Reversal of target-specific oral anticoagulants

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Target-specific oral anticoagulants (TSOACs) provide safe and effective anticoagulation for the prevention and treatment of thrombosis in a variety of clinical settings by interfering with the activity of thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban, edoxaban, betrixaban). Although TSOACs have practical advantages over vitamin K antagonists (VKAs), there are currently no antidotes to reverse their anticoagulant effect. Herein we summarize the available evidence for TSOAC reversal using nonspecific and specific reversal agents. We discuss important limitations of existing evidence, which is derived from studies in human volunteers, animal models and in vitro experiments. Studies evaluating the safety and efficacy of reversal agents on clinical outcomes such as bleeding and mortality in patients with TSOAC-associated bleeding are needed.

Introduction

Vitamin K antagonists (VKAs) have been the mainstay of long-term antithrombotic therapy for prevention and treatment of thromboembolism. VKAs have practical limitations including long half-lives, drug interactions and unpredictable pharmacokinetics necessitating routine monitoring of anticoagulant effect. Unlike VKAs, which impair the production of vitamin-K-dependent coagulation factors II, VII, IX and X, target-specific oral anticoagulants (TSOACs) exert their anticoagulant effect by inhibiting the activity of thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban, edoxaban, betrixaban), coagulation factors that mediate the final stages of coagulation.

TSOACs were developed as alternatives to VKAs owing to practical advantages including rapid onset of action, short half-lives, more-predictable pharmacokinetics, fewer drug interactions and lack of need for routine monitoring. TSOAC drug characteristics are shown in Table 1. Clinical uses of TSOACs include prevention of stroke and systemic embolism in nonvalvular atrial fibrillation, prevention of venous thromboembolism (VTE) following hip and knee arthroplasty and treatment of VTE. Based on large clinical trials and real-world post-marketing surveillance data, TSOACs are at least as effective and safe as VKAs or low molecular weight heparin for approved indications [1–3].

However, unlike VKAs for which vitamin K and coagulation factor replacement with prothrombin complex concentrate (PCC) or plasma can be used to replace coagulation factors and restore coagulation, there are no antidotes available to reverse the anticoagulant effect of TSOACs in the event of bleeding or need for an emergent procedure. Specific reversal agents are currently undergoing clinical development. In this narrative review, we summarize the current published evidence for TSOAC reversal using nonspecific and specific reversal agents.

Types of reversal agents

Coagulation factor replacement

Plasma. Plasma is the aqueous part of blood that contains dissolved proteins including coagulation factors. Plasma transfusion is associated with health risks including transfusion-associated circulatory overload, transfusion-related acute lung injury, allergy and infection [4].

Prothrombin complex concentrates. PCCs are plasma-derived concentrates of vitamin-K-dependent coagulation factors II, IX and X (3-factor PCC; 3-PCC) or factors II, VII, IX and X (4-factor PCC; 4-PCC) with variable amounts of proteins C and S. There is a
TABLE 1
Pharmacologic properties of target-specific oral anticoagulants.

<table>
<thead>
<tr>
<th>Target-specific oral anticoagulant</th>
<th>Target</th>
<th>Time to peak concentration (h)</th>
<th>Half-life (h)</th>
<th>Renal excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran [33,34]</td>
<td>Thrombin</td>
<td>1–3</td>
<td>7–9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80–85%</td>
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<td></td>
<td></td>
<td></td>
<td>7–17&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Rivaroxaban [35–37]</td>
<td>Factor Xa</td>
<td>2–4</td>
<td>7–17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12–13&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6–9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Apixaban [38–40]</td>
<td>Factor Xa</td>
<td>1–3</td>
<td>8–14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25%</td>
</tr>
<tr>
<td>Edoxaban [41]</td>
<td>Factor Xa</td>
<td>1–2</td>
<td>6–11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36–45%</td>
</tr>
<tr>
<td>Betrixaban [41,42]</td>
<td>Factor Xa</td>
<td>3–4</td>
<td>9–10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;8%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Healthy adults, single dose.  
<sup>b</sup>Healthy adults, multiple doses.

PER977 (Perosphere, Bedford, NY, USA) reversed heparin, low molecular weight heparin, fondaparinux, dabigatran, rivaroxaban, apixaban and edoxaban in preclinical studies [13]. The mechanism by which a single molecule could reverse direct and indirect inhibitors of thrombin and factor Xa is not clear and has not been reported.

Evidence for reversal of target-specific oral anticoagulants

Limitations of evidence

Ideally, evidence for reversal would be based on studies of patients with TSOAC-associated bleeding treated with various reversal strategies. Unfortunately, apart from a small number of case reports, such evidence is nonexistent. Instead, current evidence for reversal is limited to studies in human volunteer subjects, animal models and in vitro experiments, each of which carries important limitations (Fig. 1).

In vitro investigations comprise the weakest form of evidence for reversal. In these studies, anticoagulated human plasma is spiked with a reversal agent and its effect on a laboratory assay is measured. The major limitation of in vitro studies is the use of laboratory test results as a surrogate marker of efficacy as opposed to clinically relevant outcomes such as cessation of bleeding or mortality. Furthermore, widely used tests of coagulation such as the prothrombin time (PT) and activated partial thromboplastin time (aPTT) have variable sensitivity and accuracy for measuring the anticoagulant effect of the TSOACs [14].

Evidence for reversal is also derived from animal models in which bleeding is assessed following an induced injury (e.g. tail vein clipping) in the presence or absence of TSOAC treatment. These studies are limited by the need to extrapolate from animals to humans and inherent differences in artificial injury compared with clinical bleeding in patients.

Finally, several studies have been carried out in which healthy volunteers are given a TSOAC and then a reversal agent to measure the effect of the reversal agent on hemostatic laboratory assays. As with in vitro experiments, a major shortcoming of these ex vivo studies is use of a surrogate laboratory endpoint rather than a clinically relevant outcome. In addition, it is likely that the young low risk of viral transmission owing to viral inactivation during product preparation [5]. Thromboembolism is a potential complication of PCC use occurring at a rate of 1.4% when used to treat VKA-associated bleeding [5].

Prohemostatic agents

Activated prothrombin complex concentrate. Activated PCC (aPCC) contains plasma-derived activated forms of coagulation factors II, VII, IX and X. aPCC was developed as a prohemostatic agent to treat bleeding in hemophilia patients with inhibitors to factors VIII or IX [6]. There is a low risk of thromboembolism associated with aPCC use (4–8 events per 10<sup>6</sup> infusions) based on pharmacovigilance data in hemophilia patients [7,8]. However, the majority of these events (81%) occurred in patients with risk factors for thrombosis, which raises concerns regarding aPCC use in patients receiving anticoagulant therapy for prevention or treatment of thrombotic disease.

Recombinant factor VIIa. Recombinant factor VIIa (rVIIa) was also developed as a bypassing agent for bleeding complications in hemophilia patients with inhibitors. Use of rVIIa outside its approved indication is associated with an increased risk of arterial thromboembolism compared with placebo [5.5% vs 3.2%; relative risk 1.68; 95% confidence interval (CI) 1.20–2.36] [9].

Specific reversal agents

A humanized monoclonal antibody fragment against dabigatran (anti-Dabi-Fab; Boehringer Ingelheim, Biberach, Germany) is currently undergoing clinical development as a specific reversal agent [10]. It has ~350-fold greater affinity for dabigatran than it does for thrombin, and does not appear to bind endogenous thrombin substrates, activate coagulation or platelets.

A recombinant factor Xa derivative (PRT064445; Portola Pharmaceuticals, San Francisco, CA, USA) is being developed as a specific factor Xa inhibitor reversal agent [11]. The protein lacks catalytic and membrane-binding activity, but retains the ability to bind factor Xa inhibitors with subnanomolar affinity. Also in development is an inactive zymogen-like factor Xa variant, which has demonstrated reversal of the anticoagulant effect of rivaroxaban in vitro [12].
healthy subjects recruited for these studies differ from patients on anticoagulation.

**Dabigatran**

**Non-specific reversal agents.** Non-specific reversal agents have variable effects on the anticoagulant effect of dabigatran as measured by laboratory tests. Administration of 4-PCC (50 U/kg) failed to correct the aPTT, ecarin clotting time (ECT) or thrombin time (TT) in healthy volunteers receiving dabigatran (150 mg twice daily for 2.5 days) in a randomized placebo-controlled crossover study [15]. aPCC, 4-PCC and rVIIa each corrected some, but not all, abnormal thrombin generation indices when added to the plasma of healthy volunteers who had received a single oral dose of dabigatran (150 mg) [16]. When added to the plasma of dabigatran-treated patients, aPCC corrected thrombin generation parameters [17]. In another study, PCC and aPCC, but not rVIIa, corrected the PT, aPTT and some thrombin generation indices when added to plasma from dabigatran-treated patients [18]. Only PCC corrected TT prolongation. None of the reversal agents affected results of the Hemoclot® assay (HYPHEN BioMed, Neuville Sur Oise, France), a commercially available dilute TT that provides an accurate, reproducible measure of dabigatran anticoagulant activity [18,19].

In animal models, the ability of non-specific reversal agents to ameliorate dabigatran-related bleeding is inconsistent. PCC, aPCC and rVIIa did not reduce bleeding following tail transection in mice receiving dabigatran [20]. In another study, PCC, but not rVIIa or plasma, reduced intracranial hematoma expansion and 24-hour mortality in dabigatran-treated mice [21]. PCC also reduced blood loss following kidney incision in dabigatran-treated rabbits [22].

**Specific reversal agent.** Administration of anti-Dabi-Fab resulted in a rapid, dose-dependent decrease in blood loss following tail transection in rats receiving supratherapeutic doses of dabigatran [23]. In monkeys, anti-Dabi-Fab dose-dependently reversed dabigatran anticoagulant activity as measured by the dilute PT assay [24]. A Phase I clinical trial of this agent is currently enrolling (ClinicalTrials.gov identifier: NCT01955720). Table 2 summarizes evidence for reversal of dabigatran anticoagulant effect.

**Factor Xa inhibitors**

**Non-specific reversal agents.** Unlike dabigatran, administration of 4-PCC (50 U/kg) to healthy volunteers receiving rivaroxaban (20 mg twice daily for 2.5 days) corrected PT prolongation [15]. The addition of aPCC to plasma from rivaroxaban-treated volunteers corrected all thrombin generation parameters, whereas PCC and rVIIa modified only some parameters [16]. Another study showed that PCC, aPCC and rVIIa all corrected PT prolongation when added to plasma from patients receiving rivaroxaban, but only PCC and aPCC modified all abnormal thrombin generation.
### TABLE 2

**Summary of *ex vivo* and *in vivo* evidence for reversal of dabigatran anticoagulant effect.**

<table>
<thead>
<tr>
<th>Reversal strategy</th>
<th>Animal studies (dabigatran-treated animals)</th>
<th><em>Ex vivo</em> studies (dabigatran-treated healthy volunteers or patients)</th>
<th><em>Ex vivo</em> studies (dabigatran-and reversal-agent-treated healthy volunteers)</th>
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</thead>
<tbody>
<tr>
<td><strong>Non-specific reversal agents</strong></td>
<td></td>
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</tr>
<tr>
<td>PCC</td>
<td>• No reduction in blood loss after tail transection in mice [20]</td>
<td>• Corrected some TG indices in dabigatran-treated healthy volunteers [16]</td>
<td>• No correction of aPTT, ECT, TT [15]</td>
</tr>
<tr>
<td></td>
<td>• Reduced intracranial hematoma expansion and 24-hour mortality in mice [21]</td>
<td>• Corrected PT, aPTT, TT and some TG indices in dabigatran-treated patients [18]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Reduced blood loss following kidney incision in rabbits [22]</td>
<td>• No correction of Hemoclot® assay in dabigatran-treated patients [18]</td>
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<tr>
<td>aPCC</td>
<td>• No reduction in blood loss after tail transection in mice [20]</td>
<td>• Corrected some TG indices in dabigatran-treated healthy volunteers [16]</td>
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</tr>
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<td>• Corrected PT, aPTT and some TG indices in dabigatran-treated patients [17,18]</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>• No correction of Hemoclot® assay in dabigatran-treated patients [18]</td>
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<tr>
<td>rVIIa</td>
<td>• No reduction in blood loss after tail transection in mice [20]</td>
<td>• Corrected some TG indices in dabigatran-treated healthy volunteers [16]</td>
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</tr>
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<td></td>
<td></td>
<td>• No correction of Hemoclot® assay in dabigatran-treated patients [18]</td>
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</tr>
<tr>
<td><strong>Specific reversal agent</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Dabi-Fab [10]</td>
<td>• Reduced blood loss after tail transection</td>
<td>• Corrected dilute PT assay</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** Anti-Dabi-Fab, humanized monoclonal antibody fragment against dabigatran; aPCC, activated prothrombin complex concentrate; aPTT, anticoagulant partial thromboplastin time; ECT, ecarin clotting time; PCC, prothrombin complex concentrate; PT, prothrombin time; rVIIa, recombinant activated factor VII; TG, thrombin generation; TT, thrombin time.

### TABLE 3

**Summary of *ex vivo* and *in vivo* evidence for reversal of factor Xa inhibitor anticoagulant effect.**

<table>
<thead>
<tr>
<th>Reversal strategy</th>
<th>Animal studies (factor-Xa-inhibitor-treated animals)</th>
<th><em>Ex vivo</em> studies (factor-Xa-inhibitor-treated healthy volunteers or patients)</th>
<th><em>Ex vivo</em> studies (factor-Xa-inhibitor-and reversal-agent-treated healthy volunteers)</th>
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<tbody>
<tr>
<td><strong>Non-specific reversal agents</strong></td>
<td></td>
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<tr>
<td>Rivaroxaban</td>
<td>• Corrected aPTT [29]</td>
<td>Rivaroxaban</td>
<td>Rivaroxaban</td>
</tr>
<tr>
<td></td>
<td>• Variously corrected PT [29,30]</td>
<td>• Corrected PT [18]</td>
<td>• Corrected PT [15]</td>
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<tr>
<td></td>
<td>• No reduction of blood loss in rabbits [29]</td>
<td>• Variously corrected TG indices [16,18]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Reduced bleeding time in rats but not primates [30]</td>
<td>• No correction of anti-Xa activity [20]</td>
<td></td>
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<tr>
<td>aPCC</td>
<td>Rivaroxaban</td>
<td>Rivaroxaban</td>
<td>Rivaroxaban</td>
</tr>
<tr>
<td></td>
<td>• Corrected aPTT [29]</td>
<td>• Corrected PT [18]</td>
<td>• Corrected PT [15]</td>
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<td>• Reduced bleeding time in rats and primates [30]</td>
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<td></td>
<td>Edoxaban</td>
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<td></td>
<td>• Reduced bleeding time in rats [28]</td>
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<tr>
<td>rVIIa</td>
<td>Rivaroxaban</td>
<td>Rivaroxaban</td>
<td>Rivaroxaban</td>
</tr>
<tr>
<td></td>
<td>• Reduced bleeding time in rats but not primates [30]</td>
<td>• Variously corrected TG indices [16]</td>
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<td>• No correction of anti-Xa activity [18]</td>
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<td></td>
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<tr>
<td></td>
<td>• Reduced bleeding time in rats [28]</td>
<td></td>
<td></td>
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<tr>
<td><strong>Specific reversal agent</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Corrected INR, PT, aPTT, anti-Xa activity</td>
<td>• Decreased anti-Xa activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Reduced blood loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Apixaban, betrixaban</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Corrected INR, PT, aPTT, anti-Xa activity</td>
<td></td>
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</tr>
</tbody>
</table>

**Abbreviations:** aPTT, activated partial thromboplastin time; aPCC, activated prothrombin complex concentrate; INR, international normalized ratio; PCC, prothrombin complex concentrate; PT, prothrombin time; rVIIa, recombinant activated factor VII; TG, thrombin generation.
indices [18]. None of the reversal agents normalized anti-Xa activity. In separate studies, the addition of PCC to rivaroxaban-spiked plasma in vitro did not change abnormal coagulation tests, thromboelastometry or thrombin generation tests [25,26]. However, rVIIa corrected PT prolongation at a low rivaroxaban concentration (80 ng/μl) and thromboelastometry results at a high rivaroxaban concentration (200 ng/μl) [26]. When added to apixaban-spiked plasma, PCC, aPCC and rVIIa variably corrected some abnormal thrombin generation and thromboelastometry results [27]. PCC, aPCC and rVIIa corrected PT prolongation induced by edoxaban in vitro [28].

Studies in animal models also show variable results for rivaroxaban reversal using non-specific reversal agents. In a rabbit bleeding model, PCC and aPCC normalized the aPTT and partially corrected the PT, but neither agent reduced blood loss in rivaroxaban-treated rabbits [29]. Conversely, PCC (50 U/kg, but not 25 U/kg), aPCC and rVIIa corrected PT prolongation and reduced bleeding time in rivaroxaban-treated rats [30]. In the same study, aPCC and rVIIa corrected PT prolongation, but only aPCC reduced bleeding time in primates treated with rivaroxaban; aPCC and rVIIa also significantly shortened the bleeding time in edoxaban-treated rats [28].

**Specific reversal agent.** PRT064445 dose-dependently reversed the anticoagulant activity of rivaroxaban, apixaban and betrixaban in vitro as measured by anti-Xa activity assays [11]. PT prolongation induced by rivaroxaban was also corrected with the addition of PRT064445. In the same study, PRT064445 administered to rats in vivo following rivaroxaban, apixaban and betrixaban infusions rapidly reduced the international normalized ratio (INR). In rivaroxaban-treated rats, PRT064445 decreased plasma concentrations of free rivaroxaban, the fraction responsible for mediating anticoagulant activity. Abnormal PT, aPTT and anti-Xa assays were corrected and blood loss reduced when PRT064445 was given to rabbits receiving rivaroxaban in a liver laceration model.

Preliminary results of an ongoing Phase II, randomized, double-blind trial in healthy volunteers (apixaban 5 mg twice daily, 11 doses) showed that PRT064445 decreased anti-Xa activity and reduced plasma concentrations of free apixaban compared with placebo (ClinicalTrials.gov identifier: NCT01758432) [31]. Table 3 summarizes evidence for reversal of factor Xa inhibitor anticoagulant effect.

**Concluding remarks**

Although TSOAC-associated bleeding occurs with similar or reduced frequency compared to VKAs for approved indications, effective TSOAC reversal agents are likely to improve the morbidity and mortality of bleeding events when they occur. Although specific reversal agents are currently undergoing clinical development and not yet available, non-specific reversal therapies such as PCC, aPCC and rVIIa have variable efficacy and show conflicting results when used to reverse TSOAC anticoagulant effect in human volunteers, animal models and in vitro experiments. In addition, non-specific therapies are known to increase the risk of thrombosis, which must be balanced against potential efficacy before administration for TSOAC-associated bleeding.

In the absence of high-quality studies that measure clinically relevant outcomes in anticoagulated patients with bleeding, provision of evidence-based recommendations regarding the use of non-specific reversal therapies is problematic. Further studies that incorporate clinical and patient-important outcomes such as blood loss and mortality are needed to establish the efficacy and safety of putative reversal agents. In the interim, the mainstay of management of TSOAC-associated bleeding is supportive measures and urgent referral for definitive intervention [32].

**Conflicts of interest**

A.C. has served as a consultant for Baxter, Bayer and Genzyme; has served on an advisory board for CSL Behring, Daiichi Sankyo and Genzyme; and has received research support from Diagnostica Stago. D.M.S. has no conflicts of interest to declare.

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