EDUCATIONAL COMMENTARY – NEW ANTICOAGULATION THERAPIES AND REVERSAL AGENTS

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Learning Outcomes

On completion of this exercise, the participant should be able to

• list and describe currently available anticoagulant drugs;
• identify the anticoagulation pathway of these drugs;
• analyze the impact of these drugs on coagulation testing; and
• describe currently available reversal agents.

Introduction

The role of anticoagulation is to treat and prevent blood clots in patients at risk for stroke, deep vein thrombosis, and pulmonary embolism, without putting the patient at risk for bleeding. To effectively stop the effect of the anticoagulant, a reversal agent is administered.

For more than 70 years, both unfractionated heparin and vitamin K antagonists have been administered to patients for anticoagulation. Vitamin K antagonists, such as warfarin, work by rendering the vitamin K-dependent factors (II, V, VII, X, protein C, and protein S) nonfunctional. The effectiveness of this form of anticoagulation can be monitored easily and quickly in the laboratory through prothrombin time. To minimize instrument and reagent variability in monitoring patients, the international normalized ratio is calculated. The anticoagulant effect of vitamin K antagonists can be reversed by administering vitamin K or fresh frozen plasma.

Unfractionated heparin works by inactivating thrombin (factor IIa) and activated factor X (Xa) through an antithrombin-dependent mechanism. Its effects can be monitored using the activated partial thromboplastin time and anti-Xa assays. Low-molecular-weight heparin has a greater ratio of anti-factor Xa to IIa activity. The molecule is chemically degraded, and in the process loses the antithrombin binding site. As a result, low-molecular-weight heparin can only be measured by an anti-Xa assay. The anticoagulant effects of both these drugs can be neutralized by the administration of protamine sulfate.

In the past decade, a new compendium of anticoagulants has been developed. The direct oral anticoagulants (DOACs) have certain advantages over other agents by directly targeting specific coagulation factors and having a wider therapeutic window, fewer drug interactions, and a short half-life. Based on all of these advantages, especially the short half-life, these drugs were approved by the U.S. Food and Drug Administration (FDA) without requiring routine monitoring. Currently, there are no FDA-approved tests for monitoring DOACs. However, there are several situations in which detection of these
anticoagulants would benefit patients. These include patients with atrial fibrillation, acute ischemic stroke, renal insufficiency, extreme body weight, and older age; and to determine compliance, in particular before emergency surgery.\(^4\) To complicate matters, these drugs were released without reversal agents. Only recently have such agents been developed.

**Direct Oral Anticoagulants**

The direct oral anticoagulants include dabigatran, rivaroxaban, apixaban, and edoxaban. These drugs are approved for use in patients with atrial fibrillation and to prevent and treat venous thromboembolism.

Dabigatran, the prodrug of dabigatran etexilate, is a direct thrombin (IIa) inhibitor (Figure). This is a small molecule that blocks both free and clot-bound thrombin by binding to the active site of thrombin. The drug is excreted by the kidneys and may be contraindicated in patients with decreased renal function. Patients with a decreased creatinine clearance (<30 mL/min) should not receive the drug.\(^5\)

The other three drugs are Xa inhibitors (Figure). Rivaroxaban inactivates free and clot-associated factor Xa. It has a high bioavailability of 80%, and is 90% bound to protein. The drug is administered once per day and is excreted via the liver. Apixaban, another Xa inhibitor, has a bioavailability of approximately 50% and is absorbed in the stomach and small intestine. It is metabolized in the liver and does not accumulate in patients with mild to moderate renal impairment.\(^5\) Edoxaban is a selective factor Xa inhibitor that is metabolized by the kidneys and works by inhibiting free Xa without the need for antithrombin. As a result, thrombin generation is decreased, leading to decreased thrombus formation and progression.

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**Figure.** Direct oral anticoagulants' action on the coagulation cascade.
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Laboratory Assessment of Direct Oral Anticoagulants

The criterion standard for measuring the plasma concentration of DOACs is liquid chromatography / tandem-mass spectrometry (LC-MS/MS). It has better selectivity than coagulation factor assays and is not affected by preanalytic variables. This is the most specific and sensitive method, but it is a laboratory-developed test and is only implemented in select laboratories, making standardization of assays difficult. Also, there are no specific kits or international reference standards for this testing.

When screening a patient for the presence or absence of a DOAC, it is important to rule out other coagulation disorders. Dabigatran is the only anti-IIa DOAC and the activated partial thromboplastin time is more sensitive than the prothrombin time to screen for the presence of the drug. It has been demonstrated that instruments and reagents can affect the sensitivity of the estimation of drug concentration. The thrombin time assay is very sensitive to dabigatran and the degree of prolongation depends on the reagent. A normal thrombin time rules out the presence of dabigatran. In a modification of the thrombin time or a dilute thrombin time, the sample is diluted and mixed with pooled normal plasma and thrombin is added. A linear response has been observed and can be used to quantify dabigatran. The ecarin clotting time is a snake-venom–based assay with a linear correlation to dabigatran. Results using this assay showed good correlation with LC MS/MS.

Rivaroxaban, apixaban, and edoxaban represent the anti-Xa DOACs. Prothrombin time is most sensitive when screening for rivaroxaban, less for edoxaban, and is insensitive to apixaban. This assay is also reagent dependent, and some reagents may show normal results with therapeutic levels of apixaban and edoxaban. To quantify levels of these anticoagulants, a chromogenic anti-Xa assay calibrated with the DOAC being tested is the best assay.

Reversal of Direct Oral Anticoagulants

Nonspecific Reversal Agents

Until recently there have been no FDA-approved reversal agents for DOACs, and clinicians have had to rely on agents that indirectly aid in reversing the effects of this class of anticoagulants.

Prothrombin complex concentrate (PCC) is derived from plasma. There are two kinds: three-factor PCC, which contains factors II, IX, and X, and four-factor PCC, which also contains factor VII. PCC is used to reverse vitamin K antagonists. It has also been shown to correct prolonged clotting times in patients taking rivaroxaban, but not in patients receiving dabigatran. Activated PCC, also plasma derived, contains activated factors II, VII, IX, and X and has demonstrated variable effects on DOACs. Studies have demonstrated that when activated PCC is added to blood from patients taking rivaroxaban and dabigatran, the thrombin-generation assay showed corrected results. Recombinant factor VIIa has been
shown to have variable effects on DOAC coagulation test abnormalities. This recombinant product has shown correction in rivaroxaban-prolonged coagulation tests. It is important to note that, when used in patients not taking anticoagulants, all of these agents increase the risk for thrombosis.7

Specific Reversal Agents

Idarucizumab was approved by the FDA in 2015 as a reversal agent for dabigatran. It is a monoclonal antibody with the capacity to bind to dabigatran with a 350-fold higher affinity than thrombin. This complex is cleared by the kidney, with a half-life of about 45 minutes in patients with normal kidney function. Patients who are actively bleeding or those who are taking dabigatran and require an intervention are candidates for reversal. Patients are given two bolus doses within 5 to 10 minutes of each other.8 If the concentration of dabigatran is greater than 50 ng/mL, a reversal agent should be considered; however, if there is a need for urgent intervention, idarucizumab should be administered at 30 ng/mL.6

Andexanet alfa antidote is pending FDA approval as a reversal agent for oral factor Xa inhibitors. It is a recombinant human factor Xa variant that reverses the drug in a dose-dependent manner. It binds similarly to native factor Xa. It also forms a complex and binds tissue-factor pathway inhibitor, which reduces its activity, resulting in a transient rise in prothrombin fragment, D-dimer, and thrombin-antithrombin complex. Almost 90% of the anticoagulant is reversed following a bolus dose.8

Ciraparantag is under investigation as a reversal agent. This small synthetic cationic molecule binds direct thrombin inhibitors, direct Xa inhibitors, and both unfractionated and low-molecular-weight heparin through noncovalent hydrogen bonds and charge-charge interactions. In studies, edoxaban required the smallest dose, but ciraparantag also showed correction with rivaroxaban and apixaban in a dose dependent fashion using an anti-Xa assay.

Conclusion

Direct oral anticoagulants provide patients with alternatives to the standard vitamin K antagonists. These drugs have been shown to have similar or lower bleeding risks in several situations. The presence or absence of DOACs can be detected with routine coagulation tests. Although mass spectrophotometry is the criterion standard for quantitating drug levels, several laboratory tests can also quantitate DOACs. However, none of these tests are yet approved by the FDA. The recent approval of the reversal agent idarucizumab has made using dabigatran easier and minimized the need for testing. It is hoped that additional agents will be approved shortly to allow all the DOACs to be used safely and effectively.
### Table. Overview of DOACs.

<table>
<thead>
<tr>
<th>Drug; Target FDA use</th>
<th>PT/INR</th>
<th>aPTT</th>
<th>TT</th>
<th>Best Test</th>
<th>Reversal Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran IIa inhibitor NVAF, VTE</td>
<td>Prolonged at very high concentration</td>
<td>Sensitive until higher concentrations; normal aPTT can exclude presence of drug. Sensitivity based on reagents.</td>
<td>Very sensitive, results prolonged, normal TT can exclude drug</td>
<td>Dilute TT and ecarin-based assays</td>
<td>Idarucizumab Monoclonal antibody: binds and neutralizes free and bound thrombin</td>
</tr>
<tr>
<td>Rivaroxaban Xa inhibitor NVAF, VTE</td>
<td>Sensitive throughout therapeutic range, variability between assays; normal PT excludes drug.</td>
<td>Insensitive</td>
<td>Insensitive</td>
<td>Anti-FXa activity calibrated with rivaroxaban</td>
<td>Andexanet alfa Recombinant protein: binds to direct Xa inhibitors. Ciraparantag Small synthetic, watersoluble catatonic molecule: noncovalent hydrogen binding</td>
</tr>
<tr>
<td>Apixaban Xa inhibitor NVAF, VTE</td>
<td>Insensitive</td>
<td>Insensitive</td>
<td>Insensitive</td>
<td>Anti-FXa activity calibrated to apixaban</td>
<td>Andexanet alfa Recombinant protein: binds to direct Xa inhibitors Ciraparantag Small synthetic, watersoluble catatonic molecule: noncovalent hydrogen binding</td>
</tr>
<tr>
<td>Edoxaban Xa inhibitor NVAF, VTE</td>
<td>Poor sensitivity variability between assays</td>
<td>Sensitive throughout therapeutic range with less variability between assays</td>
<td>Insensitive</td>
<td>Anti-FXa activity calibrated to heparin or edoxaban</td>
<td>Andexanet alfa Recombinant protein: binds to direct Xa inhibitors Ciraparantag Small synthetic, watersoluble catatonic molecule: noncovalent hydrogen binding</td>
</tr>
</tbody>
</table>

*aAdapted from Cuker et al., 2014; Cuker and Husseinzadeh, 2015; and Gulseth, 2016.*

Abbreviations: aPTT, activated partial thromboplastin time; FDA, US Food and Drug Administration; INR, international normalized ratio; NVAF, nonvulvular atrial fibrillation; PT, prothrombin time; TT, thrombin time; and VTE, venous thromboembolism.
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References


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