

# Effects of rivaroxaban and fondaparinux on clotting assays

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## Introduction

- Rivaroxaban is an oral, direct Factor Xa (FXa) inhibitor in advanced clinical development for the prevention and treatment of venous thromboembolism<sup>1</sup>
- Unlike the indirect, subcutaneously administered FXa inhibitor fondaparinux, the mechanism of action of rivaroxaban is antithrombin independent<sup>1</sup>
- There is no need for routine monitoring of coagulation parameters with rivaroxaban
  - Rivaroxaban has predictable pharmacokinetics and pharmacodynamics<sup>2,3</sup>
  - Rivaroxaban has a low propensity for drug-drug interactions<sup>3-6</sup>
  - Dietary restrictions are not necessary in patients receiving rivaroxaban<sup>7</sup>
- An optimum clotting assay might be valuable if a physician wants to measure the pharmacodynamic effects of rivaroxaban

## Objective

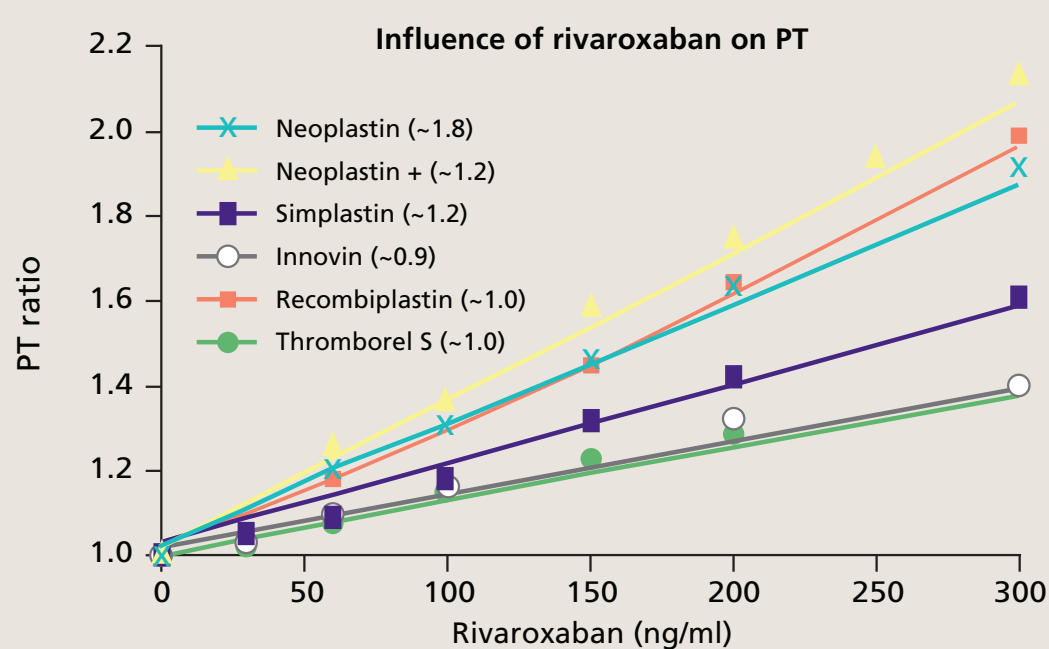
- To identify a widely available clotting assay that could be used for laboratory monitoring of rivaroxaban pharmacodynamics

## Methods

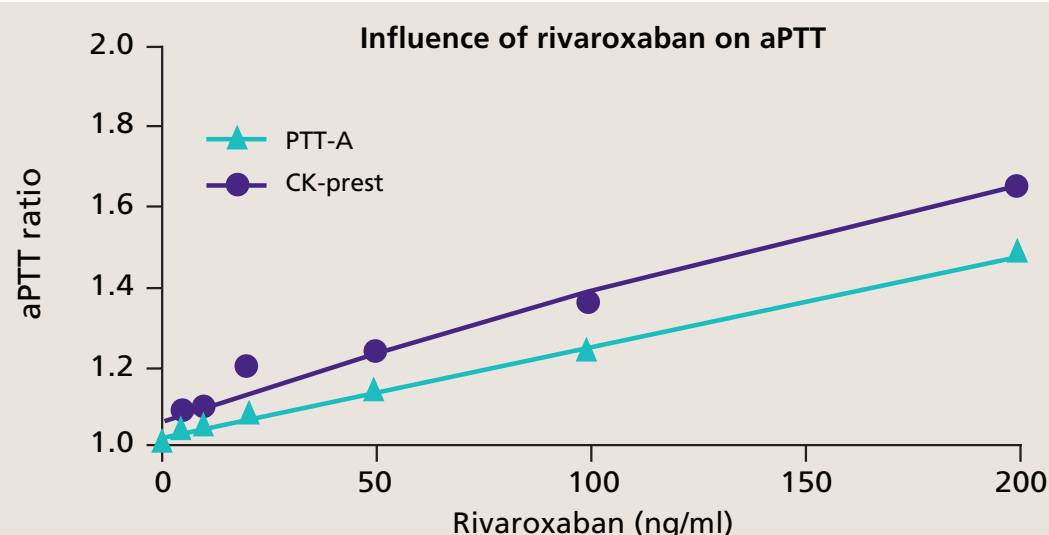
- Increasing concentrations of rivaroxaban and fondaparinux were spiked into a pool of citrated human platelet-poor plasma (PPP)
  - Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured in a coagulometer KC-4 (Amelung, Germany) with different reagents
  - The results presented are the mean value of three different experiments (maximum standard deviation: 12%)
- In a separate experiment, rivaroxaban at different concentrations (0, 50, 150 and 300 ng/ml) was spiked into PPP
  - PT was determined at baseline
  - The samples were kept at -80°C for 4, 17, 30 and 60 days, and PT was measured at each of these time points

## Results

- A concentration-dependent prolongation of both PT and aPTT was observed with rivaroxaban, although the results varied depending on the thromboplastin reagent used (Figures 1 and 2)

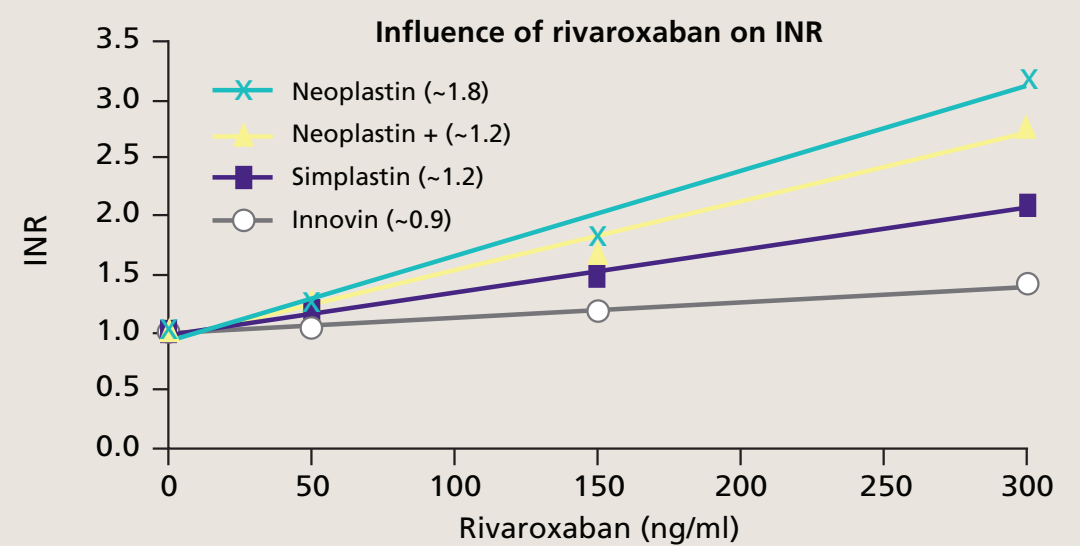


**Figure 1.** Influence of rivaroxaban on prothrombin time (PT) with six different thromboplastins (international sensitivity index value in brackets). The graphs represent linear regressions. The PT ratio observed with rivaroxaban varies depending on the thromboplastin reagent used.



**Figure 2.** Influence of rivaroxaban on activated partial thromboplastin time (aPTT) with two different cephalins.

- International normalized ratio (INR) conversion did not reduce this variation (Figure 3)
- In order to address this variability, reference plasmas with well-defined concentrations of rivaroxaban were prepared at -80°C
  - PT was tested at days 4, 17, 30 and 60 (except for Neoplastin, international sensitivity index ~1.8, which was not tested at day 60)
  - PT values did not change at any of these time points, compared with baseline (Table 1)
- Fondaparinux had no significant effect on PT (Figure 4) and aPTT (results not shown)<sup>8</sup>

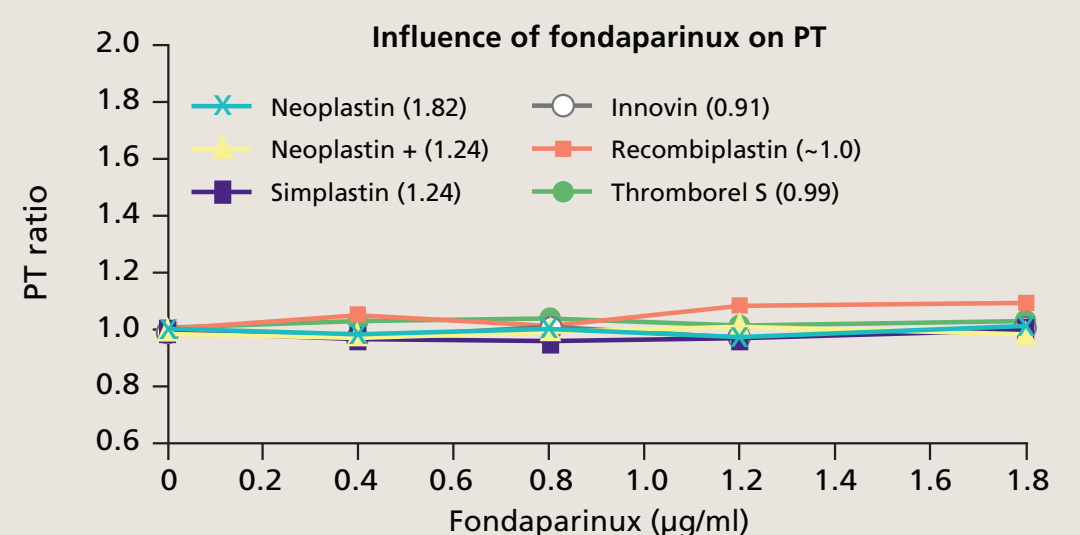


**Figure 3.** Influence of rivaroxaban on international normalized ratio (INR) with four different thromboplastins (international sensitivity index value in brackets). INR conversion did not reduce the variation due to the thromboplastin reagent used.

**Table 1.** Comparison of prothrombin time (PT) ratio at baseline ('fresh'; day 0) versus frozen plasma (median value for days 4, 17, 30 and 60) spiked with rivaroxaban

Rivaroxaban (ng/ml)	Ratio*	Neoplastin (ISI ~1.8) <sup>†</sup>	Neoplastin + (ISI ~1.2)	Simplastin (ISI ~1.2)	Innovin (ISI ~0.9)
50	Fresh	1.15	1.19	1.10	1.06
	Frozen	1.13±0.05	1.15±0.02	1.09±0.03	1.05±0.02
150	Fresh	1.47	1.50	1.37	1.21
	Frozen	1.37±0.04	1.51±0.08	1.34±0.04	1.17±0.05
300	Fresh	2.04	2.35	1.83	1.50
	Frozen	1.84±0.08	2.21±0.013	1.81±0.15	1.44±0.06

ISI, international sensitivity index. \*Compared with assays without rivaroxaban. <sup>†</sup>Not tested on day 60. The PT ratio varies with the four different thromboplastin reagents. For any given thromboplastin reagent, there is a dose-response relationship, which allows the determination of plasma concentrations expressed in ng/ml of rivaroxaban.



**Figure 4.** Influence of fondaparinux on prothrombin time (PT) with six different thromboplastins (international sensitivity index value in brackets).

## Conclusions

- Rivaroxaban prolongs PT, whereas fondaparinux does not
  - Both rivaroxaban and fondaparinux inhibit thrombin generation;<sup>9-12</sup> however, unlike fondaparinux, rivaroxaban can inhibit FXa activity in the prothrombinase complex, which is vastly more efficient than free FXa at activating prothrombin to form thrombin<sup>1</sup>
- A new method for monitoring the anticoagulant effect of rivaroxaban, based on reference plasma samples spiked with increasing concentrations of the drug, was investigated
  - The experiments showed that, by reference to a standard curve, the anticoagulant effect of rivaroxaban can be expressed in rivaroxaban (ng/ml) rather than PT ratio or INR
- PT appears to be the best test for monitoring the effect of rivaroxaban in the clinical setting, if considered necessary
- These preliminary results suggest that reference samples of PPP spiked with defined concentrations of rivaroxaban should be used in order to standardize results obtained with different thromboplastin reagents in different laboratories

## References and disclosures

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Rivaroxaban is in clinical development and not yet licensed.

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