

Facts and artefacts of coagulation assays for factor Xa inhibitors

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Direct factor Xa inhibitors are among the compounds at the forefront of clinical development for the prevention and treatment of thromboembolic disorders; of these, rivaroxaban, apixaban and edoxaban (DU-176b) are in the most advanced stages of development. Rivaroxaban is approved in several countries for the prevention of venous thromboembolism (VTE) after elective hip or knee arthroplasty, and trials are ongoing for other indications. Apixaban and edoxaban are undergoing clinical development for several indications. In phase II studies, various dosage regimens of rivaroxaban showed similar efficacy and safety to enoxaparin for the prevention of VTE after total hip and total knee arthroplasty (THA and TKA, respectively) (1–3) and for the treatment of deep-vein thrombosis when compared with overlapping treatment with enoxaparin and vitamin K antagonists (VKAs) (4, 5). Rivaroxaban inhibits factor Xa in a concentration-dependent manner and was found to have predictable pharmacokinetics and pharmacodynamics across a wide range of doses in healthy individuals (6, 7), as well as in patients undergoing THA (8, 9) or TKA (9). A fixed dosing regimen (10 mg once daily) has been shown to be suitable for the prevention of VTE after THA or TKA (10–13) and in VTE treatment in patients who have previously completed 6–12 months of anticoagulant therapy (20 mg once daily) (14).

Unlike VKAs, direct factor Xa inhibitors have a single target and have been found to have a predictable mode of action; there-

fore, they do not require regular coagulation monitoring. Although rivaroxaban and other direct factor Xa inhibitors can be administered without the need for regular coagulation monitoring, the ability to measure their pharmacodynamic effects (monitorability) might be useful (15), for example, when assessing patient compliance or in emergency situations. However, there is no specific laboratory test, and clinicians could be misled by the results of conventional coagulation tests, which are not specifically affected by the new, oral, direct factor Xa inhibitors. This may also be the case for other single-target anticoagulants such as direct thrombin inhibitors.

The study by Samama et al. (16) published in this issue of *Thrombosis Haemostasis* aimed to find the most appropriate coagulation assays to measure rivaroxaban pharmacodynamics and explored how pharmacodynamics can be measured by use of specific tests for factor Xa measurement. The investigators found that although rivaroxaban prolonged the conventional tests for prothrombin time (PT; also known as thromboplastin time or Quick test), dilute PT and activated partial thromboplastin time (aPTT) in a concentration-dependent manner, the results were reported to vary depending on the assay reagent used (16).

What causes variation of rivaroxaban effects in coagulation assays?

PT is a global coagulation test assessing the activity of several coagulation factors, such as factor V, factor VII and factor X. Furthermore, it is also sensitive to the prothrombinase complex. In principle, PT can potentially be prolonged by any drug affecting at least one of these factors. The commonly used PT clotting assays use a source of tissue factor to start the reaction in the test tube. Typically, PT reagents are

made from homogenates of brain, lung or placenta of human, rabbit or bovine origin and are often referred to as thromboplastins. Because these reagents have varying amounts of tissue factor, phospholipids and contaminants, they have varying potencies. This is also true for recombinant thromboplastin reagents that are made from purified, recombinant tissue factor that has been reconstituted into phospholipid vesicles. The major application of PT is to monitor the intensity of anticoagulation with VKAs. In fact, the sum of effects of these inhibitors is measured by this test. However, the results obtained with this assay may vary according to the sensitivity of the thromboplastin reagents because different thromboplastins have different sensitivities towards the vitamin K-dependent factors II, VII and X and also towards factor V.

To control for differing thromboplastin sensitivities, a standardisation system for these reagents has been developed, i.e. the international sensitivity index (ISI), which is used in conjunction with a normalization method, and the international normalised ratio (INR) for reporting prolongations of PT in patients receiving VKAs. Although the INR has become the parameter of choice to monitor the intensity of anticoagulation with VKAs after steady state has been obtained, it is noteworthy that INR values can be misleading during the initial phase of VKA therapy due to different half-lives of the VKA-dependent factors.

Samama et al. have provided firm evidence that the direct factor Xa inhibitor rivaroxaban induced a concentration-dependent prolongation of PT; however, the clotting time increase varied depending on the thromboplastin reagent used. Furthermore, the authors demonstrated that conversion of PT (in seconds) to INRs does not correct the difference in results due to differential assay reagent sensitivities to rivaroxaban. Thus, it is a fact that rivaroxaban prolongs PT. However, under clini-

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cal circumstances, this fact may turn into an artefact because the results are always provided as a percentage of normal and/or converted into INR values. The conventional and commercially available PT tests do not reliably reflect the intensity of anti-coagulation obtained with rivaroxaban or other factor Xa inhibitors. Therefore, these tests should not be used for monitoring the anticoagulant effects of these inhibitors unless modified test kits with specific calibrators become available.

Samama et al. have shown how to make use of this artefact (16). Modifications of PT with the use of specific calibrators may allow the results of PT measurements to be expressed as plasma concentrations of rivaroxaban in µg/ml. This would clearly have the benefit of enabling a simple and widely available test to assess the pharmacokinetics and pharmacodynamics of direct factor Xa inhibitors. The authors also conclude that other tests, such as dilute Russell's viper venom time, one-step prothrombinase-induced clotting time, Hep-Test® and factor Xa chromogenic assays, would be more appropriate for measuring the pharmacodynamic effects of rivaroxaban. However, these tests are either not specific and/or are not commercially available.

The authors compared the effects of rivaroxaban with fondaparinux (an indirect

factor Xa inhibitor that requires anti-thrombin), and differences in the results for both agents were observed. For example, rivaroxaban prolonged PT, but fondaparinux did not; this is because rivaroxaban can inhibit factor Xa in the prothrombinase complex (which is more efficient at generating thrombin than free factor Xa), but fondaparinux can only inhibit free factor Xa. The comparison of pharmacodynamic effects of indirect and direct factor Xa inhibitors makes this paper unique. It facilitates the understanding of their different modes of action, as illustrated in ► Figure 1.

For the clinical community it is important to know why rivaroxaban prolongs PT and why fondaparinux does not. Routine measurements of PT in patients treated with rivaroxaban may lead to inaccurate safety concerns, whereas the non-sensitivity of PT towards fondaparinux may mask real safety issues in these patients. There is another issue to consider in the fact that no reference ranges or cut-off levels have been described for direct factor Xa inhibitors, and this is further complicated by the fact that daily peak and trough values occur according to the dose regimens of these compounds.

The different sensitivities of assay reagents are not specific to rivaroxaban but also occur with other direct factor Xa in-

hibitors (such as apixaban and edoxaban). This is also true for thrombin inhibitors, which have a greater effect on aPTT than PT assays, again with different sensitivities towards various reagents. The results reported by Samama et al. clearly suggest that different tests and/or calibrators are needed for each compound to measure direct and indirect factor Xa inhibitor pharmacodynamics and plasma levels.

In conclusion, the findings from the study by Samama et al. provide much needed insight into which assays would be appropriate for measuring rivaroxaban in order to assess over- or under-coagulation in special clinical situations. However, in contrast to conventional anticoagulants such as heparins and VKAs, which require routine coagulation monitoring for their clinical use (17), the paper of Samama et al. addresses the issue of the specific detectability of rivaroxaban and, perhaps, other direct factor Xa inhibitors under certain clinical circumstances.

The publication provides a number of clinically relevant key messages, which should be shared with the wider clinical community that has an interest in the use of direct oral anticoagulants. Due to their mode of action, these new compounds may affect conventional coagulation tests, such as PT predominantly for direct factor Xa inhibitors and aPTT for direct thrombin

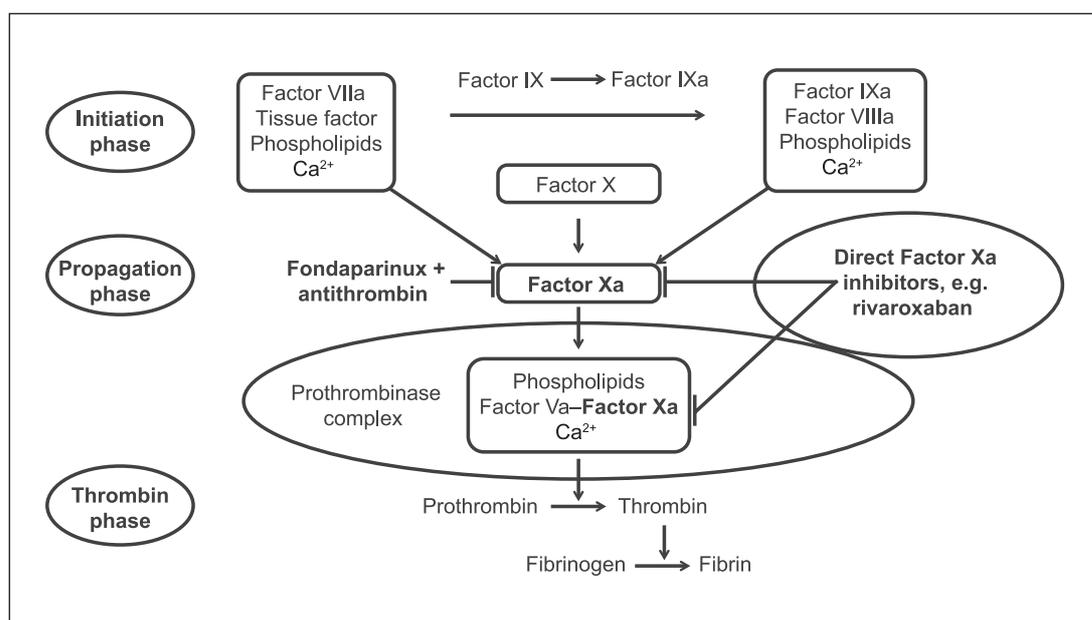


Figure 1: Different models of action of indirect factor Xa inhibitors (e.g. fondaparinux) and direct factor Xa inhibitors (e.g. rivaroxaban).

inhibitors. Depending on the PT and aPTT test reagents, these effects should be regarded as non-specific influencing variables and not as quantitative parameters to assess the intensity of anticoagulation with these new anticoagulants. It is suggested that PT or aPTT testing should be repeated if pathological results are obtained by routine testing and treatment with a direct oral inhibitor cannot be ruled out. Unlike VKAs, these inhibitors do not provide steady-state anticoagulation but yield circadian peak and trough activities.

Modified or new specific coagulation testing systems could help to identify those patients who are over- or under-dosed. However, to some extent, this is still an unmet need for some conventional anticoagulants that have been in clinical use for many years.

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