

# Hypercoagulability in rats is increased by low doses of the direct thrombin inhibitor melagatran, but is reduced by the direct Factor Xa inhibitor rivaroxaban

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## Introduction

- ◆ Rebound thrombin generation can occur upon cessation of unfractionated heparin, low molecular weight heparin, and the direct thrombin inhibitor ximelagatran – this may manifest as cardiovascular adverse events<sup>1,2</sup>
- ◆ Thrombin–antithrombin (TAT) level is a marker of a prothrombotic state and has been used as a potential tool for evaluating the presence of rebound hypercoagulability<sup>3</sup>
- ◆ Rivaroxaban is an oral, direct Factor Xa inhibitor<sup>4</sup> that has received a positive CHMP recommendation for the prevention of venous thromboembolism after elective total hip or knee replacement surgery

## Objective

- ◆ To compare the effects of rivaroxaban with the direct thrombin inhibitor melagatran in a rat model of tissue factor (TF)-induced hypercoagulability, by measuring fibrinogen and TAT levels

## Methods

- ◆ Anaesthetized, fasted male Wistar rats (n=10 per study group) were given intravenous injection of either rivaroxaban (0.0009–0.9 mg/kg) or melagatran (0.0012–1.2 mg/kg), or the appropriate vehicle (control)
- ◆ Disseminated intravascular coagulation was induced by TF. TF (8 mg/kg; RecombiPLasTin; Instrumentation Laboratory) reconstituted in 0.5 ml RecombiPLasTin Diluent and 0.5 ml 0.9% NaCl was given slowly at 0.3 ml/min as an intravenous bolus 5 minutes later; a sham group received vehicle only
- ◆ Blood was obtained 10 minutes later by puncture of the abdominal aorta for the measurements of TAT using a commercially available ELISA kit (Enzygnost, Dade Behring) and fibrinogen (Rat Fibrinogen ELISA, Immunology Consultants Laboratory). Rivaroxaban and melagatran levels were measured by liquid chromatography–tandem mass spectrometry
- ◆ In the rivaroxaban-treated animals, fibrinogen was also measured using a commercially available clotting assay (STA-Fibrinogen, Diagnostica Stago) based on the addition of an excess of thrombin to the plasma according to the method of Claus

## Statistics

One-way ANOVA followed by Tukey's multiple comparison test was used for statistical analysis. A  $p < 0.05$  was considered to be significant. Results are shown as mean  $\pm$  standard error of the mean.

## Results

### Rivaroxaban

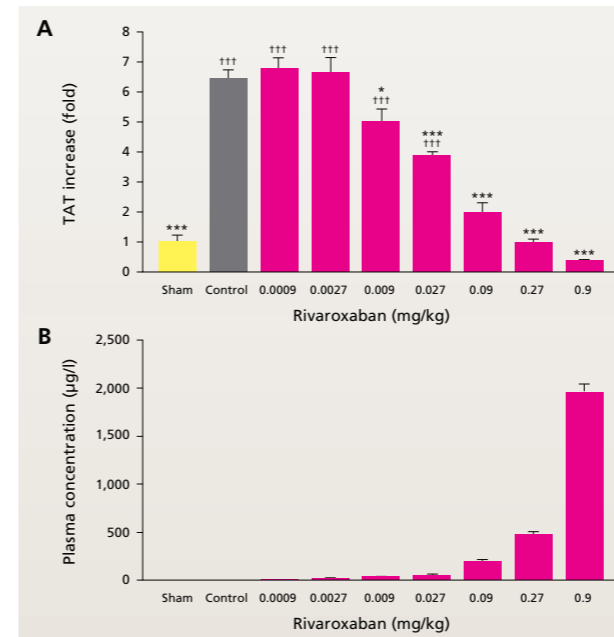
- ◆ Rivaroxaban dose-dependently inhibited TF-induced TAT generation over the dose range of 0.009–0.9 mg/kg (corresponding to plasma levels of 24–1952  $\mu\text{g/l}$ ); TAT increase was completely inhibited at a dose of 0.27 mg/kg (Figure 1). Rivaroxaban showed a flat dose–response curve
- ◆ Lower doses of rivaroxaban (0.0009–0.0027 mg/kg) did not affect TAT generation (Figure 1)
- ◆ Rivaroxaban did not affect fibrinogen level. The small decrease of fibrinogen after 0.0027 and 0.009 mg/kg rivaroxaban was significant only when compared with the sham group by ELISA and was not confirmed using the fibrinogen clotting assay (Figure 2)

### Melagatran

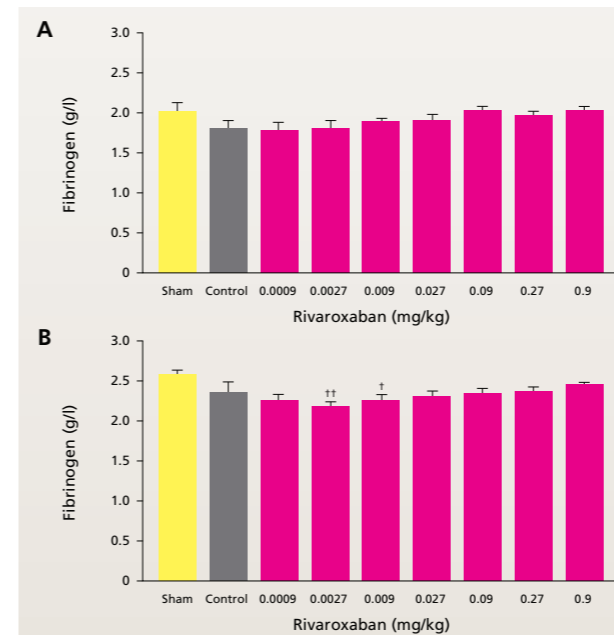
- ◆ Melagatran also reduced TF-induced TAT generation at higher doses (0.35–1.2 mg/kg; corresponding to plasma levels of 525–989  $\mu\text{g/l}$ ; Figure 3), and showed a steep dose–response curve in contrast to rivaroxaban
- ◆ Melagatran potentiated TF-induced hypercoagulability at lower doses (0.012–0.035 mg/kg; corresponding to plasma levels of 19–54  $\mu\text{g/l}$ ); TAT increased from  $2.7 \pm 0.4$ -fold to  $6.6 \pm 0.6$ -fold vs TF control at 0.012 mg/kg (Figure 3)
- ◆ In addition, fibrinogen was significantly decreased from  $2.30 \pm 0.07$  g/l (TF control) to  $1.28 \pm 0.24$  g/l at 0.035 mg/kg melagatran (Figure 4)

## Conclusions

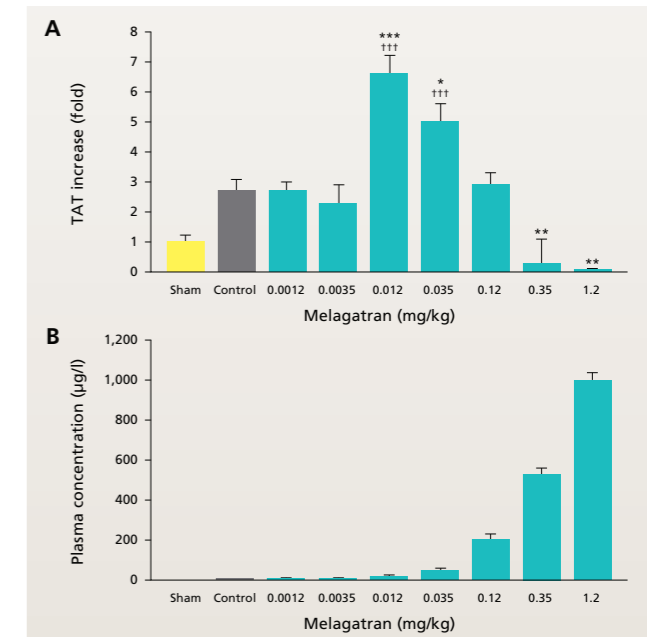
- ◆ Rivaroxaban significantly reduced TF-induced hypercoagulability over a wide dose range
- ◆ Melagatran also significantly reduced TF-induced hypercoagulability, but over a small dose range
- ◆ The direct thrombin inhibitor melagatran increased hypercoagulability at low plasma levels, which was not observed with the direct FXa inhibitor, rivaroxaban
- ◆ Similar results were described comparing the direct FXa inhibitor DX-9065a and melagatran in a similar rat disseminated intravascular coagulation model after oral administration<sup>5</sup>
- ◆ The results suggest that low plasma concentrations of the direct thrombin inhibitor melagatran may increase thrombin generation and thus, may cause coagulation activation. Furthermore, low drug levels are present upon drug cessation and thus may lead to rebound coagulation



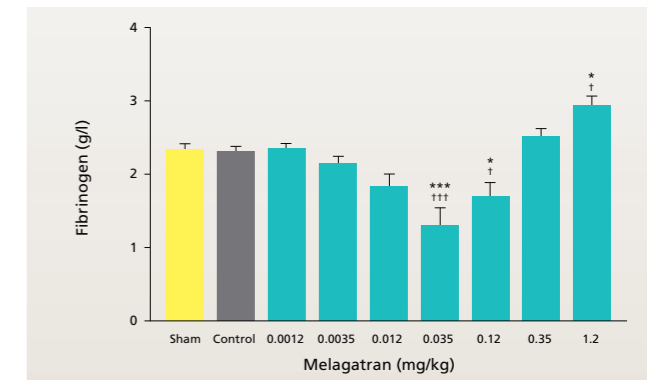
**Figure 1.** Effects of rivaroxaban on thrombin–antithrombin (TAT) concentrations 10 minutes after tissue factor injection (A); plasma levels of rivaroxaban 15 minutes after rivaroxaban administration (B). \* $p < 0.05$ , \*\*\* $p < 0.001$  vs tissue factor control group (vehicle + tissue factor); ††† $p < 0.001$  vs sham group (vehicle + vehicle).



**Figure 2.** Effects of rivaroxaban on fibrinogen levels 10 minutes after tissue factor injection. Fibrinogen was determined according to the method of Claus (A); or by ELISA (B). † $p < 0.05$ , †† $p < 0.01$  vs sham group (vehicle + vehicle).



**Figure 3.** Effects of melagatran on thrombin–antithrombin (TAT) concentrations 10 minutes after tissue factor injection (A); plasma levels of melagatran 15 minutes after administration of melagatran (B). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs tissue factor control group (vehicle + tissue factor); ††† $p < 0.001$  vs sham group (vehicle + vehicle).



**Figure 4.** Effects of melagatran on fibrinogen levels 10 minutes after tissue factor injection. Fibrinogen was determined using an ELISA. \* $p < 0.05$ , \*\*\* $p < 0.001$  vs tissue factor control group (vehicle + tissue factor); † $p < 0.05$ , ††† $p < 0.001$  vs sham group (vehicle + vehicle).

## References

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## Disclosure of Conflict of Interest

This study and production of this poster was supported by Bayer HealthCare AG and J&JPRD. Rivaroxaban is in clinical development and not yet licensed.

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