

# Concomitant administration of rivaroxaban – an oral, direct Factor Xa inhibitor – with clopidogrel and acetylsalicylic acid enhances antithrombosis in rats

Elisabeth Perzborn, Elke Fischer, Uwe Lange  
Global Drug Discovery, Cardiology Research, Bayer HealthCare AG, Wuppertal, Germany

## Introduction

Rivaroxaban is a novel, oral, once-daily, direct Factor Xa inhibitor in advanced clinical development for the prevention and treatment of venous thromboembolic disorders. Patients likely to receive rivaroxaban include those with cardiovascular diseases, who may also take clopidogrel (Plavix®), acetylsalicylic acid (ASA), or a combination of these drugs.<sup>1-3</sup>

## Objective

To assess the effects of rivaroxaban, clopidogrel, ASA and their combinations on arterial thrombosis and haemostasis, using a rat arteriovenous (AV)-shunt model and tail-transection bleeding-time model, respectively.

## Methods

◆ Anaesthetized male Wistar rats received intravenous rivaroxaban (0.1 mg/kg), oral clopidogrel (1 mg/kg) and oral ASA (10 mg/kg), alone or in combination with appropriate solvents

### In vivo studies

#### AV-shunt model (arterial thrombosis)

- ◆ The right common carotid artery was isolated and cannulated with an 80-mm polyethylene catheter (PE-60; internal diameter [ID], 0.76 mm) connected with a 60-mm Tygon® tube (ID, 3.2 mm) containing a rough thrombogenic nylon thread (60 × 0.26 mm) folded into a double string
- ◆ The left jugular vein was isolated and cannulated with a 20-mm polyethylene tube (PE-60; ID, 0.76 mm) connected with a 60-mm polyethylene catheter (PE-160; ID, 1.14 mm)
- ◆ The two tubes were then filled with saline and connected to form an AV shunt
- ◆ The shunt was opened for 15 minutes. The nylon thread covered with the thrombus was then withdrawn and weighed immediately

#### Tail-transection bleeding-time model (haemostasis)

- ◆ The tail was transected 2 mm from the tip by a dorsoventral vertical cut using a razor blade, and immediately immersed into pre-warmed (37°C) physiological saline solution
- ◆ Bleeding time was recorded from tail transection until permanent cessation of bleeding (defined as no bleeding for at least 30 seconds). Bleeding times exceeding 30 minutes were recorded as 1800 seconds

### Ex vivo studies

- ◆ Prolongation of prothrombin time (PT) was measured using blood collected at the end of the *in vivo* studies in both models

### Statistical analysis

- ◆ Tukey's multiple comparison test (one-way ANOVA) was used for statistical analysis, with a significance level of  $p < 0.05$ . Results are shown as means ± standard error of the mean

## Results

### In vivo studies

#### Arterial thrombosis

- ◆ Rivaroxaban, clopidogrel and ASA alone significantly inhibited thrombus formation vs control ( $p < 0.001$ ) (Figure 1, Table 1)
- ◆ When rivaroxaban was combined with clopidogrel, there was a significant increase in antithrombotic effect compared with each drug given alone ( $p < 0.01$  vs clopidogrel or rivaroxaban alone) (Figure 1)
- ◆ A combination of rivaroxaban plus ASA also showed a significantly increased antithrombotic effect compared with rivaroxaban or ASA given alone ( $p < 0.05$  vs ASA or rivaroxaban alone) (Figure 1)
- ◆ In contrast, a combination of ASA and clopidogrel did not show a significant increase in antithrombotic activity over the drugs given alone (Figure 1)
- ◆ Addition of rivaroxaban to the combination of clopidogrel and ASA further and significantly enhanced the antithrombotic effect ( $p < 0.001$ ) (Figure 1)

#### Haemostasis

- ◆ Bleeding time was not prolonged by rivaroxaban, clopidogrel or ASA alone (Figure 2, Table 1)
- ◆ Addition of rivaroxaban to the combination of clopidogrel plus ASA produced a small, non-significant prolongation of bleeding time beyond the slight increase observed with all combinations containing clopidogrel (Figure 2)

### Ex vivo studies

- ◆ The anticoagulant effect of rivaroxaban, as shown by PT, was not influenced by clopidogrel and ASA alone or in combination (Figure 3, Table 1)

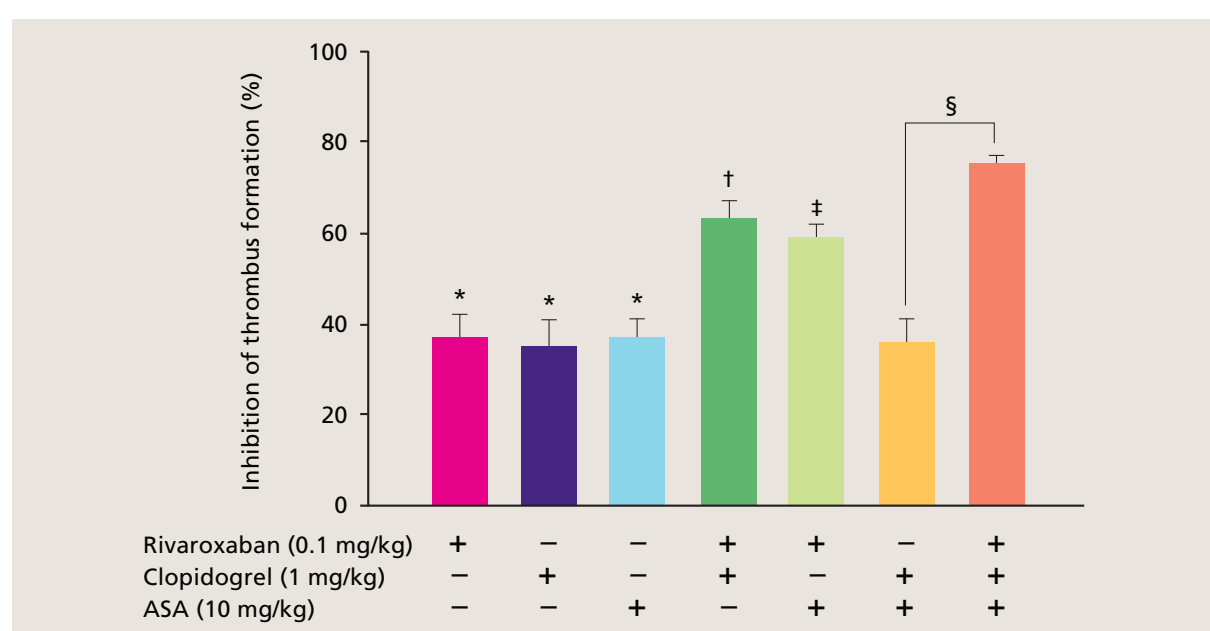


Figure 1. Inhibition of arterial thrombus formation in a rat arteriovenous-shunt model with rivaroxaban, clopidogrel and acetylsalicylic acid (ASA) alone, and their combinations (n=6). \* $p < 0.001$  vs control, † $p < 0.01$  vs clopidogrel or rivaroxaban, ‡ $p < 0.05$  vs ASA or rivaroxaban, § $p < 0.001$ .

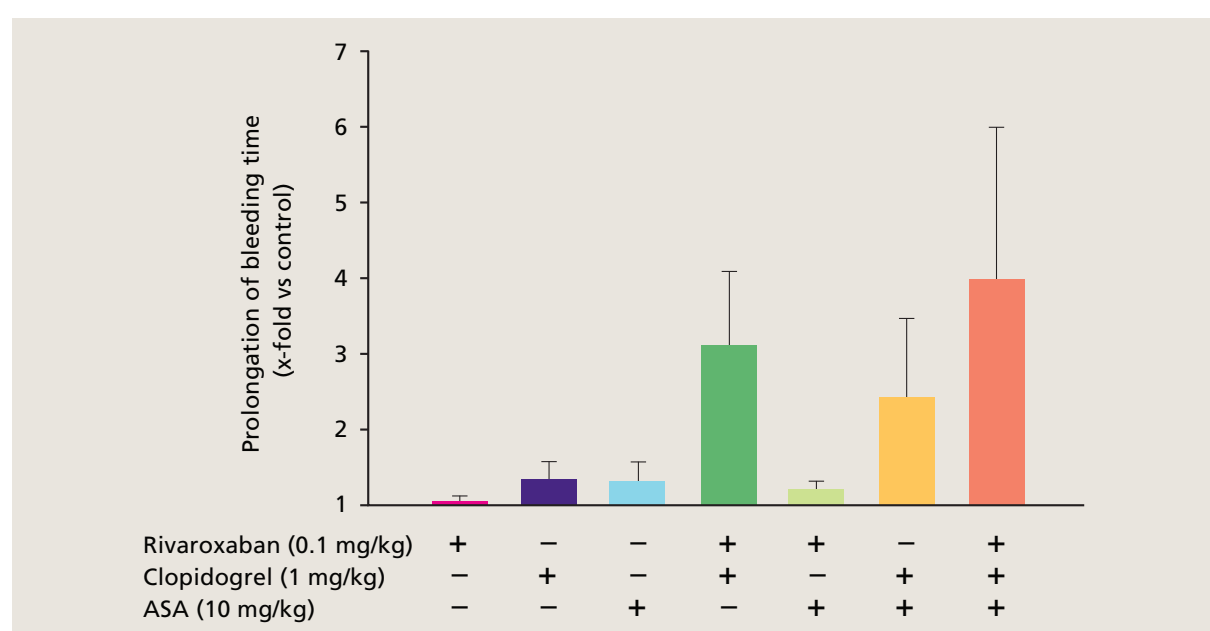


Figure 2. Bleeding time in a rat tail-transection bleeding-time model with rivaroxaban, clopidogrel and acetylsalicylic acid (ASA) alone, and in combination (n=10).

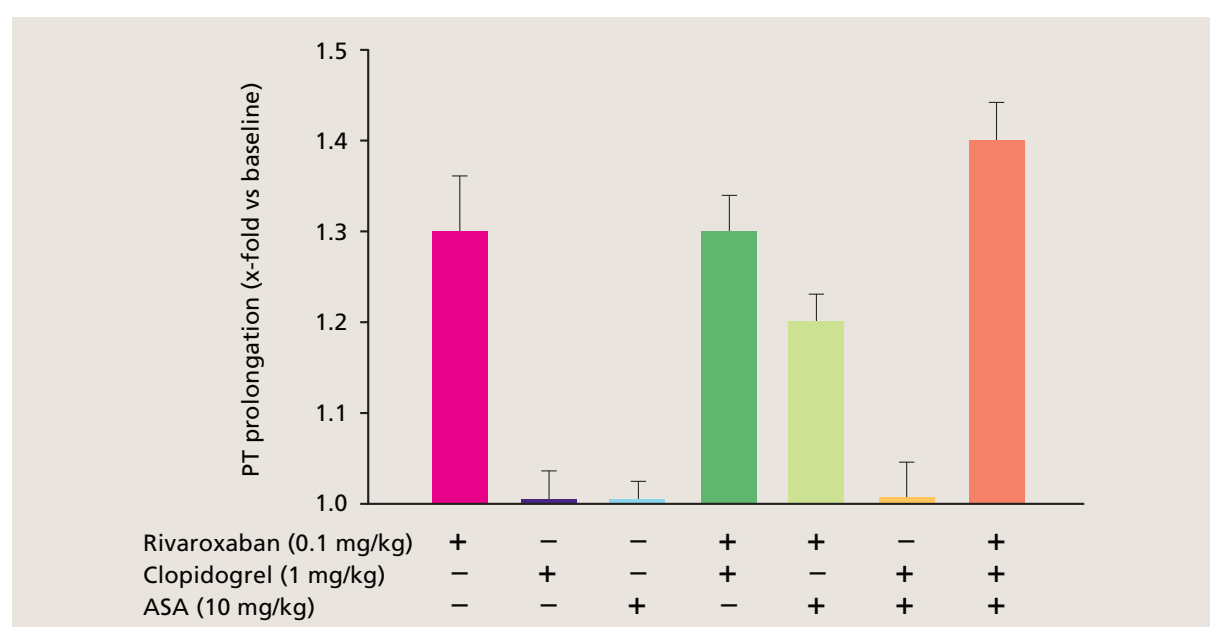


Figure 3. The effect of rivaroxaban, clopidogrel and acetylsalicylic acid (ASA) alone, and in combination on prothrombin time (PT).

Table 1. Effect of rivaroxaban, clopidogrel and acetylsalicylic acid (ASA), alone and in combination, on thrombus formation, bleeding time and prothrombin time (PT). Results are shown as mean ± standard error of the mean

|                                 | Thrombus reduction (%; n=6) | Bleeding time (x-fold vs control; n=10) | PT prolongation (x-fold; n=10) |                             |
|---------------------------------|-----------------------------|---|--------------------------------|-----------------------------|
|                                 |                             |   | AV-shunt model                 | Tail-transection time model |
| Rivaroxaban                     | 37±5                        | 1.0±0.1                                 | 1.3±0.06                       | 1.4±0.04                    |
| Clopidogrel                     | 35±6                        | 1.3±0.1                                 | 1.0±0.03                       | 1.0±0.03                    |
| ASA                             | 37±4                        | 1.3±0.2                                 | 1.0±0.02                       | 1.0±0.02                    |
| Rivaroxaban + clopidogrel       | 63±4                        | 3.1±1.0                                 | 1.3±0.04                       | 1.5±0.05                    |
| Rivaroxaban + ASA               | 59±3                        | 1.2±0.1                                 | 1.2±0.03                       | 1.4±0.10                    |
| Clopidogrel + ASA               | 36±5                        | 2.4±1.0                                 | 1.0±0.04                       | 1.0±0.02                    |
| Rivaroxaban + clopidogrel + ASA | 75±2                        | 4.0±2.0 <sup>a</sup>                    | 1.4±0.04                       | 1.4±0.10                    |

AV, arteriovenous. <sup>a</sup>>1800 seconds (n=1).

## Conclusions

- ◆ The study suggests that rivaroxaban, clopidogrel and ASA may be co-administered, and that this triple combination may have a greater antithrombotic effect than each drug administered alone
- ◆ The addition of rivaroxaban to the combination of clopidogrel plus ASA may offer greater antithrombotic effects than the clopidogrel plus ASA combination alone

### References and disclosures

- Bertrand ME *et al.* *Eur Heart J* 2002;23:1809–1840.
- Braunwald E *et al.* *J Am Coll Cardiol* 2002;40:1366–1374.
- Geerts WH *et al.* *Chest* 2004;126:3385–4005.

This study was supported by Bayer HealthCare AG and Scios, Inc. All authors are employees of Bayer HealthCare AG. Rivaroxaban is in clinical development and not yet licensed.

Poster P060 presented at the 20th International Congress on Thrombosis (ICT), Athens, Greece; 25–28 June 2008