

Concomitant administration of low-dose rivaroxaban – an oral, direct Factor Xa inhibitor – with clopidogrel and acetylsalicylic acid enhances antithrombotic efficacy in rats

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Introduction

- An important part of the secondary prevention of recurrent ischaemic events in acute coronary syndrome (ACS) patients is the inhibition of coagulation and of platelet aggregation^{1,2}
- Rivaroxaban is an oral, direct Factor Xa inhibitor³ that has recently been shown to prevent venous thromboembolism after total hip and knee replacement surgery⁴⁻⁷
- The ATLAS ACS TIMI 46 phase II dose-escalation study in patients with ACS has shown that rivaroxaban may be able to reduce the risk of important clinical events (death, myocardial infarction, or stroke compared with placebo) while dose-dependently increasing clinically significant bleeding⁸
- Rivaroxaban is currently undergoing phase III study for the secondary prevention of ACS in patients receiving acetylsalicylic acid (ASA) or ASA and a thienopyridine in the ATLAS ACS TIMI 51 study

Objective

- To assess if a synergistic effect exists between low doses (sub-efficacious doses and a moderately effective dose) of rivaroxaban in combination with the thienopyridine clopidogrel and/or ASA on arterial thrombosis and haemostasis in rat models

Methods

- Anaesthetized male Wistar rats received intravenous rivaroxaban (0.01, 0.03, and 0.1 mg/kg; studies A, B, and C, respectively), oral clopidogrel (1 mg/kg), and oral ASA (3 mg/kg), alone or in combination with appropriate solvents

Arteriovenous shunt model (arterial thrombosis)

- The right common carotid artery and left jugular vein were isolated and cannulated with two catheters connected by a Tygon® (Saint-Gobain Performance Plastics) tube containing a rough thrombogenic nylon thread (of known weight), folded into a double string
 - The right common carotid artery was cannulated with an 80 mm polyethylene catheter (internal diameter [ID] 0.76 mm) connected to the 60 mm Tygon tube (ID 3.2) containing the nylon thread (60 mm × 0.26 mm)
 - The left jugular vein was cannulated with a 20 mm polyethylene tube (ID 0.76 mm) connected to a 60 mm polyethylene catheter (ID 1.14 mm). The tubes were filled with saline and then connected
- The shunt was opened for 15 minutes. The nylon thread covered with the thrombus was then withdrawn and immediately weighed
- Rivaroxaban, ASA, or clopidogrel (or their appropriate vehicles) was given 15, 40, or 120 minutes, respectively, before the shunt was opened

Tail-transection bleeding time model (haemostasis)

- The tail was transected 2 mm from the tip, and the distal part was immersed into prewarmed (37°C) physiological saline solution
- Bleeding time was recorded as the time taken for continuous blood flow to cease for >30 seconds. Bleeding times exceeding 30 minutes were recorded as 1,800 seconds

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 mean ± standard error of the mean

Results

Arterial thrombosis

- Rivaroxaban at doses of 0.01 and 0.03 mg/kg (sub-efficacious doses; studies A and B) had no effect on thrombus formation, whereas the 0.1 mg/kg dose moderately reduced thrombus formation by 28% ($p < 0.01$ versus the control; study C; Table 1, Figures 1, 2, and 3)
- Thrombus formation was not inhibited by ASA (3 mg/kg; Table 1)
- Thrombus formation was moderately inhibited by clopidogrel (1 mg/kg) by 28–35% ($p < 0.01$; Table 1)
- The combinations of either sub-efficacious doses (0.01 or 0.03 mg/kg; studies A and B) of rivaroxaban with ASA resulted in a moderate reduction of thrombus formation (24%, $p < 0.05$, and 37%, $p < 0.001$, respectively, versus control; Table 1, Figures 1 and 2)
- The addition of ASA or sub-efficacious doses of rivaroxaban (0.01 and 0.03 mg/kg; studies A and B) to clopidogrel did not enhance the antithrombotic effect of clopidogrel (Table 1, Figures 1 and 2)
- The addition of sub-efficacious doses (0.01 and 0.03 mg/kg) of rivaroxaban to the combination of clopidogrel and ASA (triple combination) resulted in an increase in the antithrombotic effect (studies A and B: 43%; $p < 0.001$ versus control; $p < 0.05$ versus rivaroxaban; $p < 0.01$ versus ASA; Table 1, Figures 1 and 2)
- Combining a moderately effective dose of rivaroxaban (0.1 mg/kg; study C) with either ASA or clopidogrel, or with the combination of ASA and clopidogrel, increased the antithrombotic effect to 39%, 52%, and 51%, respectively ($p < 0.001$ versus control; Table 1, Figure 3)
 - The combination of rivaroxaban (0.1 mg/kg) with clopidogrel (52%; $p < 0.05$ versus rivaroxaban) or the triple combination (51%; $p < 0.05$ versus rivaroxaban) was more effective than the combination of ASA and clopidogrel (37%)

Haemostasis

- Rivaroxaban and ASA alone did not affect bleeding times significantly in all three studies. There was a slight, non-significant trend towards an increase in bleeding time with clopidogrel in all studies (1.2–1.5-fold versus control)

Table 1. Effects of rivaroxaban (0.01–0.1 mg/kg i.v.), acetylsalicylic acid (3 mg/kg p.o.), or clopidogrel (1 mg/kg p.o.) and their combinations on thrombus formation in anaesthetized rats

Study and rivaroxaban concentration	Inhibition of thrombus formation (%)						
	Rivaroxaban	ASA	Clopidogrel	Rivaroxaban + ASA	Rivaroxaban + clopidogrel	ASA + clopidogrel	Rivaroxaban + ASA + clopidogrel
Study A 0.01 mg/kg	18±4	15±5	35±4***	24±6*	37±7***	20±2	43±5***†§
Study B 0.03 mg/kg	20±7	17±4	28±3**	37±4***	27±7**	26±5**	43±3***†§
Study C 0.1 mg/kg	28±3**	22±6*	30±5**	39±7***	52±4***†§	37±7***	51±2***†§

Results are mean ± standard error of the mean (n=6). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control; † $p < 0.05$ versus rivaroxaban; ‡ $p < 0.01$ versus ASA; § $p < 0.05$ versus ASA + clopidogrel. ASA, acetylsalicylic acid.

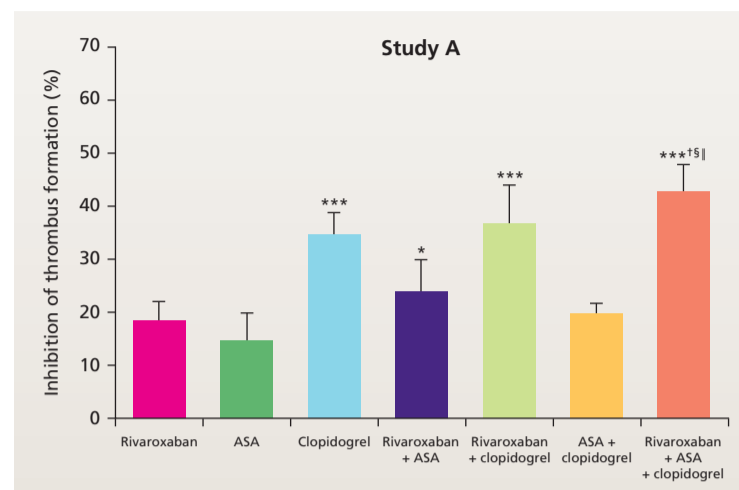


Figure 1. Effects of rivaroxaban (0.01 mg/kg i.v.), ASA (3 mg/kg p.o.), clopidogrel (1 mg/kg p.o.), or their combination on thrombus formation in anaesthetized rats (study A). Results are mean ± standard error of the mean (n=6). * $p < 0.05$; *** $p < 0.001$ versus control; † $p < 0.05$ versus rivaroxaban; ‡ $p < 0.01$ versus ASA; § $p < 0.05$ versus ASA + clopidogrel. ASA, acetylsalicylic acid; i.v., intravenous; p.o., oral.

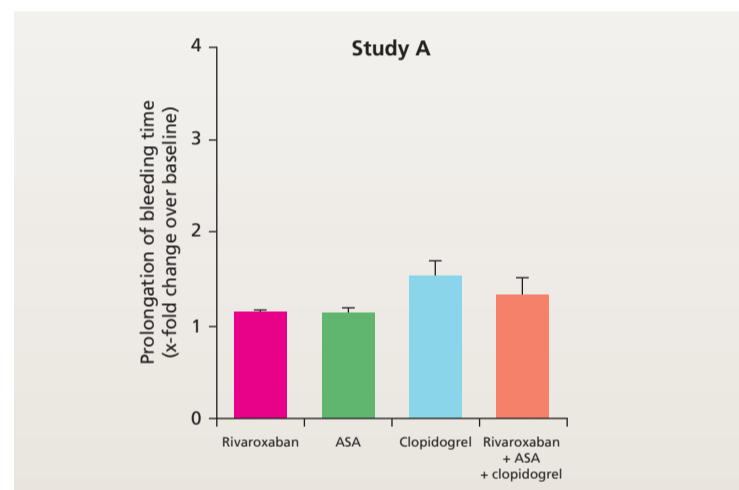


Figure 4. Prolongation of bleeding time after the administration of rivaroxaban (0.01 mg/kg i.v.), ASA (3 mg/kg p.o.), clopidogrel (1 mg/kg p.o.), or their combination in anaesthetized rats (study A). Results are mean ± standard error of the mean (n=10). ASA, acetylsalicylic acid; i.v., intravenous; p.o., oral.

- The addition of the lowest dose of rivaroxaban (0.01 mg/kg; study A) to the combination of clopidogrel and ASA did not further increase bleeding time (Figure 4)
- The addition of rivaroxaban (0.03 mg/kg; study B; 0.1 mg/kg; study C) to the combination of clopidogrel and ASA increased bleeding time to 2.8-fold and 2.5-fold of the control value (beyond the increase observed with clopidogrel alone), but this effect did not reach statistical significance (Figures 5 and 6)

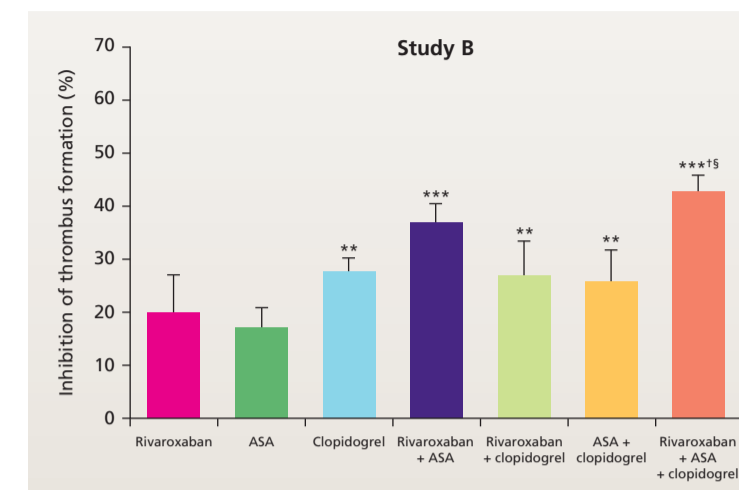


Figure 2. Effects of rivaroxaban (0.03 mg/kg i.v.), ASA (3 mg/kg p.o.), clopidogrel (1 mg/kg p.o.), or their combination on thrombus formation in anaesthetized rats (study B). Results are mean ± standard error of the mean (n=6). ** $p < 0.01$; *** $p < 0.001$ versus control; † $p < 0.05$ versus rivaroxaban; ‡ $p < 0.01$ versus ASA. ASA, acetylsalicylic acid; i.v., intravenous; p.o., oral.

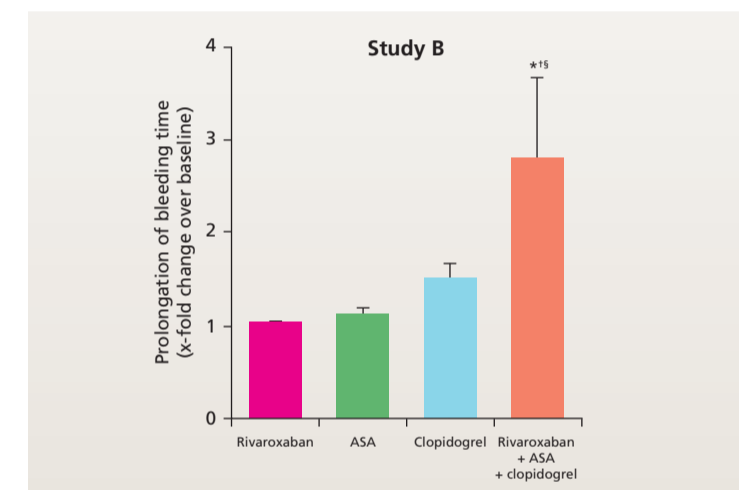


Figure 5. Prolongation of bleeding time after the administration of rivaroxaban (0.03 mg/kg i.v.), ASA (3 mg/kg p.o.), clopidogrel (1 mg/kg p.o.), or their combination in anaesthetized rats (study B). Results are mean ± standard error of the mean (n=10). * $p < 0.05$ versus control; † $p < 0.05$ versus rivaroxaban; ‡ $p < 0.05$ versus ASA. ASA, acetylsalicylic acid; i.v., intravenous; p.o., oral.

Conclusions

- These results show that low doses (sub-efficacious and moderately effective doses) of rivaroxaban co-administered with either ASA or the combination of clopidogrel and ASA may have a greater antithrombotic effect than the individual treatments, without excessive bleeding
- Bleeding time was, however, slightly increased by the addition of either a sub-efficacious (0.03 mg/kg) or a moderately effective dose (0.1 mg/kg) of rivaroxaban to the combination of ASA and clopidogrel
- If these findings translate into the clinic, the co-administration of a low dose of rivaroxaban with ASA, or clopidogrel, or their combination may have therapeutic advantages

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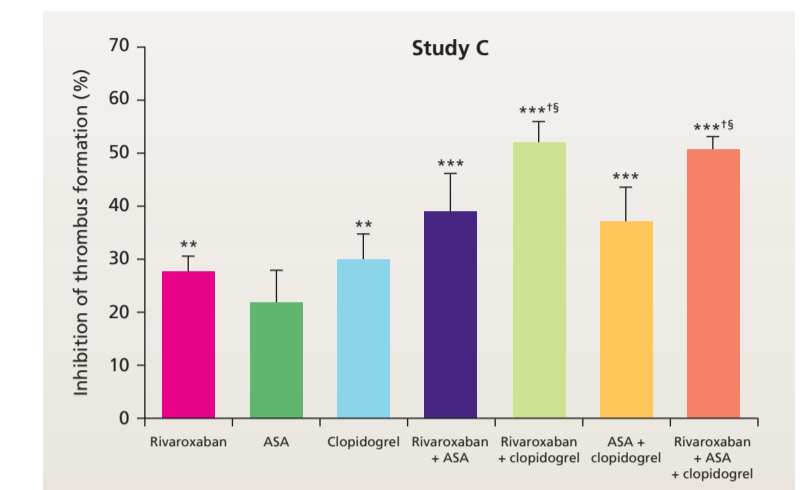


Figure 3. Effects of rivaroxaban (0.1 mg/kg i.v.), ASA (3 mg/kg p.o.), clopidogrel (1 mg/kg p.o.), or their combination on thrombus formation in anaesthetized rats (study C). Results are mean ± standard error of the mean (n=6). ** $p < 0.01$; *** $p < 0.001$ versus control; † $p < 0.05$ versus rivaroxaban; ‡ $p < 0.01$ versus ASA. ASA, acetylsalicylic acid; i.v., intravenous; p.o., oral.

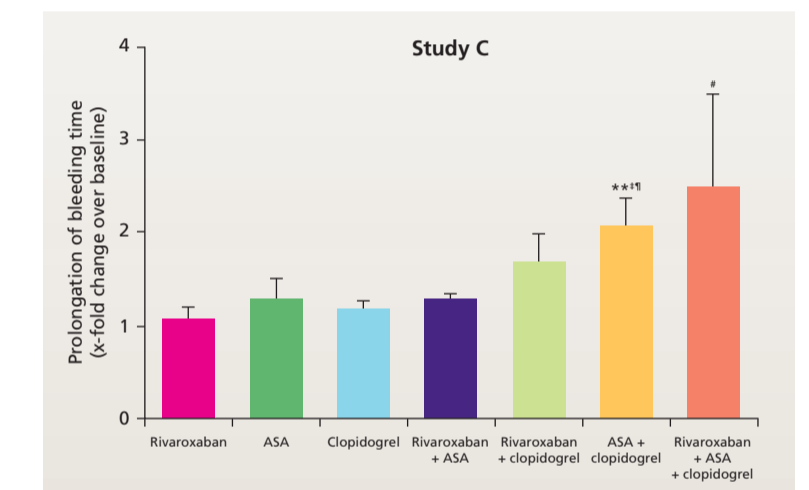


Figure 6. Prolongation of bleeding time after the administration of rivaroxaban (0.1 mg/kg i.v.), ASA (3 mg/kg p.o.), clopidogrel (1 mg/kg p.o.), or their combination in anaesthetized rats (study C). Results are mean ± standard error of the mean (n=10). ** $p < 0.01$ versus control; † $p < 0.01$ versus rivaroxaban; ‡ $p < 0.01$ versus clopidogrel; §in one animal bleeding time >1,800 seconds. ASA, acetylsalicylic acid; i.v., intravenous; p.o., oral.

Disclosure of conflict of interest

This study was supported by Bayer Schering Pharma AG and Johnson & Johnson Pharmaceutical Research & Development, L.L.C. E Perzborn, E Fischer, U Lange, and M Harwardt are shareholders of Bayer AG and employees of Bayer Schering Pharma AG. 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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