Oral Factor Xa Inhibitors and Clinical Laboratory Monitoring

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ABSTRACT

Oral anticoagulation therapy is currently undergoing great changes with the development and use of several new medications. The newer drugs have a more specific mechanism of action for inhibiting coagulation and do not require continuous monitoring like their predecessor. Their efficacy and safety has been proven, but problems arise in the clinical laboratory with developing accurate procedures to measure the therapy when needed. Methods being used are the prothrombin time and anti-factor Xa chromogenic assay which require calibration with the drug being measured. The prothrombinase-induced clotting test shows promise as a measure for Xa inhibitors. The article reviews the oral Factor Xa inhibitor's clinical usefulness, effects on current laboratory coagulation tests and methods for measuring their anticoagulant activity.

ABBREVIATIONS: CYP – cytochrome; DOAC – direct oral anticoagulant; DVT – deep venous thrombosis; INR – international normalized ratio; PE – pulmonary embolism; PiCT – prothrombinase-induced clotting time; PT – prothrombin time; APTT – activated partial thromboplastin time.

INDEX TERMS: Anticoagulants, rivaroxaban, apixaban, edoxaban

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INTRODUCTION

Oral anticoagulant therapy is necessary for the risk reduction and treatment of venous thromboembolic events. Therapy with the vitamin K antagonist, warfarin, has historically been the effective treatment choice. Warfarin targets the vitamin K conversion cycle which is necessary for the carboxylation of coagulation factors II, VII, IX, and X. The enzyme vitamin K epoxide reductase is inhibited by warfarin, blocks the carboxylation of these factors, and results in the formation of biologically inactive coagulation molecules.[1] The coagulation process is inhibited, since the treated patient is missing effective forms of these factors. The prothrombin time (PT) can be used to monitor therapy because the assay shows a response to the decreased effectiveness of the vitamin K dependent coagulation factors.

Warfarin treatment is effective but there are several challenges. The drug has an 8- 12 hour delayed onset, depending on the half-lives of the four vitamin K dependent coagulation factors. These half-lives range from 6 hours for factor VII to 60 hours for factor II, creating a delay of 5 days for full therapeutic effect. The therapeutic range is narrow and requires frequent monitoring to prevent under or over dosage. Dietary restrictions are necessary to maintain a steady intake of vitamin K and there are many known drug interactions. The mechanism of action is effective, but nonspecific. While blocking the formation of functional coagulation factors, warfarin also blocks the formation of vitamin K dependent anticoagulant proteins C and S.[2] The reduction of coagulation control proteins and slow therapeutic onset causes the need for concurrent use of a fast acting anticoagulant, such as heparin, until full coagulation suppression is achieved.

The recent development of alternatives to warfarin for oral anticoagulant therapy have created a wide range of options for clinicians. The newer drugs have direct targets for treating and preventing thrombosis, therefore the term used for these drugs is direct oral anticoagulant (DOAC). The mechanism for one DOAC group is the inhibition of Xa. Another group works by directly

inhibiting thrombin. The differences in the DOAC pharmacologic profiles and large amounts of clinical trial data present a challenge to clinicians when trying to choose the best patient–specific anticoagulant.[3]

Oral factor Xa inhibitors

Factor Xa inhibitors function as anticoagulants by binding the active site of both free and fibrin bound Xa. The traditional coagulation cascade has long been used to represent the activation of coagulation factors, but the cell-based coagulation model is currently the more accurate representation of *in vivo* coagulation. Both models show the importance of Xa in the production of thrombin. The Xa inhibitors block thrombin generation and thrombin amplification of coagulation factors and platelets.[4,5] The drugs: rivaroxaban, apixaban, and edoxaban are oral Xa inhibitors. Unlike heparin, the drugs inhibit Xa without the need for antithrombin. They are specific for Xa and unlike warfarin, do not inhibit other coagulation factors or control proteins. (Figure 1)

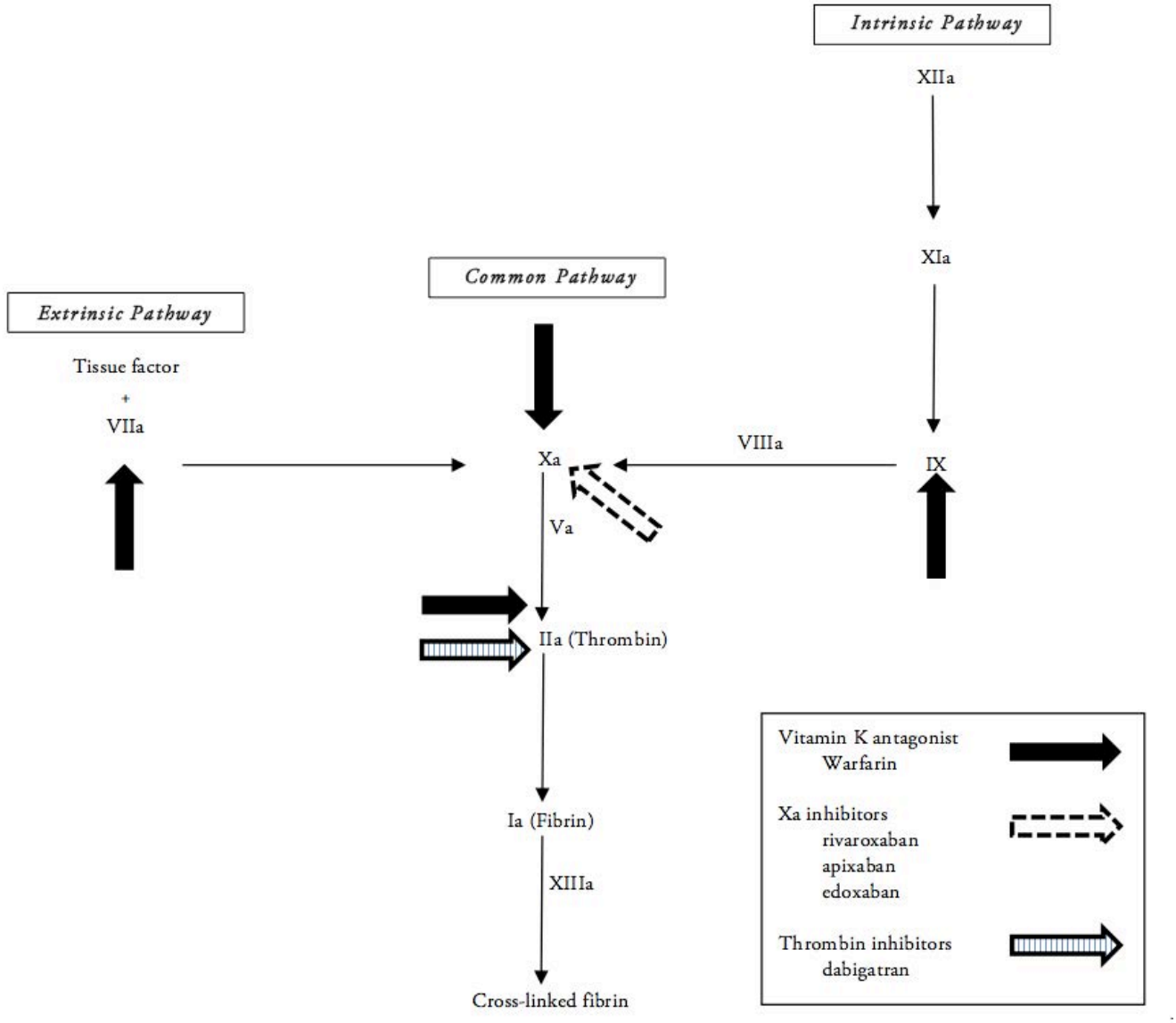

Figure 1. Mechanism of action for oral anticoagulants. Coagulation cascade demonstrating sites of active-site inhibition by oral anticoagulants.

Rivaroxaban was the first approved Xa inhibitor. Currently rivaroxaban (Xarelto®) is FDA-approved for patients recovering from knee or hip replacement surgery to reduce the risk of deep venous thrombosis (DVT) and pulmonary embolism (PE); patients with nonvalvular atrial fibrillation to reduce stroke risk; and the treatment of DVT or PE and to reduce recurrence risk following initial treatment.[6] Later apixaban (Eliquis®) was approved for DVT prophylaxis in patients who have had hip or knee replacement surgery and to reduce stroke and embolism risk in nonvalvular atrial fibrillation patients.[7] Edoxaban (Savaysa®) was approved in January, 2015 for the reduction of stroke risk and systemic embolism in patients with nonvalvular atrial fibrillation. It is also approved to treat DVT and PE in patients who have been receiving anticoagulants by injection or infusion for five to ten days.[8] The three drugs represent a sampling of the Xa inhibitors currently being developed or studied for expanded usage.

Pharmacology

Rivaroxaban is manufactured in tablets with dosage of 10, 15 and 20 mg. The bioavailability, fraction of the dose of unchanged drug that reaches the systemic circulation, is dose dependent. The oral bioavailability of the 10 mg dosage is 80-100% and the 20 mg dosage is 66%. Bioavailability is not decreased by ingestion with food or by drugs which lower gastric pH. However, the 20 mg dosage shows increased bioavailability and less patient response variability when administered with food.[4] Maximum concentrations of the drug appear within 4 hours and the half-life is 5 to 9 hours. The liver metabolizes rivaroxaban with two major enzymes called cytochromes (CYP); CYP3A4 and CYP2J2. Drugs which inhibit these enzymes have shown an increase in rivaroxaban exposure and bleeding risk. For this reason the use of CYP inhibitor drugs with rivaroxaban is not recommended. The excretion route is predominately renal (33% unchanged, 33% post metabolic degradation) with some fecal excretion. The dosage needs adjusted in patients with a creatinine clearance of less than 50 mL/min.[4, 9]

Apixaban is manufactured in 2.5 mg tablets and has 50% bioavailability which is not affected by food. The maximum concentration appears within 4 hours of tablet ingestion and the half-life is 12 hours. The CYP enzyme metabolizes apixaban, but at the rate of 15% compared to the 30% for rivaroxaban metabolism. Dosage of apixaban is reduced for patients using CYP inhibitor drugs, such as ketoconazole. Excretion is by renal and biliary routes. Apixaban clearance is not as renal dependent as rivaroxaban, so dose adjustment is not needed with mild renal impairments.[4, 9]

Edoxaban is manufactured in tablet and powder for oral solutions. The bioavailability is approximately 60%, with the drug primarily excreted by the kidneys. Edoxaban was found to be less effective in patients with normal renal function. In atrial fibrillation patients with renal clearance greater than 95 milliliters per minute, the risk of stroke was greater compared to similar patients given warfarin.[4, 8]

Clinical efficacy

The clinical usefulness of direct Xa inhibitors has been proven by phase III trial data.[9] Rivaroxaban was proven as effective as warfarin for preventing thrombosis in atrial fibrillation patients and superior in the treatment of acute venous thrombosis. Rivaroxaban shows better performance for thrombosis risk reduction after orthopedic surgery in comparison to enoxaparin. Similar findings support the use of apixaban for DVT risk reduction after hip or knee replacement surgery and in atrial fibrillation patients. Bleeding is the most severe risk with the use of anticoagulant therapy. The intracranial bleeding risk for the direct oral Xa inhibitors is lower, but the extracranial bleeding risk is higher with rivaroxaban; particularly gastrointestinal bleeding. Apixaban has a decreased risk of GI bleed when compared with warfarin. Clinical trials evaluating the use of apixaban for treatment of acute coronary syndrome had to be terminated due to major bleeding risks.[9] The clinical efficacy and bleeding risk of edoxaban are currently being generated from trial data.

A clear method for reversing the anticoagulant activity of rivaroxaban, apixaban and edoxaban in an emergency bleeding crisis is not determined. The DOAC patient with bleeding problems has effective coagulation factors, but needs the Xa inhibition reversed. Prothrombin complex concentrates, fresh frozen plasma, and recombinant FVIIa have all been studied as effective options.[5] The prothrombin complex concentrates are the preferred therapy of the current available treatments.

Laboratory Monitoring

The DOACs have rapid onset of anticoagulant activity, short half–lives, limited drug and food interactions and fixed dosage guidelines.[10] These properties eliminate the requirement for routine therapeutic monitoring. There are clinical situations which would require the evaluation of the anticoagulant activity and drug level.[11] Patients presenting with acute hemorrhage would require evaluation for anticoagulant identification and reversal monitoring. Recurrent thrombosis would require testing to determine noncompliance or under dosing. Monitoring would be necessary for patient groups excluded from clinical trials and in surgical patients. Any condition reducing drug clearance would be cause for laboratory evaluation. There is disagreement on a single standardized test for the measurement of the Xa inhibitors' anticoagulant effect.[12, 13] (Table 1)

Table 1. Comparison of laboratory assays for monitoring oral Xa inhibitor drugs.

Assay	Advantages	Disadvantages
Prothrombin time	Readily available	Nonspecific for anti-Xa
	Linear dose-response curve for rivaroxaban with some reagents	Minimally influenced by apixaban
		Traditional INR cannot be used
Chromogenic anti-Xa assay	Less sensitive to sample collection conditions	Responsive to other anticoagulants (LMWH*, fondaparinux)
	Sensitive measure of anti-Xa activity	Must be standardized using drug specific calibrators and controls
	Directly proportional to drug concentration	
Prothrombinase-induced clotting time	One assay to measure heparin, fondaparinux, oral Xa inhibitors, and direct thrombin inhibitors	Studies reported for rivaroxaban only
		Test modification necessary for the direct Xa inhibitors
	Clotting assay measurable by mechanical or optical detection	

* *LMWH* low molecular weight heparin

Rivaroxaban and apixaban prolong the standard PT and activated partial thromboplastin time (APTT) tests. The APTT response is extremely variable depending on the type of reagent used. The PT prolongation is different than what occurs with warfarin; and even differs between rivaroxaban and apixaban. The current international normalized ratio (INR) cannot be used because it is formulated from warfarin treated patient specimens. For these reasons, the anticoagulant effect must be evaluated with a PT that utilizes a dose-response curve for the specific drug concentration. The laboratory would need to know before testing which anticoagulant the patient was taking, so the correct PT curve could be used. The inexpensive nature and familiarity of the PT would make it a preferred indirect test for rivaroxaban monitoring.[12]

The anti-Xa chromogenic assay would be a direct test for the quantification of the Xa inhibitors. The anti-Xa assay would require standardization for rivaroxaban, apixaban or edoxaban with drug specific calibrators. Due to the very minimal effect of apixaban on the PT, the anti-Xa assay is necessary. A commercial kit (BIOPHEN® DiXal, Aniara) containing calibration plasmas for standardizing the anti-Xa tests is available for rivaroxaban and apixaban. In the United States the kits are for research use only. The anti-Xa assay is more specific for Xa inhibitors than the PT, which is prolonged by many factors. However the anti-Xa assay is also responsive to heparin, fondaparinux and any of the future Xa inhibitor medications. The test is not as readily available in small hospitals as the PT, which could cause problems in emergency situations. The process of standardizing the anti-Xa assay to every available Xa inhibitor could prove daunting for even progressive laboratories.[12, 13]

The prothrombinase-induced clotting test (PiCT) is performed by adding reagent Xa and Russell viper venom V to patient plasma. The mixture is incubated for 180 seconds and calcium chloride added to promote clot formation. The test is a two-step PiCT used to measure fondaparinux. The fondaparinux-antithrombin complex, when present, will inhibit the added reagent Xa and prolong the clotting time. The prolonged time is compared to a standard curve to determine therapeutic effect. The PiCT has been studied as an assay for rivaroxaban since it is a Xa inhibitor, but works independent of antithrombin. The PiCT is

modified by omitting the incubation time when testing for rivaroxaban.[14] The one step PiCT could prove useful in measuring rivaroxaban, apixaban, and edoxaban, especially if the standard curves are comparable and not drug specific. (Figure 2)

The direct Xa inhibitors affect common laboratory tests, however. The thrombin time is not affected, so the assay could provide a quick differentiating test between direct thrombin and Xa inhibitors in emergency situations. The DOAC, dabigatran, is a thrombin inhibitor instead of a Xa inhibitor and would show a prolonged thrombin time. Tests for thrombotic disorders which are based on clotting methods should not be performed when taking DOAC. These include assays for protein S activity, activated protein C resistance, and lupus anticoagulant. The PT-derived fibrinogen method will be falsely prolonged, but the Clauss fibrinogen method is thrombin dependent and not affected.[15]

CONCLUSIONS

The development of alternative anticoagulant therapies is giving valuable treatment options. The Xa inhibitors have less drug interactions, proven clinical effectiveness and comparable safety to warfarin. The oral intake, lack of required continuous monitoring and lower diet restrictions make them attractive to patients. The effectiveness, direct mechanisms of action and standard dosing guidelines are encouraging to physicians. Obstacles remain in developing laboratory assays for monitoring the Xa inhibitors. The chromogenic anti-Xa assay shows accurate and reproducible results for rivaroxaban, apixaban, and edoxaban. The PiCT, once studied with each drug, could serve as a marker of anticoagulant activity. The development of a specific therapeutic reversal for the oral Xa inhibitors during an emergency would further their appeal. As the clinical use of these drugs increases, the clinical laboratory scientist will have to be vigilant to assure test quality.

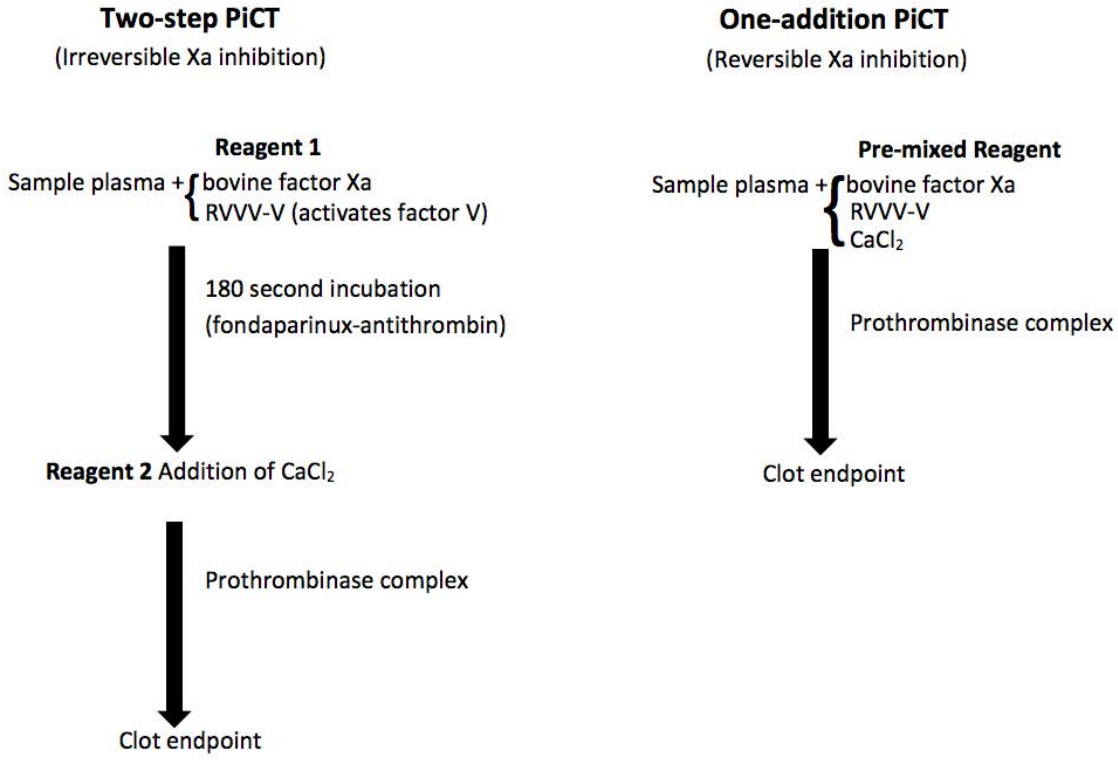

Figure 2. Comparison of PiCT methods. Two-step PiCT method for the measure of fondaparinux. The one-addition PiCT method for the measurement of rivaroxaban.

**PiCT* prothrombinase-induced clotting time
***RVV-V* Russell's viper venom V

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