

Effects of Rivaroxaban, a Novel, Oral, Direct Factor Xa Inhibitor, on Coagulation Assays

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Introduction

- Newly developed anticoagulants target specific components of the coagulation cascade; among the new agents, those targeting thrombin or Factor Xa are showing promising results^{1,2}
- Rivaroxaban is an oral, direct Factor Xa inhibitor in advanced clinical development for the prevention and treatment of venous and arterial thromboembolism
- There is no requirement for coagulation monitoring with rivaroxaban in routine clinical practice. However, in certain clinical circumstances (e.g. severe overdose, hemorrhagic event), measurement of the pharmacodynamic effects of rivaroxaban may be of value

Objective

- To identify suitable assays that could potentially be used in clinical practice for determining the pharmacodynamics of rivaroxaban

Methods

- Increasing concentrations of rivaroxaban were spiked into citrated pooled human platelet-poor plasma (PPP)
- Global and specific clotting assays were performed. In addition, the inhibition of Factor Xa activity was measured using a coagulometric assay

Prothrombin time

- Prothrombin time (PT) was measured with a steel ball coagulometer KC-4 (Amelung, Lemgo, Germany) by using different thromboplastin reagents: Neoplastin[®], Neoplastin Plus[®] (Diagnostica Stago, Asnières, France), Innovin[®], Thromborel[®] S (Siemens Healthcare Diagnostics, Deerfield, IL, USA), Simplastin (Biomérieux, Marcy l'Etoile, France) and Recombiplastin (Instrumentation Laboratory, Lexington, KY, USA)
- In a separate experiment, rivaroxaban was spiked into PPP. Samples were kept at -80°C. PT was measured at baseline and after 4, 17, 30, and 60 days

Dilute prothrombin time

- To increase the sensitivity of the test, experiments were performed with the same thromboplastin reagents, but diluted in CaCl₂ 25 mM to obtain an initial clotting time of approximately 30 seconds

Activated partial thromboplastin time

- The activated partial thromboplastin time (aPTT) was assessed by using different reagents: PTT-A, STA-C.K. Prest[®], PTT-LA (Diagnostica Stago, Asnières, France), or Actin FS (Siemens Healthcare Diagnostics, Deerfield, IL, USA)
- Clotting time was measured after the addition of 50 µL CaCl₂ 25 mM with a STA-R[®] coagulometer (Diagnostica Stago, Asnières, France)

HepTest

- The HepTest[®] (American Diagnostica, Stamford, CT, USA) consisted of incubating undiluted PPP with bovine Factor Xa for 30 seconds (one-step test) or 120 seconds (two-step test) at 37°C. Clotting time was measured with a steel ball coagulometer KC-4

Prothrombinase-induced clotting time

- For the prothrombinase-induced clotting time (PiCT[®] [Pentapharm, Basel, Switzerland]) test, undiluted PPP was mixed with PiCT activator reagent and incubated at 37°C for 0, 30, or 180 seconds. Clotting time was measured after the addition of CaCl₂ 25 mM

Thrombin generation test

- The thrombin generation test was assessed by a calibrated automated thrombogram with the Thromboscope[™] software (Thromboscope, Maastricht, The Netherlands)
- Thrombin generation was triggered by the addition of 5 pM tissue factor, 4 µM phospholipids, and a 100 mM solution of fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Weil am Rhein, Germany)

Dilute Russell's viper venom time test

- For the dilute Russell's viper venom time (dRVVT) test, undiluted PPP was mixed with Factor Xa, CaCl₂, dRVV, and low or high phospholipid concentrations for lupus anticoagulant, as recommended by the manufacturer. Clotting time was measured with a STA-R coagulometer

Inhibition of Factor Xa activity

- Factor Xa activity was measured using a coagulometric assay, the STA-Staclo[®] heparin kit (Diagnostica Stago, Asnières, France). After the addition of an excess of Factor Xa, the clotting activity of the residual Factor Xa was measured by adding phospholipids and Ca²⁺
- All tests have been repeated recently with the same batch of reagents on six different days, using nine different concentrations of rivaroxaban dissolved in 100% dimethyl sulfoxide (DMSO)

Results

- Rivaroxaban induced a concentration-dependent prolongation of PT over a broad rivaroxaban concentration range (Figure 1A). However, clotting time increase varied depending on the thromboplastin reagent used. The concentrations of rivaroxaban required to double clotting times are shown in Table 1
- It is very important to note that in contrast to PT measurements after anticoagulation with vitamin K antagonists, international normalized ratio conversion did not reduce the variation associated with different thromboplastin reagents. In addition, data in Table 1 also show that rivaroxaban-induced PT prolongations do not correlate with the international sensitivity indices of the various thromboplastin reagents
- Frozen storage of plasma spiked with rivaroxaban did not alter its effect on PT compared with that of 'fresh' plasma (data not shown)⁴

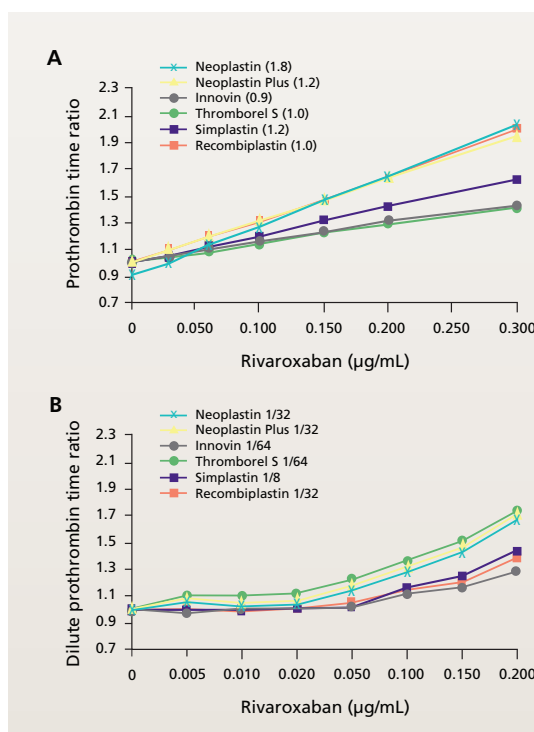


Figure 1. (A) Influence of rivaroxaban on prothrombin time and (B) dilute prothrombin time with six different thromboplastins (expressed as ratio versus baseline – international sensitivity index values are in brackets).

Table 1. Concentrations of rivaroxaban required to double clotting time in different coagulation assays

Test	Rivaroxaban (µg/mL)
Prothrombin time*	
Neoplastin (1.8)	0.35
Neoplastin Plus (1.2)	0.25
Innovin (0.9)	0.65
Thromborel S (1.0)	0.50
Simplastin (1.2)	0.45
Recombipastin (1.0)	0.30
Activated partial thromboplastin time	
PTT-A	0.75
STA-C.K. Prest	0.55
PTT-LA	0.65
Actin FS	0.50

*Expressed as ratio versus baseline – international sensitivity index values are in brackets.

- Rivaroxaban induced a concentration-dependent prolongation of dilute PT (Figure 1B), aPTT, and dRVVT, and the increase in clotting time also varied depending on the reagent used
- A concentration-dependent prolongation of aPTT was also observed (Figure 2)
- A paradoxical response was observed using the two-step HepTest and two-step PiCT test (incubation times of 120 seconds and 180 seconds, respectively)
 - Clotting time was prolonged in a concentration-dependent manner at higher rivaroxaban levels; however, low concentrations of rivaroxaban reduced clotting times
 - This response was not observed with antithrombin-immunodepleted plasma (Figure 3A and B),³ or with shorter incubation times (30 seconds for HepTest and 0 or 30 seconds for one-step PiCT test; data not shown)⁴

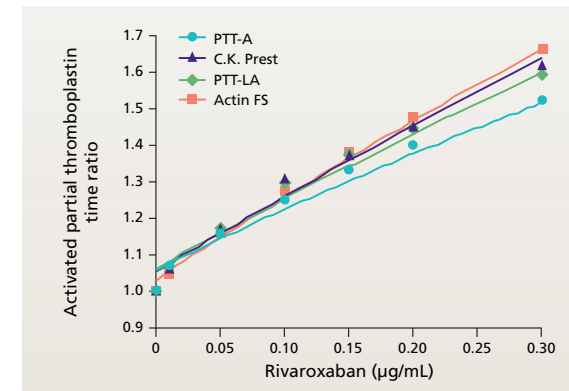


Figure 2. Influence of rivaroxaban on activated partial thromboplastin time (aPTT) with four different cephalins (expressed as ratio versus baseline).

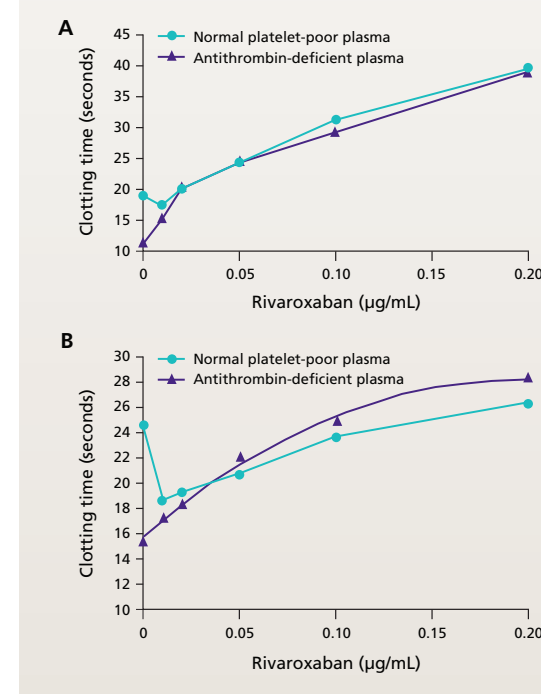


Figure 3. (A) Influence of rivaroxaban on two-step HepTest (incubation time 120 seconds) and (B) two-step prothrombinase-induced clotting time (incubation time 180 seconds), with or without antithrombin.

- Rivaroxaban inhibited Factor Xa activity in a concentration-dependent manner in the Staclo[®] coagulometric assay when rivaroxaban concentrations were derived from a rivaroxaban calibration curve

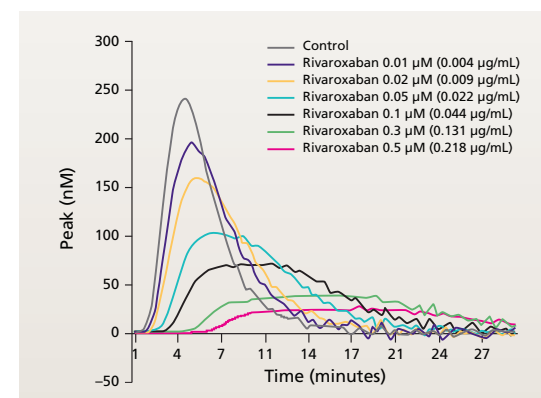


Figure 4. Influence of rivaroxaban on the thrombin generation test.

Conclusions

- Although there is no requirement for routine coagulation monitoring with rivaroxaban, an appropriate assay may be useful in certain clinical circumstances
- The international normalized ratio correction for prothrombin time assays cannot be used with rivaroxaban
- Certain assays exhibit a concentration-dependent response and show promise as candidate assays for assessing rivaroxaban pharmacodynamics
- Further development will be required to establish one of these assays as a simple, reliable, and accurate test for rivaroxaban plasma concentration
- Interestingly, the search for lupus anticoagulants with the dilute Russell's viper venom time test in patients receiving rivaroxaban can be falsely positive

- Rivaroxaban prolonged thrombin generation test parameters concentration dependently – high concentrations of rivaroxaban were shown to suppress thrombin generation almost completely (Figure 4)
- The results from tests repeated recently on six different days are consistent with the findings reported here

References

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Chromogenic anti-Factor Xa assays are also under evaluation and may also be of potential value (relevant posters being presented at this congress: Samama et al. [abstract PP-WE-199]; Perzborn et al. [abstract PP-MO-185]; Karst et al. [abstract PP-MO-162]).

Disclosure of conflict of interest

This study was supported by Bayer Schering Pharma AG and Johnson & Johnson Pharmaceutical Research & Development, L.L.C. The data contained within this poster do not support or recommend the use of rivaroxaban in indications or countries in which it is not licensed.

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