A PoTENtial Antidote

A Prohemostatic Factor Xa Variant for Reversal of Direct Oral Anticoagulants

James C. Fredenburgh, Jeffrey I. Weitz

A Rapid Pro-Hemostatic Approach to Overcome Direct Oral Anticoagulants
Thalji et al
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Direct oral anticoagulants have revolutionized long-term anticoagulant therapy because they are at least as effective, but safer and easier to administer than vitamin K antagonists. Nonetheless, when life-threatening bleeding occurs, reversal of the direct oral anticoagulants is necessary. A prohemostatic factor Xa variant was developed to address this unmet need.

The direct oral anticoagulants (DOACs), which include dabigatran, rivaroxaban, apixaban, and edoxaban, can be given in fixed doses without routine coagulation monitoring. In clinical trials, the DOACs have been shown to be at least as effective as vitamin K antagonists (VKAs), such as warfarin, for stroke prevention in nonvalvular atrial fibrillation and for treatment of venous thromboembolism, and to produce less serious bleeding. With similar efficacy, better safety, and greater convenience, the DOACs are now replacing VKAs for these indications. Although the risk of serious bleeding, particularly intracranial bleeding, is reduced with the DOACs, drug reversal is needed when major bleeding occurs or if patients require urgent surgery. Idarucizumab is widely available for dabigatran reversal, but there are no licensed reversal agents for rivaroxaban, apixaban, or edoxaban. FXa^{I16L} is a recombinant factor (F) Xa variant with a novel mechanism of action. A prohemostatic agent, FXa^{I16L}, shows promise for controlling bleeding not only with the DOACs, but also in hemophilia patients with inhibitors.

Thrombosis is the underlying cause of venous thromboembolism, which includes deep vein thrombosis and pulmonary embolism, and most heart attacks and strokes. On a global basis, thrombosis accounts for 1 of 4 deaths, and this number is likely to rise with the aging population.[1] With this burden of disease, it is not surprising that more and more patients are on long-term anticoagulant therapy for the prevention or treatment of thrombosis.

For over 65 years, VKAs such as warfarin were the only available oral anticoagulants. Although effective, VKAs are difficult to manage because the dose varies from patient to patient depending on dietary vitamin K intake, drug–drug interactions, and common polymorphisms that influence the metabolism of the VKAs. Consequently, frequent coagulation monitoring and dose adjustments are needed to ensure that a therapeutic concentration has been achieved. Such monitoring is inconvenient for patients and physicians and costly for healthcare systems.

The limitations of VKAs prompted development of the DOACs, which were designed to simplify anticoagulation therapy because they can be administered in fixed doses without the need for routine coagulation monitoring. Since 2009, 4 DOACs have been licensed: dabigatran, which inhibits thrombin, and rivaroxaban, apixaban and edoxaban, which inhibit FXa. In randomized clinical trials that included over 100 000 patients, the DOACs were at least as effective as VKAs, but produced less serious bleeding, particularly less intracranial bleeding.[2,3] These findings have been confirmed in large observational studies, suggesting that the results of the randomized trials can be translated to the community.[4] With similar efficacy, less bleeding, and greater convenience, most guidelines now give preference to the DOACs over VKAs for stroke prevention in atrial fibrillation and for venous thromboembolism treatment. It is not surprising, therefore, that the prescriptions for DOACs are outnumbering those for VKAs in some countries.[5]

Although bleeds with the DOACs tend to be less severe than those with VKAs,[6,7] serious bleeding still occurs, and patients taking DOACs may require urgent surgery. Consequently, the lack of specific reversal agents for the DOACs has been a concern. Idarucizumab, a monoclonal antibody fragment against dabigatran, has recently been licensed for dabigatran reversal in patients with serious bleeding or in those requiring urgent surgery.[8] However, reversal agents for rivaroxaban, apixaban, and edoxaban are lacking. The reversal agent in the most advanced stage of development is andexanet. Andexanet is a recombinant FXa variant with its active site serine residue replaced with an alanine residue to eliminate catalytic activity and its membrane-binding Gla domain removed to prevent incorporation into the prothrombinase complex.[9] By competing with FXa for binding of rivaroxaban, apixaban, or edoxaban, andexanet sequesters the drugs until they can be

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cleared from the circulation. Andexanet is undergoing phase III evaluation in patients taking FXa inhibitors who present with serious bleeding.[10] As an alternative, Thalji et al[11] present data on FXa^{I16L}, another recombinant FXa variant with an entirely different mechanism of action (Figure), as a potential reversal agent.

Originally designed as a prohemostatic agent for treating hemophilia patients with inhibitors,[12] the investigators now set out to determine whether FXa^{I16L} would control bleeding with the DOACs. Development of FXa^{I16L} was predicated on the observation that the catalytic triad of FXa, which is composed of serine, aspartate, and histidine residues, also is properly oriented in zymogen FX, indicating that other transitions capacitate the enzyme. One such transition occurs when FX is cleaved at residue 15 (using the chymotrypsin numbering system), thereby exposing a new amino-terminal isoleucine residue at position 16 (I16) that reorients itself into the active site pocket. Because this reorientation creates an oxyanion hole that is critical for substrate binding and catalytic activity, it was anticipated that replacement of I16 with leucine (I16L) would short circuit the active site and inactivate the enzyme.[13] As expected, FXa^{I16L} had limited catalytic activity on its own. Surprisingly, however, its capacity to activate prothrombin was restored to near wild-type FXa levels when FXa^{I16L} assembled into the prothrombinase complex with factor Va, thus limiting its activity to the surface of activated platelets. Moreover, unlike native FXa, FXa^{I16L} was resistant to inhibition by antithrombin.[14] These features render FXa^{I16L} an attractive option for managing bleeding in hemophilia patients with inhibitors, a concept supported by the demonstration that FXa^{I16L} attenuated tail bleeding in a mouse model of hemophilia.[13]

Does it make sense to use a prohemostatic agent such as FXa^{I16L} for management of bleeds with the DOACs? FXa^{I16L} reversed rivaroxaban-induced inhibition of thrombin generation in vitro and reversed the inhibition of large-vessel thrombosis and the tail bleeding produced by rivaroxaban in mice. Compared with mouse Gla-domainless Ser195Ala FXa (GD-S195A-FXa), the murine equivalent of andexanet, FXa^{I16L} was 300- and 50-fold more potent at reversing inhibition of thrombin generation and large-vessel thrombosis, respectively.[11] The differences in potency highlight the distinct mechanisms of action of the 2 FXa variants (Table). FXa^{I16L} is more potent because once it incorporates into the prothrombinase complex, it activates prothrombin in a catalytic manner. In contrast, higher concentrations of GD-S195A-FXa are needed to sequester rivaroxaban in a 1:1 stoichiometric complex. Therefore, FXa^{I16L} is a potent prohemostatic agent that has the potential to reverse rivaroxaban-induced bleeding.

How does FXa^{I16L} fit in the competitive landscape of reversal agents for the DOACs? Idarucizumab is now available in many hospitals worldwide. Therefore, even though Thalji et al[11] show that FXa^{I16L} can reverse dabigatran and rivaroxaban, there is no need for additional reversal agents for dabigatran. Andexanet is undergoing phase III evaluation for the management of serious bleeding in patients taking oral FXa inhibitors, and the results in the first 67 patients were recently reported.[10] Although andexanet only partially and transiently reversed the anti-FXa activity of rivaroxaban and apixaban, clinical

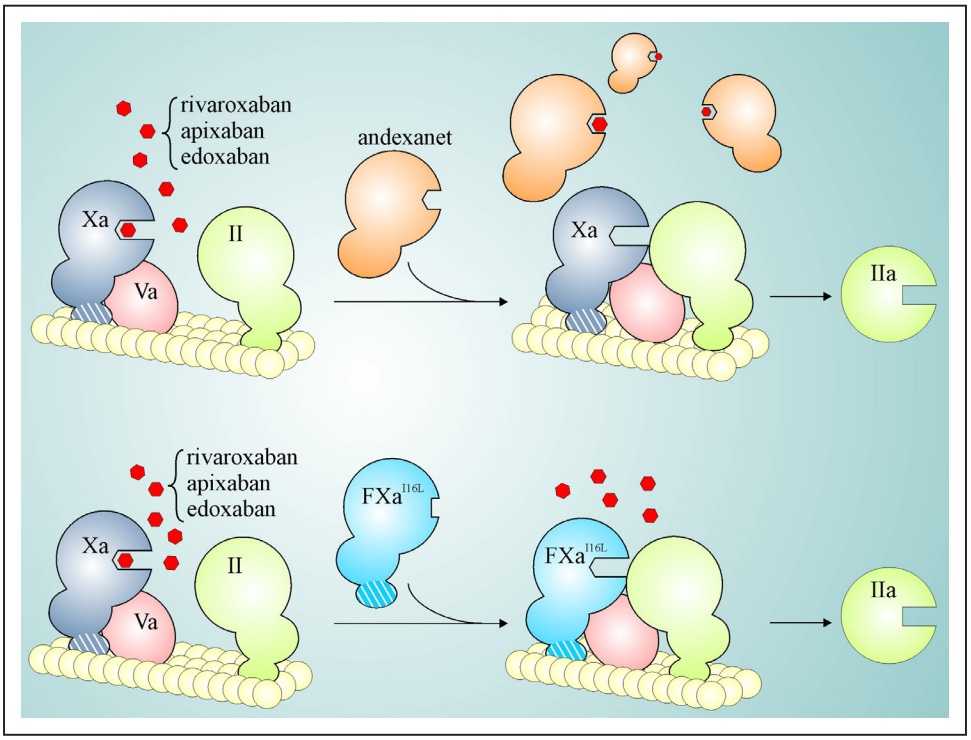

Figure. Mechanism of action of andexanet and FXa^{I16L}. Rivaroxaban, apixaban, and edoxaban (hexagons) inhibit prothrombin (II) activation by factor Xa (Xa) bound to factor Va (Va) in the prothrombinase complex on the surface of activated platelets. Andexanet (**Upper**) is an active site-disabled variant of Xa that lacks the membrane-binding Gla domain (hatched oval). Andexanet competes with Xa for stoichiometric binding of rivaroxaban, apixaban, or edoxaban and sequesters them so they are no longer inhibitory. FXa^{I16L} (**Lower**) is an attenuated Xa variant that retains its Gla domain and whose activity is recovered when incorporated into prothrombinase. FXa^{I16L} bypasses the effects of the inhibitors by restoring prothrombinase function.

Table. Comparison of Factor Xa^{I16L} With Other Reversal Agents for the Direct Oral Anticoagulants

Feature	Factor Xa^{I16L}	Andexanet	Ciraparantag	Idarucizumab
Structure	Recombinant factor Xa variant with attenuated activity	Truncated inactive recombinant factor Xa variant	Diarginyl synthetic molecule	Monoclonal antibody fragment
Mass (Daltons)	46 000	39 000	512	47 776
Target	Dabigatran, rivaroxaban, apixaban, and edoxaban	Rivaroxaban, apixaban, edoxaban, and heparins	Dabigatran, rivaroxaban, apixaban, edoxaban, and heparins	Dabigatran only
Mechanism	Prohemostatic; activates prothrombin when incorporated into prothrombinase with factor Va	Competes with factor Xa for binding rivaroxaban, apixaban, edoxaban, and the antithrombin–heparin complex	Binds drugs via charge-dependent hydrogen bonding	Binds dabigatran with high affinity and specificity
Administration	Likely by intravenous bolus	Intravenous bolus followed by a 2-h infusion	Intravenous bolus	Intravenous bolus
Laboratory monitoring of reversal	Uncertain	Calibrated anti-factor Xa assays	Whole-blood clotting time	Activated partial thromboplastin time, diluted thrombin time, or ecarin clot time
Stage of development	Preclinical	Phase III	Phase II	Approved

hemostasis was judged to be good or excellent 12 hours after andexanet administration in 37 of the 47 patients (70%) included in the efficacy analysis. However, thrombotic events occurred in 12 of the 67 patients (18%) in the 30-day follow-up period; 4 of these thrombotic events occurred within 3 days of andexanet administration. Would FXa^{I16L} be more effective than andexanet in this setting, and would it also be associated with thrombotic events? FXa^{I16L} is certainly more potent than andexanet in preclinical studies, so it is likely that lower doses of FXa^{I16L} would be required. Furthermore, because FXa^{I16L} is latent until it incorporates into the prothrombinase complex, its activity should be restricted to sites of injury, thus limiting systemic thrombin generation. Supporting this concept, FXa^{I16L} administration in mice did not induce platelet or fibrinogen consumption regardless of whether or not rivaroxaban was given.[11] Clinical trials are needed to confirm these findings, but such trials will be challenging if andexanet, which is on a fast-track for approval, is licensed for reversal of rivaroxaban, apixaban, and edoxaban. Thus, if andexanet is licensed, the unmet need for a reversal agent for these drugs will be addressed and head-to-head comparison of FXa^{I16L} with andexanet will be required.

Ciraparantag (PER977) is another reversal agent that is at an even earlier stage of development than andexanet.[15] A synthetic diarginine molecule, ciraparantag, binds dabigatran, rivaroxaban, apixaban, and edoxaban. When given as an intravenous bolus, ciraparantag attenuated edoxaban-induced tail bleeding in a rat model and restored the whole-blood clotting time to baseline values in volunteers given a single oral dose of edoxaban. Although not yet tested in patients, development of ciraparantag is more advanced than that of FXa^{I16L}. Therefore, FXa^{I16L} will need to compete with ciraparantag and andexanet for reversal of oral FXa inhibitors.

In summary, the DOACs have streamlined long-term anticoagulation therapy, and observational studies have confirmed the efficacy and safety of these agents in the community setting. The majority of bleeds with the DOACs can be managed conservatively and because of the short half-life of the DOACs, time is often the best antidote. Nonetheless, reversal is needed in patients with life-threatening bleeds, such as an intracranial bleed, or in those requiring urgent surgery or intervention. Idarucizumab is licensed for dabigatran reversal in both of these situations. However, there remains an urgent need for reversal agents for rivaroxaban, apixaban, and edoxaban. Together with andexanet and ciraparantag, FXa^{I16L} has now joined this race. Neither andexanet nor ciraparantag has been tested in patients requiring urgent surgery, but clinicians will be reluctant to administer a prohemostatic agent such as FXa^{I16L} before surgery for fear of inducing thrombotic events. Therefore, the search for reversal agents for the oral FXa inhibitors continues.

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