhances the activity of the plasma cofactor antithrombin that in turn inhibits thrombin and factor Xa. While efficacious, UFH, like warfarin, has a
number of limitations which restrict its clinical use (Tab. 1), including its parenteral route of administration, frequent laboratory monitoring and the development of potentially life-threatening heparin-induced thrombocytopenia (HIT) Type II.

LMWHs, derived from UFH by enzymatic or chemical depolymerization resulting in shorter heparin chains, have an enhanced affinity for antithrombin-mediated inhibition of factor Xa relative to thrombin inhibition. LMWHs have overcome several of the limitations of UFH, including a more predictable anticoagulant effect resulting in no requirement for routine coagulation monitoring, but their use is still associated with a risk of HIT (though to a lesser extent than that seen with UFH), while the need for parenteral administration limits their use in the outpatient setting (18).

### Improving anticoagulation

How can anticoagulation therapy be improved? By considering the shortcomings of the current anticoagulants the following characteristics required for the ideal anticoagulant can be defined:
- oral and parenteral administration,
- no requirement for close coagulation monitoring and individual dose adjustments,
- a wide therapeutic window,
- an appropriate elimination half-life,
- rapid onset of action,
- rapid offset of action,
- minimal interactions with food or drugs,
- low, non-specific plasma protein binding,
- ability to inhibit free and clot-bound coagulation factors.

In the search for new agents matching the ideal anticoagulant profile, a number of steps in the coagulation cascade have been targeted, including direct thrombin inhibition, and inhibition of factor Xa, factor IXa, the factor VIIa-tissue factor complex and the factor Va-factor VIIa complex (Tab. 2). The mode of action of targeted factor Xa- and factor IIa-inhibitors is shown in Figure 1.

### Thrombin inhibitors

Due to its central role in the coagulation cascade, thrombin is an attractive target for the development of new anticoagulants. The procoagulant effects can be blocked by several ways:
- Indirect inhibitors act by catalyzing the physiologic thrombin inhibitors, e.g. heparins require antithrombin as a cofactor.
Direct thrombin inhibitors (DTIs) are small molecules that inhibit thrombin by directly binding to the active catalytic site.

Thus DTIs inhibit clot-bound as well as free thrombin without requiring a cofactor—a potential advantage over the indirect thrombin inhibitors (33). Furthermore, they do not bind to plasma proteins, producing a more consistent anticoagulant response, and are not neutralized by platelet factor 4, thereby posing no risk of HIT.

Hirudin was the first DTI available for clinical use. Although more effective than heparin, it is associated with an increased risk of bleeding and is parenterally administered. Other DTIs include bivalirudin and argatroban, both of which have a more favourable safety profile than hirudin due to reversible instead of irreversible binding to thrombin (5). Argatroban has been approved as alternate anticoagulant in patients with heparin induced thrombocytopenia type II whereas bivalirudin has been licensed for percutaneous coronary intervention (7). After intravenous administration, bivalirudin shows reversible anticoagulant effects, with coagulation time returning to baseline in approximately 60 min (5).

However, the clinical use of these DTIs is also limited by the need for parenteral administration which makes them not suitable for longterm use. This has led to further advances in the development of oral DTIs such as dabigatran etexilate, which is in phases II and III of clinical development.

**Dabigatran etexilate**

After oral administration, dabigatran etexilate is rapidly bioconverted to the active form dabigatran which exerts antithrombotic effects with maximal plasma concentrations being reached 1.25 to 1.75 h, followed by biphasic decline with terminal elimination half-lives of 8.2 to 10.4 h after doses of 100 to 400 mg (29).

Dabigatran etexilate is undergoing evaluation for the prevention of venous thromboembolism (VTE) following orthopedic surgery. In a multicenter, parallel-group, double-blind study, 1973 patients undergoing total hip or knee replacement were randomized to 6-10 days of oral dabigatran etexilate (50, 150 mg twice daily, 300 mg once daily, 225 mg twice daily), starting 1-4 h after surgery, or subcutaneous enoxaparin (40 mg once daily) starting 12 h prior to surgery.

The primary efficacy outcome was the incidence of VTE (detected by bilateral venography or symptomatic events) during treatment. Of the 1949 treated patients, 1464 (75%) patients were evaluable for the efficacy analysis. VTE occurred in 28.5%, 17.4%, 16.6%, 13.1%, and 24% of patients assigned to dabigatran etexilate 50, 150 mg twice daily, 300 mg once daily, 225 mg twice daily and enoxaparin, respectively. A significant dose-dependent decrease in VTE occurred with increasing doses of dabigatran etexilate (p <0.0001). Compared with enoxaparin, VTE was significantly lower in patients receiving 150 mg twice daily [odds ratio (OR) 0.65, p = 0.04], 300 mg once daily (OR 0.61, p = 0.02) and 225 mg twice daily (OR 0.47, p = 0.0007).

Compared with enoxaparin, major bleeding was significantly lower with 50 mg twice daily (0.3% vs. 2.0%, p = 0.047) but elevated with higher doses, nearly reaching statistical significance with the 300 mg once-daily dose (4.7%, p = 0.051). Thus, it can be concluded that oral administration of dabigatran etexilate, commenced early in the postoperative period, was effective and safe across a range of doses. Further optimization of the efficacy/safety balance will be addressed in future studies (9).

The safety and efficacy of dabigatran etexilate (50, 150 or 300 mg per os b.i.d. for 12 weeks) was compared with enoxaparin (40 mg/day) or warfarin adjusted to INR 2.0 to 3.0, were examined in a randomized, dose-finding trial conducted in 502 patients with atrial fibrillation on stable warfarin treatment and with at least one additional risk factor for thromboembolic events. Of the patients enrolled, 464 completed the 12 weeks of treatment and 29 and 9 discontinued due to adverse events and other causes, respectively. Acetylsalicylic acid was terminated prematurely in the 300 mg dabigatran etexilate group due to excessive bleeding events (11% of patients). However, coadministration of acetylsalicylic acid in the other dose groups did not significantly increase bleeding events; only 2% and 8% of the patients, respectively, on 50 and 150 mg developed major or relevant bleeding events. The rate of bleeding events...
in the highest dose group in the absence of acetylsalicylic acid was comparable to in the other groups.

Thromboembolic events were seen in two, none and one patient, respectively, in the 50, 150 and 300 mg b.i.d. dose groups and in none patient in the warfarin group. The 150 mg b.i.d. dose of dabigatran etexilate was concluded to have the same anticoagulant activity as the higher dose and warfarin. None of the dabigatran etexilate doses significantly altered liver function tests. From the results of this study, dabigatran etexilate doses of 200 to 300 mg daily were recommended for phase III testing for the prevention of thromboembolic events in patients with atrial fibrillation (32).

The promising results from these trials suggest that dabigatran etexilate may represent a significant improvement over existing therapies for the management of various thromboembolic complications. The convenience of oral administration, without the need for individualized dosing or routine coagulation monitoring, mean that the management of patients will be made considerably easier.

**Factor Xa inhibitors**

Factor Xa inhibitors include the synthetic pentasaccharides fondaparinux and idraparinux, which act indirectly via activating antithrombin, as well as direct inhibitors such as the synthetic agents DX-9065a, rivaroxaban, apixaban and other compounds. Fondaparinux, which is discussed in more detail below, is the most advanced of the factor Xa inhibitors (Tab. 2), having been approved in 2001 for thromboprophylaxis in patients undergoing major orthopaedic surgery and in 2004 for treatment of venous thromboembolism, and recent trials have confirmed its efficacy and safety in acute coronary syndrome (23, 35).

**Fondaparinux, idraparinux**

Fondaparinux is the first in a new class of antithrombotic compounds, the synthetic pentasaccharides. Synthetic pentasaccharides are analogues of the pentasaccharide sequence of heparin that mediates the binding of heparin to antithrombin, accelerating the rate at which it inactivates factor Xa. In contrast to heparins, however, the small size of fondaparinux means that it does not form complexes with platelet factor 4 and thereby will not cause HIT. Fondaparinux is 100% bioavailable and has a highly predictable pharmacokinetic profile. It is administered subcutaneously once daily and can be used without coagulation monitoring (12). The pharmacodynamic profile of idraparinux is comparable to fondaparinux except for the significantly longer half life requiring only once weekly injections.

The benefit-to-risk ratio of fondaparinux in preventing venous thromboembolism has been investigated in four randomized, phase III trials in patients undergoing surgery for hip fracture, hip replacement, and major knee surgery. A metaanalysis of these trials showed that fondaparinux reduced the incidence of venographically proven venous thromboembolism by 55.2% compared with enoxaparin. The superior profile of fondaparinux over enoxaparin was demonstrated for proximal DVT with a reduction of 57.4% (30). Fondaparinux has also been investigated for the initial treatment of venous thromboembolism. The results of the MATISSE DVT trial (6) and the MATISSE PE (7) trial suggest that fondaparinux is as effective and safe as low-molecular-weight heparin or unfractionated heparin for initial treatment of patients with deep vein thrombosis or pulmonary embolism, respectively.

Fondaparinux is an improvement over heparin and LMWHs in terms of efficacy, its use is associated with a higher transfusion requirement and its long-term use may be limited by the parenteral route of administration.

Once-weekly subcutaneously administered idraparinux was compared with warfarin in a phase II dose-finding trial involving 659 patients with proximal deep vein thrombosis. The rates of normalization and deterioration of ultrasonographic and perfusion lung scanning were similar in all idraparinux dosing groups, and did not differ from that in the warfarin control group. However, there was a clear dose-response with respect to major bleeding in patients given idraparinux, with an unacceptably high frequency of bleeding in those given 10 mg. Two patients, both of whom received 5 mg idraparinux, suffered a fatal bleeding. Patients given the lowest dose of 2.5 mg had less bleeding than those randomized to warfarin (p = 0.029) (27).

Phase III trials comparing 2.5 mg of idraparinux subcutaneously once-weekly with enoxaparin followed by warfarin for treatment of patients with deep vein thrombosis or pulmonary embolism are ongoing, as is another phase III study of once-weekly idraparinux versus warfarin in patients with atrial fibrillation.

**DX-9065a**

DX-9065a is a synthetic, non-peptidic, small-molecule inhibitor of factor Xa that binds reversibly to the active site. It needs to be administered intravenously and prolongs prothrombin time and aPTT as well as inhibiting factor Xa activity. Overall, the changes in clotting parameters correlate well with the plasma concentrations. In the phase II XaNADU-ACS study, conducted in patients with NSTEMI, subjects were randomized to low- or high-dose intravenous DX-9065a, or UFH (3). The composite of death, MI, urgent revascularization and ischemia on continuous ST-segment monitoring occurred in 34.3%, 31.3%, and 34.3% of patients receiving low-dose DX-9065a, high-dose DX-9065a and UFH, respectively. The composite of death, MI and urgent revascularization occurred in 11.9% of the patients receiving high-dose DX-9065a, compared with 19.5% of patients receiving UFH; however, this difference was not significant.

Higher plasma DX-9065a concentrations were associated with a significantly lower rate of ischaemic events, but also a tendency towards increased major bleeding (3). Large-scale, adequately powered studies in patients with cardiovascular disease are warranted to further evaluate this drug.

**Otamixaban**

Otamixaban is a small-molecule, direct factor Xa inhibitor that is administered intravenously. Healthy subjects received escalating intravenous otamixaban doses of 1.7–183 µg/kg/hour as 6-hour infusions, or a bolus of
30 or 120 µg/kg body weight, followed by a 6-hour infusion of 60 or 140 µg/kg/hour, respectively. $C_{\text{max}}$ and AUC increased dose dependently, and were slightly more than dose proportional (25). There was a close correlation between plasma concentrations and pharmacodynamic parameters, and otamixaban was well tolerated without any consistent changes in bleeding time.

Anti-Xa activity correlated well with plasma concentrations (26). Prothrombin time (PT), aPTT, HepTest and Russell’s viper venom-induced clotting time (RVVT) displayed dose-dependent prolongations at otamixaban doses $>27$ µg/kg/hour. PT, aPTT, and RVVT times returned to baseline within 2-4 hours after cessation of the infusion, whereas HepTest remained slightly above baseline for longer. Inhibition of thrombin generation, as measured by endogenous thrombin potential, decreased dose dependently 2-6 hours after the otamixaban bolus + 6 hour infusion, and returned to baseline 10 hours after the start of infusion (26).

Subsequent investigations revealed that the combination of an otamixaban bolus of 25-140 µg/kg body weight followed by a 3-hour infusion of 35–200 µg/kg/hour would achieve the target effective otamixaban concentrations necessary for a phase II study (15). A study conducted in healthy men indicated that there was no pharmacokinetic interaction between otamixaban and aspirin; the combination had no additive effects on coagulation or platelet parameters, and no clinically relevant effect on bleeding (16). These promising pharmacodynamic effects require correlation with outcomes in clinical studies.

### Rivaroxaban

Rivaroxaban is a member of a new class of orally available, small-molecule, active-site-directed factor Xa inhibitors (28). Rivaroxaban was well tolerated over the whole dose range when administered to healthy male subjects at single oral doses of 1.25-80 mg or multiple doses up to 30 mg twice daily. After multiple dosing, when taken with food, the $C_{\text{max}}$ and AUC of rivaroxaban were dose proportional. Rivaroxaban was rapidly absorbed, reaching $C_{\text{max}}$ 2-4 hours after oral administration, with a $t_{1/2}$ of 5-9 hours, and no undue accumulation under steady-state conditions (20). The relative bioavailability of rivaroxaban was high, reaching approximately 80%. Rivaroxaban has a dual mode of excretion, via the renal (66%) and faecal/biliary (28%) routes, and is mainly excreted as unchanged drug (29, 21).

The onset of inhibition of factor Xa activity with rivaroxaban was rapid and similar to LMWHs, with the pharmacodynamic effects occurring in parallel to the pharmacokinetics at all doses. Furthermore, inhibition of factor Xa activity was dose dependent across all dosing regimens, and maximum inhibition of factor Xa activity did not differ significantly between initial drug administration and steady state (20).

Results from phase I studies suggested that dose adjustment of rivaroxaban would not be required for gender or in patients with extreme body weight. Co-administration of rivaroxaban with food increased the peak plasma concentrations slightly, regardless of the type of food eaten; however, due to the limited degree of this effect and the wide therapeutic window of rivaroxaban observed in clinical studies, no dose adjustment is likely to be required. No additive effects on platelet aggregation were observed when rivaroxaban was co-administered with either aspirin or the non-steroidal anti-inflammatory drug naproxen (20, 21).

Three phase II double-blind, double-dummy, dose-ranging studies comparing rivaroxaban with enoxaparin for the prevention of VTE after hip or knee replacement surgery have been reported. Two studies in hip or knee replacement patients used twice-daily (bid) rivaroxaban dosing, and a further study in hip replacement patients used once-daily (od) rivaroxaban dosing. Drug therapy continued until mandatory bilateral venography for the assessment of DVT was performed 5-9 or 6-10 days after surgery (11, 19, 31).

The bid studies examined total daily rivaroxaban doses of 5-60 mg, and the od study tested 5-40 mg. The total numbers of patients treated were 704, 613, and 845 in the hip bid, knee bid and hip od studies, respectively. The incidence of total VTE ranged from 6.9% to 18.2% with rivaroxaban compared with 17.0% for enoxaparin in the hip bid study (11), from 23.3% to 40.4% with rivaroxaban compared with 44.3% for enoxaparin in the knee bid study (31), and from 6.4% to 14.9% compared with 25.2% in the hip od study (19).

It should be noted that rates of VTE after total knee replacement compared with those after total hip replacement are generally accepted as being slightly higher. There was a flat dose-response relationship for efficacy (total VTE) in all of the studies. However, a significant dose-response relationship for major VTE (proximal DVT, PE and VTE-related death) was observed with rivaroxaban in the hip od study ($p = 0.0072$) (19). The incidence of major bleeding increased dose dependently with increasing rivaroxaban dose in all studies. No rivaroxaban dose group had a significantly higher incidence of major bleeding than enoxaparin; however, the studies were not powered to detect differences between treatment groups (11, 19, 31).

When the efficacy and safety of rivaroxaban were considered together after the bid studies, a total daily rivaroxaban dose of 5-20 mg was found to have similar efficacy and safety to enoxaparin, indicating that rivaroxaban has a wide therapeutic window (11, 31). The results of the hip od study showed that a rivaroxaban dose of 10 mg od was the optimal dose in this indication, and this is the dose being investigated in phase III studies (the RECORD studies) (19). Full publication of the results of studies assessing the efficacy and safety of rivaroxaban for the treatment of thrombosis are expected in 2007.

### Apixaban

Apixaban is a small molecule inhibitor with a molecular weight of 460 that targets the active site of factor Xa. It is a selective and reversible inhibitor of factor Xa and, like rivaroxaban, it inhibits factor Xa bound within the prothrombinase complex as well as the free enzyme. The drug is well absorbed from the gastrointestinal tract and peak plasma levels are achieved in about three hours. With repeated doses, the terminal half-life is between nine and 14 hours. Therefore, once-daily administration may be possible.
Apixaban is oxidized to a phenol metabolite in the liver and CYP3A4 may be involved in this metabolism. However, the potential for drug-drug interactions with apixaban is expected to be low. Like rivaroxaban, apixaban exhibits a dual mechanism of excretion. About 25% is excreted via the kidneys, while the remainder appears in the feces. Apixaban prolongs the INR and the aPTT in a concentration-dependent fashion. However, its effect on these tests is minimal at concentrations that are likely to be therapeutic (34).

After an extensive phase I trial program the compound is in clinical development and a large phase II study has recently been completed in patients undergoing major orthopaedic surgery. Several phase II and phase III trials are ongoing in various patient populations including primary prevention of venous thromboembolism, treatment of deep venous thrombosis and stroke prevention in atrial fibrillation.

**LY517717**

LY517717 is an orally active, direct factor Xa inhibitor with a Kᵢ of 4.4-6.6 nmol/l. The elimination half-life of LY517717 was 25 hours in healthy subjects, and the primary route of elimination appeared to be gastrointestinal. The safety and efficacy of LY517717 were evaluated in patients undergoing hip or knee replacement surgery. A total of 507 patients received oral LY517717 doses of 25, 50, 75, 100, 125, or 150 mg od or enoxaparin 40 mg od for 6-10 days. The three lower doses were halted prematurely due to lack of efficacy.

Total VTE rates were 24.0%, 19.1%, 17.1% for 100, 125, and 150 mg LY517717, respectively, compared with 22.2% for enoxaparin. Proximal DVT rates were 6.4%, 2.4%, and 0% for 100, 125, and 150 mg LY517717, respectively, compared with 4.2% for enoxaparin. Incidences of bleeding events were similar between the higher LY517717 doses and enoxaparin.

Dose-related prolongation of PT was observed, reaching a mean prolongation of 4.1 seconds after 150 mg of LY517717. No other haematological or chemistry abnormalities were associated with LY517717. These data suggested that LY517717 doses of 100-150 mg were as safe and efficacious as a standard enoxaparin regimen for the prophylaxis of VTE. However, as the number of patients included in this study was small, large-scale studies are necessary to further support this conclusion (2).

**YM150**

YM150 is a once-daily, orally active factor Xa inhibitor. It was investigated in a phase IIa study at doses of 3, 10, 30, or 60 mg for thromboprophylaxis in 174 patients undergoing hip replacement surgery; the comparator was enoxaparin 40 mg od. Study drugs were administered for 6-10 days.

Total VTE occurred in 52%, 39%, 23%, and 19% of patients receiving 3, 10, 30, and 60 mg YM150, respectively, compared with 39% of patients receiving enoxaparin. There was a statistically significant dose-response relationship with YM150 for the primary efficacy endpoint.

No major bleeding was observed in this study; non-major, clinically relevant bleeding events occurred in 2.9% of patients in the 3 mg group and 5.7% of patients in the 10 mg group. No dose-response relationship was observed for minor bleeding, and the incidence of minor bleeding was similar for the highest YM150 dose and enoxaparin. These promising results warrant further investigations in large-scale studies (10).

**Du-176b**

Du-176b is an orally available, direct FXa inhibitor that competitively inhibited FXa with a Kᵢ of 0.56 nmol/l. It was ~10 000-fold more selective for FXa than thrombin, and dose-dependently prolonged PT and aPTT in human plasma, with concentrations of 0.26 and 0.51 µmol/l doubling the clotting times, respectively (24).

A phase I study in healthy humans has recently been presented, in which a 60 mg dose of Du-176b showed antithrombotic effects in plasma samples, in a Badimon chamber under venous and arterial conditions. Du-176b had a rapid onset of action with anti-FXa activity peaking 1.5 hours after dosing, and returning to baseline 12 hours post-dose; PT and aPTT followed a similar pattern (36). It will be interesting to see how the drug will perform in clinical studies.

**Other new anticoagulants**

Other agents in development include the factor IXa inhibitors, which are either active site-blockers of factor IXa or monoclonal antibodies against factor IXa. A human antibody that inhibits factor IX/IXa function potently inhibits arterial thrombosis without increasing bleeding. While results in preclinical animal models appear promising, these approaches have not been evaluated in clinical trials.

The endogenous tissue factor pathway inhibitor (TFPI) blocks initiation of the clotting process by inhibiting the factor VIIa-tissue factor complex. Three phase II trials investigating a recombinant form of TFPI (rTFPI), tifacogin, found it safe and effective in patients with severe sepsis. Recently, however, a large phase III trial suggested that tifacogin reduced mortality during the first nine months of study enrolment; however, this trend was reversed in the last seven months of the study in favour of the placebo (1). Recombinant nematode anticoagulant peptide c2 (rNAPc2) has a similar mechanism of action to TFPI and has shown encouraging results in clinical studies. In phase II trials, subcutaneous administration of rNAPc2 in total knee arthroplasty patients showed low rates of DVT and an acceptable rate of major bleeding (22).

A recombinant form of thrombomodulin (rTH), which leads to the degradation of factors V and VIIIa via activated protein C (APC), is being evaluated for thromboprophylaxis in patients undergoing elective hip arthroplasty, and a recombinant form of APC has been approved in patients with severe sepsis (4).

**Conclusion**

The development of a single targeted ideal anticoagulant has turned out as a challenge. However, there are currently a host of promising new agents at various stages of development and clinical evaluation. With potential benefits including predictable efficacy, rapid onset of action, ability to bind clot-bound coagulation factors and no requirement for therapeutic monitoring, these new
agents are set to improve the management of thromboembolic disorders.

References


