

Inhibition of Thrombin Generation in Human Plasma by Rivaroxaban, an Oral, Direct Factor Xa Inhibitor

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Introduction

- ◆ Factor Xa is pivotal in the blood coagulation cascade
- ◆ Free Factor Xa is responsible for the generation of the first trace amounts of thrombin, and Factor Xa within the prothrombinase complex (bound to the surface of activated platelets) induces the propagation phase of thrombin generation from prothrombin, the conversion of fibrinogen to fibrin, and eventually the formation of a thrombus
- ◆ Rivaroxaban is a highly selective, reversible, oral, direct Factor Xa inhibitor ($K_i=0.4$ nM) that dose-dependently inhibits Factor Xa activity, thrombin generation, and thrombus formation^{1,2}
- ◆ Phase III studies of rivaroxaban have recently been completed for the prevention of venous thromboembolism after total hip and knee replacement surgery;³⁻⁶ rivaroxaban is also in advanced clinical development for other thromboembolic disorders

Objective

- ◆ To compare the effects of rivaroxaban on thrombin generation after activation of the tissue factor pathway in platelet-poor plasma (PPP) and platelet-rich plasma (PRP) from healthy subjects

Methods

- ◆ Blood was collected by venipuncture from healthy subjects who had not received medication in the previous 10 days. Blood was collected into vacutainer tubes containing 3.2% trisodium citrate (in 1/10 of the tube volume). PPP and PRP were obtained by immediate centrifugation at 1,000 g and 140 g, respectively, for 20 minutes at 20°C. The platelet count of PRP was adjusted to $3.5 \times 10^5/\mu\text{L}$ by dilution with autologous PPP
- ◆ PPP (n=14) and PRP (n=13) were spiked with increasing concentrations of rivaroxaban or the appropriate solvent
- ◆ Thrombin generation was assessed in PPP and PRP by measuring the cleavage of the fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Weil am Rhein, Germany) using the Calibrated Automated Thrombogram (Thrombinoscope, Maastricht, The Netherlands) method.⁷ The assays were performed according to the manufacturers' instructions for thrombin generation, with a mixture of 5 pM tissue factor and 4 μM phospholipids for PPP, and 0.5 pM tissue factor for PRP. Thrombin generation was assessed using a fluorometer (Fluoroskan® Ascent; ThermoFisher Scientific, Waltham, MA, USA)

- ◆ The following parameters were assessed: lag time, time to peak thrombin generation (T_{max}), peak thrombin generation (C_{max}), and endogenous thrombin potential (ETP)

Results

- ◆ The baseline rate and amount of thrombin generation in PPP and PRP control samples are presented in Table 1. Representative experiments of the kinetics of thrombin generation for the control samples are shown in Figure 1
- Thrombin generation was more efficient under the experimental conditions in the PPP assay than in the PRP assay
- ◆ Rivaroxaban inhibited thrombin generation in a concentration-dependent manner in both PPP and PRP after activation of the tissue factor pathway (Figures 1 and 2)
- ◆ Rivaroxaban had a greater effect on lag time, T_{max} , and C_{max} than on ETP in both PPP and PRP (Table 2)
- ◆ Lower rivaroxaban concentrations had greater effects on lag time, T_{max} , and C_{max} in PPP than in PRP (Figures 2A–C)
- ◆ The inhibition of ETP by rivaroxaban was more effective in PRP than in PPP, with the inhibition of ETP (85±3%) being almost complete with 475 ng/mL rivaroxaban in PRP (Table 2, Figure 2D)

Table 1. Baseline rate and amount of thrombin generation in control samples

Parameter	Platelet-poor plasma	Platelet-rich plasma
Lag time (minutes)	1.9±0.1	10.8±0.8
T_{max} (minutes)	4.3±0.2	21.5±1.5
C_{max} (nM thrombin)	350±17	90±6
ETP (nM × minutes)	1,870±84	1,435±65

Values are mean ± standard error of the mean obtained in platelet-poor plasma from 14 subjects and in platelet-rich plasma from 13 subjects. C_{max} , peak thrombin generation; ETP, endogenous thrombin potential; T_{max} , time to peak thrombin generation.

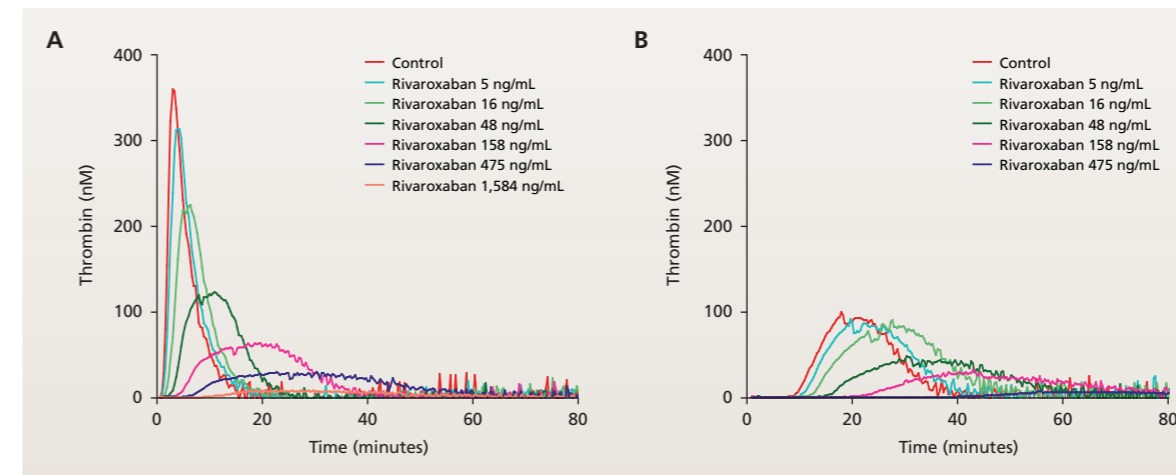


Figure 1. Representative thrombograms showing the kinetics of thrombin generation in (A) platelet-poor plasma (one of 14) and (B) platelet-rich plasma (one of 13) after activation of the tissue factor pathway.

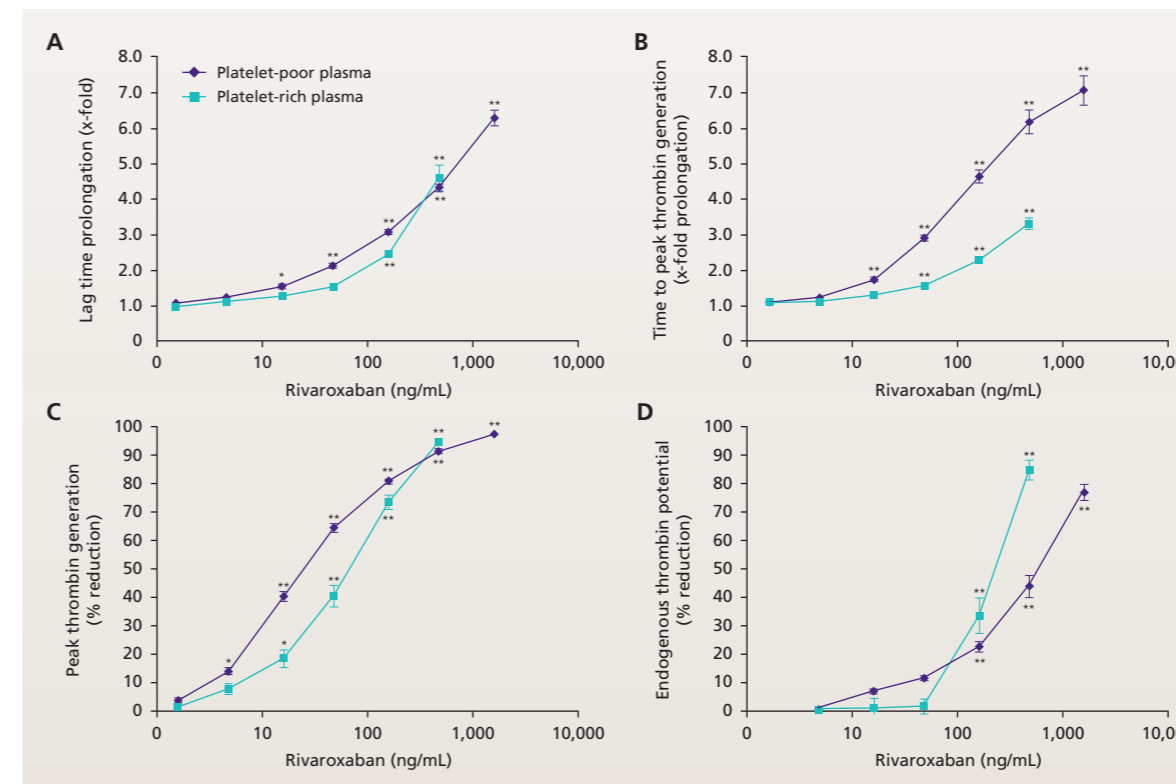


Figure 2. Effects of rivaroxaban on (A) lag time, (B) time to peak thrombin generation, (C) peak thrombin generation, and (D) endogenous thrombin potential of thrombin generation in human platelet-poor plasma and platelet-rich plasma. Each data point represents the mean ± standard error of the mean of 14 (platelet-poor plasma) and 13 (platelet-rich plasma) separate experiments. * $p<0.05$; ** $p<0.001$.

Conclusions

- ◆ Rivaroxaban inhibited thrombin generation in a concentration-dependent manner across all measured parameters in both platelet-poor and platelet-rich plasma. This was achieved via the inhibition of free Factor Xa and Factor Xa within the physiologically relevant prothrombinase complex bound to the surface of activated platelets
- ◆ Thus, rivaroxaban could be effective at reducing uncontrolled thrombin generation and thrombus formation in thromboembolic diseases

Table 2. Inhibition of thrombin generation by rivaroxaban in platelet-poor and platelet-rich plasma

Parameter	Platelet-poor plasma	Platelet-rich plasma
Doubling lag time (ng/mL)	37±3	92±7
Doubling T_{max} (ng/mL)	21±1	112±12
C_{max} IC_{50} (ng/mL)	25±2	71±7
ETP IC_{50} (ng/mL)	644±79	252±34

Values are mean ± standard error of the mean obtained in platelet-poor plasma from 14 subjects and in platelet-rich plasma from 13 subjects. C_{max} , peak thrombin generation; ETP, endogenous thrombin potential; IC_{50} , half-maximal inhibitory concentration; T_{max} , time to peak thrombin generation.

References

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Disclosure of conflict of interest

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