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

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. FXaI16L 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. By competing with FXa for binding of rivaroxaban, apixaban, and edoxaban, FXaI16L 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. As an alternative, Thalji et al present data on FXa\textsuperscript{16L}, 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, the investigators now set out to determine whether FXa\textsuperscript{16L} would control bleeding with the DOACs. Development of FXa\textsuperscript{16L} 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 (I\textsubscript{16}) 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 I\textsubscript{16} with leucine (I\textsubscript{16L}) would short circuit the active site and inactivate the enzyme. As expected, FXa\textsuperscript{16L} had limited catalytic activity on its own. Surprisingly, however, its capacity to activate prothrombin was restored to near wild-type FXa levels when FXa\textsuperscript{16L} assembled into the prothrombinase complex with factor Va, thus limiting its activity to the surface of activated platelets. Moreover, unlike native FXa, FXa\textsuperscript{16L} was resistant to inhibition by antithrombin. These features render FXa\textsuperscript{16L} an attractive option for managing bleeding in hemophilia patients with inhibitors, a concept supported by the demonstration that FXa\textsuperscript{16L} attenuated tail bleeding in a mouse model of hemophilia.

Does it make sense to use a prohemostatic agent such as FXa\textsuperscript{16L} for management of bleeds with the DOACs? FXa\textsuperscript{16L} 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\textsuperscript{16L} was 300- and 50-fold more potent at reversing inhibition of thrombin generation and large-vessel thrombosis, respectively. The differences in potency highlight the distinct mechanisms of action of the 2 FXa variants (Table). FXa\textsuperscript{16L} is more potent because once it incorporates into the prothrombinase complex, it activates thrombin 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\textsuperscript{16L} is a potent prohemostatic agent that has the potential to reverse rivaroxaban-induced bleeding.

How does FXa\textsuperscript{16L} 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 show that FXa\textsuperscript{16L} 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. Although andexanet only partially and transiently reversed the anti-FXa activity of rivaroxaban and apixaban, clinical

![Figure](https://circres.ahajournals.org/)

**Figure.** Mechanism of action of andexanet and FXa\textsuperscript{16L}. 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\textsuperscript{16L} (Lower) is an attenuated Xa variant that retains its Gla domain and whose activity is recovered when incorporated into prothrombinase. FXa\textsuperscript{16L} bypasses the effects of the inhibitors by restoring prothrombinase function.
Thus limiting systemic thrombin generation. Supporting this complex, its activity should be restricted to sites of injury, therefore, FXaI16L will need to compete with ciraparantag and andexanet will be required. Will be addressed and head-to-head comparison of FXaI16L licensed, the unmet need for a reversal agent for these drugs which is on a fast-track for approval, is licensed for reversal of rivaroxaban, apixaban, and edoxaban. Although not yet tested in patients, development of andexanet 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 ciraparantag (PER977) is another reversal agent that is at an even earlier stage of development than andexanet. 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 FXaI16L. Therefore, FXaI16L 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, FXaI16L has been tested in patients requiring urgent surgery, but clinicians will be reluctant to administer a prohemostatic agent such as FXaI16L before surgery for fear of inducing thrombotic events. Therefore, the search for reversal agents for the oral FXa inhibitors continues.

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References

Table. Comparison of Factor XaI16L With Other Reversal Agents for the Direct Oral Anticoagulants

<table>
<thead>
<tr>
<th>Feature</th>
<th>Factor XaI16L</th>
<th>Andexanet</th>
<th>Ciraparantag</th>
<th>Idarucizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>Recombinant factor Xa variant with attenuated activity</td>
<td>Truncated inactive recombinant factor Xa variant</td>
<td>Diargyl synthetic molecule</td>
<td>Monoclonal antibody fragment</td>
</tr>
<tr>
<td>Mass (Daltons)</td>
<td>46000</td>
<td>39000</td>
<td>512</td>
<td>47776</td>
</tr>
<tr>
<td>Target</td>
<td>Dabigatran, rivaroxaban, apixaban, and edoxaban</td>
<td>Rivaroxaban, apixaban, edoxaban, and heparins</td>
<td>Dabigatran, rivaroxaban, apixaban, edoxaban, and heparins</td>
<td>Dabigatran only</td>
</tr>
<tr>
<td>Mechanism</td>
<td>Prohemostatic; activates prothrombin when incorporated into prothrombinase with factor Va</td>
<td>Competes with factor Xa for binding rivaroxaban, apixaban, edoxaban, and the antithrombin–heparin complex</td>
<td>Binds drugs via charge-dependent hydrogen bonding</td>
<td>Binds dabigatran with high affinity and specificity</td>
</tr>
<tr>
<td>Administration</td>
<td>Likely by intravenous bolus</td>
<td>Intravenous bolus followed by a 2-h infusion</td>
<td>Intravenous bolus</td>
<td>Intravenous bolus</td>
</tr>
<tr>
<td>Laboratory monitoring of reversal</td>
<td>Uncertain</td>
<td>Calibrated anti–factor Xa assays</td>
<td>Whole-blood clotting time</td>
<td>Activated partial thromboplastin time, diluted thrombin time, or ecarin clot time</td>
</tr>
<tr>
<td>Stage of development</td>
<td>Preclinical</td>
<td>Phase III</td>
<td>Phase II</td>
<td>Approved</td>
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</tbody>
</table>

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