Journal of Thrombosis and Haemostasis, **7**: 1313–1320 DOI: 10.1111/j.1538-7836.2009.03503.x

ORIGINAL ARTICLE

Favorable therapeutic index of the direct factor Xa inhibitors, apixaban and rivaroxaban, compared with the thrombin inhibitor dabigatran in rabbits

P. C. WONG, E. J. CRAIN, C. A. WATSON and B. XIN
Thrombosis Research, Bristol-Myers Squibb Company, Pennington, NJ, USA

To cite this article: Wong PC, Crain EJ, Watson CA, Xin B. Favorable therapeutic index of the direct factor Xa inhibitors, apixaban and rivaroxaban, compared with the thrombin inhibitor dabigatran in rabbits. *J Thromb Haemost* 2009; **7**: 1313–20.

Summary. *Background:* Apixaban is an oral, direct factor Xa (FXa) inhibitor in late-stage clinical development. This study assessed effects of the direct FXa inhibitors, apixaban and rivaroxaban, vs. the direct thrombin inhibitor, dabigatran, on venous thrombosis (VT), bleeding time (BT) and clotting times in rabbits. *Methods:* We induced the formation of non-occlusive thrombus in VT models by placing threads in the vena cava, and induced bleeding by the incision of cuticles in anesthetized rabbits. Apixaban, rivaroxaban and dabigatran were infused IV to achieve a stable plasma level. Clotting times, including the activated partial thromboplastin time (aPTT), prothrombin time (PT), modified PT (mPT) and thrombin time (TT), were measured. *Results:* Apixaban, rivaroxaban and dabigatran exhibited dose-related efficacy in preventing VT with EC_{50} of 65, 33 and 194 nM, respectively. At doses for 80% reduction of control thrombus, apixaban, rivaroxaban and dabigatran prolonged BT by 1.13 ± 0.02-, 1.9 ± 0.1-* and 4.4 ± 0.4-fold*, respectively (*$P < 0.05$, vs. apixaban). In the treatment model, these inhibitors equally prevented growth of a pre-formed thrombus. Antithrombotic doses of apixaban and rivaroxaban prolonged aPTT and PT by < 3-fold with no effect on TT. Dabigatran was ≥50-fold more potent in prolonging TT than aPTT and PT. Of the clotting assays studied, apixaban, rivaroxaban and dabigatran responded the best to mPT. *Conclusion:* Comparable antithrombotic efficacy was observed between apixaban, rivaroxaban and dabigatran in the prevention and treatment of VT in rabbits. Apixaban and rivaroxaban exhibited lower BT compared with dabigatran at equivalent antithrombotic doses. The clinical significance of these findings remains to be determined.

Correspondence: Pancras C. Wong, Bristol-Myers Squibb Company, 311 Pennington-Rocky Hill Road, Pennington, NJ 08534, USA.
Tel.: +1 609 818 5572; fax: +1 609 818 7877.
E-mail: pancras.wong@bms.com

Presented in part at the 50th Annual Meeting of the American Society of Hematology, 6–9 December 2008, San Francisco, CA, USA (abstr 3025).

Received 2 April 2009, accepted 27 May 2009

© 2009 International Society on Thrombosis and Haemostasis

Keywords: apixaban, blood coagulation, dabigatran, direct factor Xa inhibitor, hemostasis, oral anticoagulant, rivaroxaban, thrombosis.

Introduction

Venous thromboembolism (VTE), which includes deep vein thrombosis and pulmonary embolism, is a major cause of morbidity and mortality. It has been estimated that over 900 000 recurrent, non-fatal and fatal VTE events occur in the United States alone, with approximately 300 000 deaths per year [1]. Further, it is anticipated that the burden of this disease will rise significantly in the near future as the baby boom generation ages. Although numerous classes of anticoagulants, such as heparin derivatives, vitamin K antagonists and thrombin inhibitors, are available for VTE treatment, they still have significant limitations. For instance, the oral vitamin K antagonists exhibit a narrow therapeutic index, a slow onset of therapeutic effect and many dietary and drug interactions requiring frequent monitoring and dose adjustment. The heparin derivatives and direct thrombin inhibitors require parenteral administration, which is a major obstacle for the long-term use of these agents. Therefore, novel oral anticoagulants, targeting the selective inhibition of thrombin activity or factor Xa (FXa), have been developed [2].

Apixaban is one of the promising, oral anticoagulants in late-stage clinical development. It is a reversible, direct and highly selective inhibitor of FXa, and does not require antithrombin III for antithrombotic activity [3,4]. Further, it inhibits free as well as prothrombinase-bound and clot-bound FXa activity *in vitro* [5,6]. Preclinical studies of apixaban in animal models have demonstrated considerable antithrombotic efficacy in the prevention of arterial and venous thrombosis at doses that preserved hemostasis [4,7]. In clinical trials, apixaban has shown predictable and consistent anticoagulation as well as promising safety and efficacy results for the prevention and treatment of venous thrombosis [8–10]. Favorable clinical results have also been observed with the direct thrombin inhibitor dabigatran etexilate, a prodrug (the active drug is dabigatran), [11,12] and the direct FXa inhibitor rivaroxaban [13–17].

In the absence of head-to-head clinical trials evaluating apixaban, rivaroxaban and dabigatran, it is difficult to compare and contrast their efficacy and safety profiles. Similarly, no comparative preclinical efficacy and safety studies of these inhibitors in the same experimental models and species have been reported. As head-to-head clinical trials comparing these inhibitors are costly and unlikely to be performed in the near future, it prompted us to conduct a full dose–response comparison among these compounds in experimental models of thrombosis and hemostasis. In the evaluation of their antithrombotic activity, we characterized the efficacy of apixaban, rivaroxaban and dabigatran in a rabbit model of venous thrombosis (VT) [4,18]. Specifically, we focused on both the prevention of VT and the treatment of preformed thrombus, because few studies have examined the efficacy of these inhibitors for the treatment of existing intravascular thrombus. To evaluate the antihemostatic activity of these inhibitors, we used a standardized rabbit model of cuticle bleeding time (BT) [19,20]. Finally, we also measured *ex vivo* clotting times to identify a potential coagulation biomarker assay that will best reflect the anticoagulant activity of these inhibitors.

Materials and methods

Reagents

The following drugs and chemicals were used in this study: activated partial thromboplastin time (aPTT) reagent Actin®, prothrombin time (PT) reagent Thromboplastin C Plus and thrombin time (TT) reagent from Dade-Behring (Marburg, Germany). Apixaban, rivaroxaban and dabigatran were synthesized at Bristol–Myers Squibb.

Animals

Studies were conducted in male New Zealand White rabbits weighing 2–4 kg and were obtained from Covance (Denver, PA, USA). Experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the regulations of the Animal Care and Use Committee of Bristol–Myers Squibb.

Venous thrombosis model

The rabbit VT model, described by Hollenbach *et al.* [18] with modification [4], was used in this study. Briefly, male New Zealand White rabbits were anesthetized with ketamine (50 mg kg^{-1} + 50 mg kg^{-1} h^{-1} i.m.) and xylazine (10 mg kg^{-1} + 10 mg kg^{-1} h^{-1} i.m.). The right jugular vein and left femoral artery were isolated and catheterized for IV infusion and the collection of blood samples, respectively. Silk threads attached to a copper wire were placed in the vena cava to induce the formation of a non-occlusive thrombus.

Prevention model The inhibitors or vehicle were administered IV 30 min prior to the placement of the prosthetic VT device. The test inhibitor was given as a bolus injection (1 mL kg^{-1}) and supplemented with a constant IV infusion (1 mL kg^{-1} h^{-1}) to quickly achieve an effective and stable plasma level of the compound. Ninety minutes after the placement of the threads, the abdominal vena cava was isolated and the threads with associated thrombus were removed, blotted twice on a weighing paper and weighed as described previously [4]. The thrombus weight was calculated by subtracting the average weight of eight pieces of 4–0 silk threads, 3 cm in length, from the total weight of thrombus and threads.

In the VT prevention study ($n = 6$ per group), the apixaban group consisted of vehicle (10% *N*-*N*-dimethylacetamide: 90% of 5% dextrose) and apixaban (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.006 + 0.009, 0.018 + 0.026, 0.06 + 0.09, 0.18 + 0.26 and 0.6 + 0.9. The rivaroxaban group consisted of vehicle (10% *N*-*N*-dimethylacetamide:30% polyethylene glycol 400:60% water) and rivaroxaban (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.006 + 0.009, 0.018 + 0.026, 0.06 + 0.09, 0.18 + 0.26 and 0.6 + 0.9. The dabigatran group consisted of vehicle (10% *N*-*N*-dimethylacetamide:90% of 5% dextrose) and dabigatran (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.006 + 0.009, 0.018 + 0.026, 0.06 + 0.09, 0.18 + 0.26 and 0.6 + 0.9.

Treatment model The antithrombotic activity of compounds was measured in a pre-existing environment of active thrombosis. Inhibitors or vehicle were administered 30 min after the placement of the threads to allow for significant thrombus growth prior to antithrombotic treatment. The test inhibitor was given as a bolus injection and supplemented with a constant IV infusion as described above. After the compound was infused for 2 h, the thrombus was removed from the abdominal vena cava and was weighed as described above.

In the VT treatment study, a separate group of rabbits was used in which the clot size was measured 30 min after the placement of the VT device. The apixaban group consisted of vehicle and apixaban (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.018 + 0.026, 0.06 + 0.09, 0.18 + 0.26 and 0.6 + 0.9. The rivaroxaban group consisted of vehicle and rivaroxaban (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.018 + 0.026, 0.06 + 0.09, 0.18 + 0.26 and 0.6 + 0.9. The dabigatran group consisted of vehicle and dabigatran (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.006 + 0.009, 0.018 + 0.026, 0.06 + 0.09, 0.18 + 0.26 and 0.6 + 0.9. The same vehicles were used as described above and the *n* is 6 per group.

Cuticle bleeding time model

The rabbit cuticle BT model used in this study has been described previously [19,20]. Briefly, rabbits were anesthetized and cut at the apex of the hindpaw cuticle with a razor blade. Blood was allowed to flow freely by keeping the bleeding site in contact with 37 °C lactated Ringer's solution. BT was defined as the time after transection when bleeding ceased, and was measured by averaging the bleeding time of three nail cuticles.

© 2009 International Society on Thrombosis and Haemostasis

The maximum bleeding recorded was 20 min. Bleeding durations that exceeded 20 min were recorded as a BT of 20 min. Inhibitors or vehicle were administered IV 30 min prior to cuticle transection. BT was measured before and after treatment in opposite hindlimbs, and was expressed as a ratio of treated over the control value.

The apixaban group consisted of vehicle and apixaban (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.18 + 0.26 and 0.6 + 0.9. The rivaroxaban group consisted of vehicle and rivaroxaban (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.06 + 0.09, 0.18 + 0.26 and 0.6 + 0.9. The dabigatran group consisted of vehicle and dabigatran (mg kg^{-1} + mg kg^{-1} h^{-1}) at 0.06 + 0.09, 0.18 + 0.26 and 0.6 + 0.9. The same vehicles were used as described above and the *n* is 6 per group.

Clotting assays and plasma concentrations

Ex vivo clotting times and plasma concentration were determined using blood samples collected from the femoral artery in rabbits from the VT studies. Briefly, blood was collected in tubes containing 1/10 volume of 3.8% sodium citrate, and platelet-poor plasma was obtained after centrifuging at $>2000 \times g$ for 10 min. Clotting times in platelet-poor plasma were measured with an automated coagulation analyzer (Sysmex®, Dade Behring Inc., Deerfield, IL,USA) as described previously [4]. aPTT, PT and TT reagents were reconstituted and assays were performed according to the manufacturer's instructions. The modified PT (mPT) assay was performed by diluting 1 mL of thromboplastin C Plus with 1.25 mL of 100 mmol l^{-1} calcium chloride and using this diluted reagent in place of the normal PT reagent [4]. Data points were the mean of duplicate measurements and were expressed as a ratio of treated vs. baseline control from the same animal. The concentrations required to prolong clotting time by 2-fold (EC_{2X}) were expressed as total plasma concentrations, not final assay concentrations after addition of clotting assay reagents. Concentrations of apixaban, rivaroxaban and dabigatran in plasma samples were determined by liquid chromatography-tandem mass spectrometry. As there was no difference among the VT groups in plasma drug levels or clotting times, the data from these studies were combined for analysis. The plasma concentrations from the VT studies were also used as approximation of the exposure levels for the BT studies.

Statistical analysis

The statistical analyses used were analysis of variance and Student–Newman–Keuls test using the SAS system (SAS for Windows release 8.02A, Cary, NC, USA). EC_{50} values were determined using the four-parameter logistic equation, $y = A + [(B - A)/(1 + (C/x)^D)]$, where A = minimum y value, B = maximum y value, $C = EC_{50}$, x = dose or concentration and D = Hill slope, and the logistic fit was analyzed by PRISM-4 for Windows (GraphPad Software, San Diego, CA, USA). Antithrombotic EC_{50} values were determined using a maximum value of 100 and a minimum value of 0, whereas BT EC_{50} values were determined using a maximum value (BT treated/BT control) of 7 and a minimum value of 1. A value of $P < 0.05$ was considered statistically significant. All data are means ± standard error (SE).

Results

Prevention model of venous thrombosis

Mean thrombus weights in the vehicle-treated VT rabbits from the apixaban, rivaroxaban and dabigatran groups were 78 ± 6, 65 ± 3 and 70 ± 3 mg, respectively ($n = 6$ per group). In this model, apixaban, rivaroxaban and dabigatran produced dose-dependent antithrombotic effects (Fig. 1). At the doses studied, apixaban, rivaroxaban and dabigatran produced dose-proportional increases in plasma concentrations, and their estimated antithrombotic EC_{50}s were 65, 33 and 194 nM, respectively (Fig. 1). These inhibitors, at their highest dose, decreased thrombus formation similarly by about 80%, relative to their corresponding vehicle group (Fig. 1).

Cuticle bleeding time model

Mean cuticle BT in the vehicle-treated rabbits from the apixaban, rivaroxaban and dabigatran groups was 173 ± 8, 179 ± 9 and 168 ± 8 s, respectively ($n = 6$ per group). Dabigatran produced dose-dependent increases in BT with estimated EC_{50} of 1079 nM (Fig. 1). In contrast, apixaban and rivaroxaban at antithrombotic doses increased BT slightly with EC_{50} of >1146 and >360 nM, respectively (Fig. 1). The highest administered dose of apixaban, rivaroxaban and dabigatran resulted in approximately an 80% reduction of control in thrombus weight, and prolonged BT by 1.13 ± 0.02-, 1.9 ± 0.1-* and 4.4 ± 0.4-fold*, respectively (*$P < 0.05$, vs. apixaban, $n = 6$ per group, Fig. 2). As shown in Fig. 2, the antithrombotic efficacy-to-bleeding profile of dabigatran was less favorable compared with those of apixaban and rivaroxaban in the models of prevention VT and BT.

Treatment model of venous thrombosis

The mean preformed thrombus weight was 38 ± 2 mg ($n = 6$, Fig. 3). Mean thrombus weights formed from 30 to 150 min in the vehicle-treated VT rabbits from the apixaban, rivaroxaban and dabigatran groups were 90 ± 5, 73 ± 4 and 76 ± 5 mg, respectively ($n = 6$ per group). In this model, apixaban, rivaroxaban and dabigatran were equally effective in preventing growth of a preformed thrombus and produced dose-dependent antithrombotic effects with estimated EC_{50} of 316, 84 and 124 nM, respectively (Fig. 3). Clot regression, as judged by the thrombus weight below the preformed thrombus weight, was observed following administration of apixaban, rivaroxaban and dabigatran at top doses (Fig. 3). As shown in Fig. 3 (bottom panel), the antithrombotic efficacy-to-bleeding profile of dabigatran was less favorable compared with those of

© 2009 International Society on Thrombosis and Haemostasis

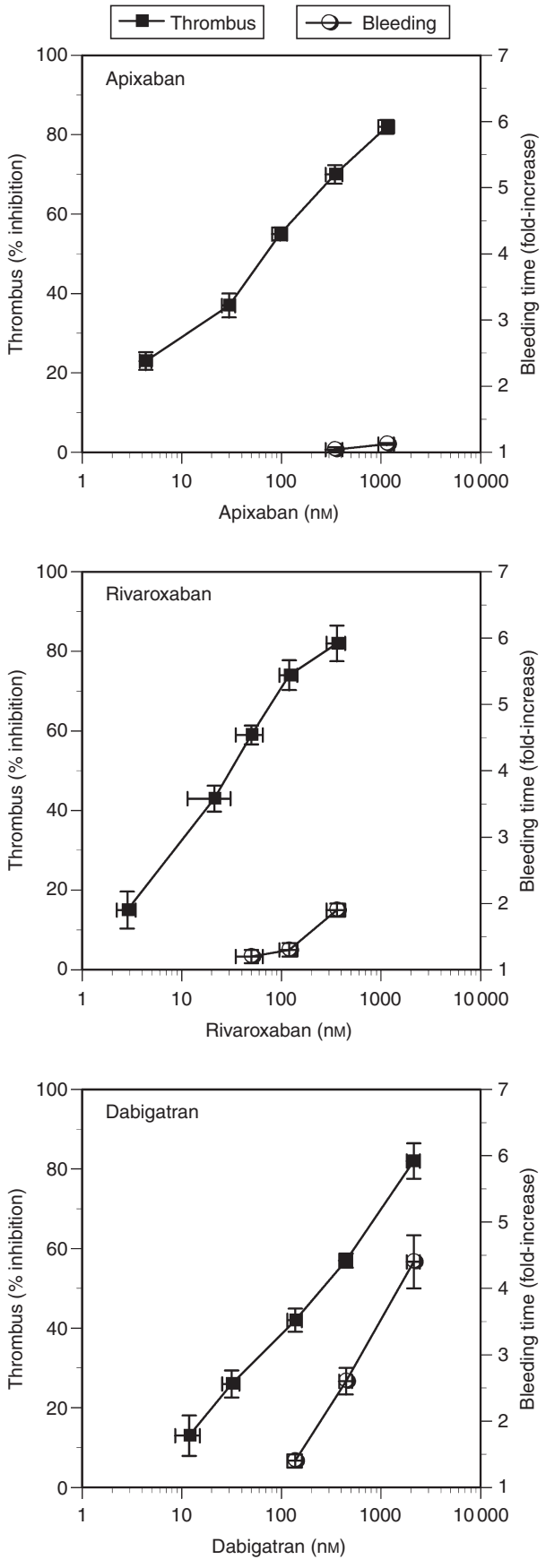

Fig. 1. Plots of thrombus reduction and bleeding time vs. plasma concentration in apixaban-, rivaroxaban- and dabigatran-treated rabbits. Thrombus reduction, measured in the prevention model of venous thrombosis, was expressed as a percentage reduction of treated relative to the mean vehicle thrombus weight. Bleeding time effect was expressed as a ratio of treated vs. the mean vehicle value. Data are mean ± SE (n = 6 per group for the thrombosis and bleeding time studies and n = 12 per dose for plasma concentrations).

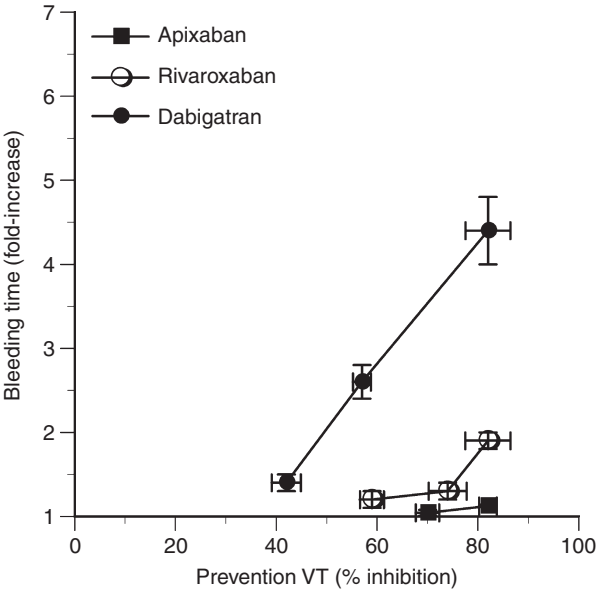

Fig. 2. Plot of bleeding time vs. the corresponding thrombus reduction in apixaban-, rivaroxaban- and dabigatran-treated rabbits. Thrombus reduction, measured in the prevention model of venous thrombosis (VT), was expressed as a percentage reduction of treated relative to the mean vehicle thrombus weight. Bleeding time effect was expressed as a ratio of treated vs. the mean vehicle value. Data are mean ± SE (n = 6 per group).

apixaban and rivaroxaban in rabbit models of treatment VT and BT.

Ex vivo *coagulation markers*

The control values for coagulation markers were the following: 21 ± 1 s for aPTT, 9 ± 0.1 s for PT, 19 ± 0.4 s for mPT and 23 ± 1 s for TT. As shown in Fig. 4, antithrombotic doses of apixaban and rivaroxaban did not prolong the TT. TT was more sensitive to dabigatran being increased by 1.7 ± 0.2-fold at a non-antithrombotic dose. Apixaban and rivaroxaban did not prolong aPTT or PT at approximately 50% of their antithrombotic dose. The aPTT, but not PT, was moderately sensitive to dabigatran, with a 2.2 ± 0.2-fold increase at 50% of the antithrombotic dose. The mPT was the most sensitive of the clotting assays to apixaban, rivaroxaban and dabigatran, with respective increases of 1.6 ± 0.1-, 3.3 ± 0.5- and 3.8 ± 0.5-fold at 50% antithrombotic doses, and 5.5 ± 0.4-, 5.4 ± 0.1- and 6.8 ± 0.4-fold at 80% antithrombotic doses. EC_{2X} (nM) of *ex vivo* aPTT, PT, mPT and TT was >1146,

© 2009 International Society on Thrombosis and Haemostasis

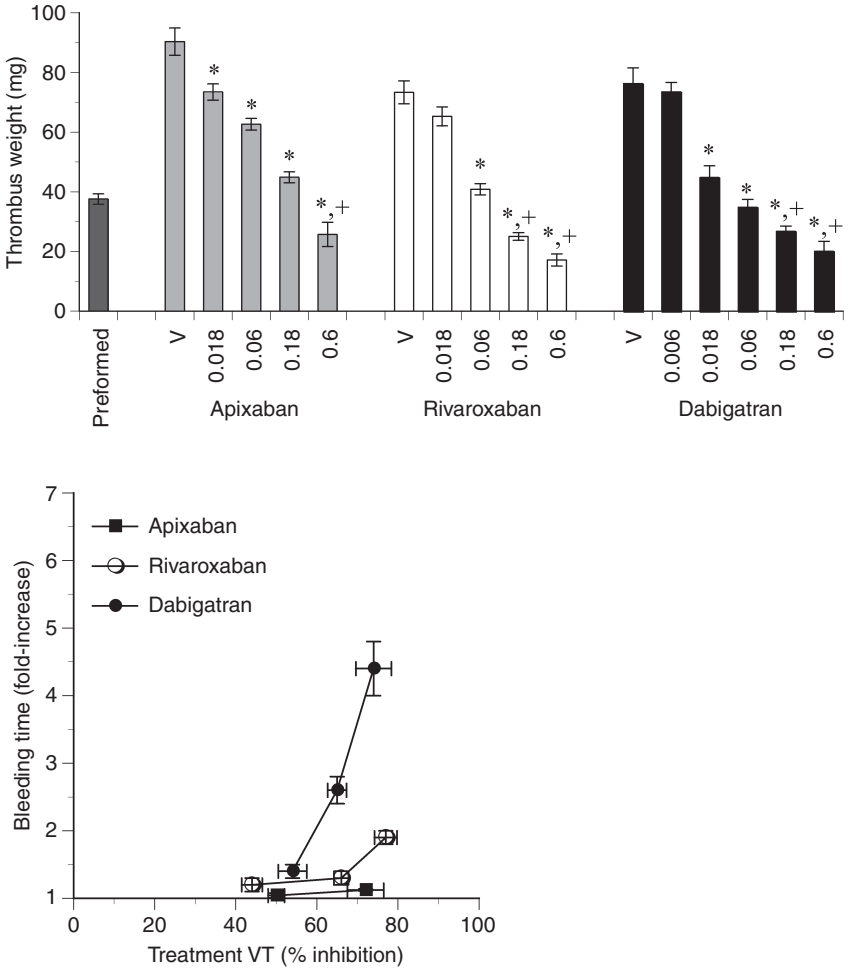

Fig. 3. Top panel plots effects of apixaban, rivaroxaban and dabigatran on thrombus formation in the treatment model of venous thrombosis (VT) in rabbits. For clarity, only the bolus doses (mg/kg) are indicated on the *x*-axis. $^{*}P < 0.05$ vs. the corresponding vehicle (V). $^{+}P < 0.05$ vs. the preformed thrombus. Bottom panel plots bleeding time vs. the corresponding thrombus reduction in apixaban-, rivaroxaban- and dabigatran-treated rabbits. Thrombus reduction, measured in the treatment model of VT, was expressed as a percentage reduction of treated relative to the mean vehicle thrombus weight. Bleeding time effect was expressed as a ratio of treated vs. the mean vehicle value. Data are mean ± SE ($n = 6$ per group).

> 1146, 142 and > 1146 for apixaban, 155, 360, 10 and > 360 for rivaroxaban, and 580, 1500, 57 and 12 for dabigatran, respectively.

Discussion

This is the first comparative dose–response study with the direct FXa inhibitors, apixaban and rivaroxaban, vs. the direct thrombin inhibitor, dabigatran, in rabbit models of VT and hemostasis. Comparable antithrombotic efficacy was observed between apixaban, rivaroxaban and dabigatran in the prevention and treatment of VT. Apixaban and rivaroxaban exhibited lower BT compared with dabigatran at equivalent antithrombotic doses.

Rabbits were used as the animal model because these inhibitors have similar anti-FXa potency (apixaban and rivaroxaban) or anticoagulant potency (dabigatran) in humans and rabbits *in vitro* [4,21,22]. The rabbit VT model was utilized to evaluate antithrombotic effects as this model has frequently been used to characterize the antithrombotic profiles of anticoagulant drugs [4,18,23]. Furthermore, a thrombus formed in this model consists of mainly fibrin and trapped red cells, which mimics clinical venous thrombosis [18]. The rabbit model of cuticle bleeding was utilized to assess BT because this model has been well characterized in previous studies with antiplatelet and anticoagulant drugs [4,7,18–20,23].

This study confirms previous results, which demonstrated the efficacy of apixaban for the prevention of VT in rabbits [4]. This study also adds a new finding by showing that apixaban effectively inhibited the growth of a preformed intravascular thrombus in rabbits, suggesting the potential opportunities for apixaban in the treatment of VT. Together, these preclinical findings support the rationale for evaluating apixaban in the clinic for the prevention and treatment of VT. Indeed initial data from phase II trials with apixaban show promising safety and efficacy results for the prevention and treatment of VT [9,10]. Similar antithrombotic results for the prevention and treatment of VT were shown with

© 2009 International Society on Thrombosis and Haemostasis

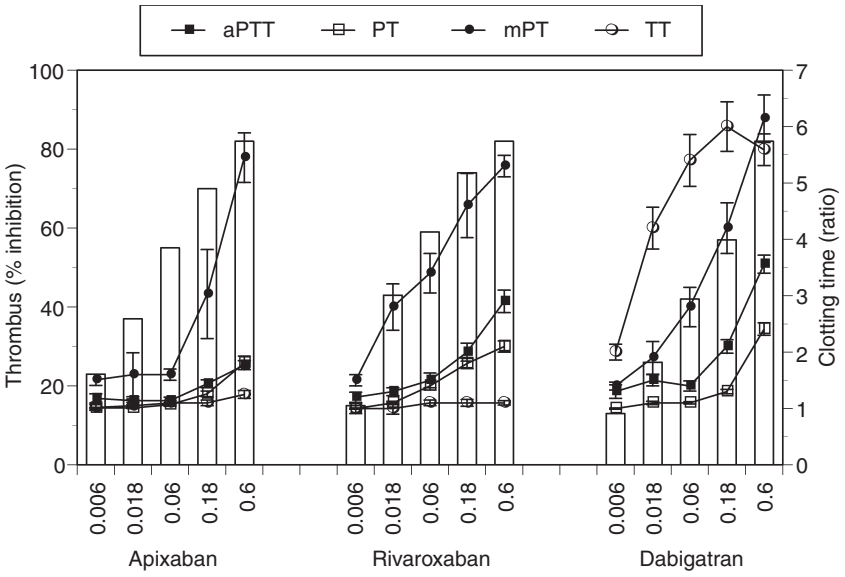

Fig. 4. Plots of thrombus reduction (bar graph) and *ex vivo* clotting times (line graph) in apixaban-, rivaroxaban- and dabigatran-treated rabbits. Thrombus reduction, measured in the prevention model of venous thrombosis, was expressed as a percentage reduction of treated relative to the mean vehicle thrombus weight (data from Fig. 1). For clarity, only the data for the mean thrombus reduction, and the bolus dose (mg/kg) on the x-axis are shown. Activated partial thromboplastin time (aPTT), prothrombin time (PT), modified prothrombin time (mPT) and thrombin time (TT) were expressed as a ratio of treated vs. the control value. Data are mean ± SE (n = 6 per group for thrombus reduction and n = 12 per group for clotting times).

rivaroxaban in this study as well as in other preclinical and clinical studies [13–17,21,24]. Regarding dabigatran, only positive preclinical and clinical findings for the prevention of VT, but not the treatment of VT, have been reported [11,12,22,25]. This study is the first report describing the effectiveness of dabigatran for the treatment of VT in rabbits. In the treatment VT model, we observed clot regression following administration of apixaban, rivaroxaban and dabigatran at higher doses. The reduction of the clot size below the preformed clot weight at these high doses may be related to the lysis of the clot by the endogenous fibrinolytic system and/or a destabilization of the clot matrix.

The antithrombotic plasma levels of rivaroxaban and dabigatran observed in the prevention VT model in rabbits are within the clinical effective plasma levels reported by these drugs [26–28], whereas no such information has yet been reported for apixaban. These results suggest that this study employed clinically relevant doses of these inhibitors. The antithrombotic (EC_{50}) dose of apixaban and rivaroxaban in rabbits appeared to be 3- to 5-fold higher in the treatment than in the prevention VT model, whereas dabigatran appeared to be similar in both models. These results are not unexpected, because higher doses of apixaban and rivaroxaban have been selected in clinical trials for the treatment than for the prevention of VT, whereas similar doses of dabigatran have been chosen for these VT indications [29–34]. Moreover, the recommended dose of the marketed indirect FXa inhibitor fondaparinux is also higher for the treatment than for the prevention of VT in patients [35].

This study compared the bleeding potential of apixaban, rivaroxaban and dabigatran. Similar to our previous study [4], apixaban achieved antithrombotic efficacy with a minimum effect on BT in rabbits. This study extended the previous finding and showed that at doses for 80% reduction of control thrombus in the prevention VT model, apixaban and rivaroxaban increased BT by 1.1- and 1.9-fold, respectively, whereas dabigatran increased BT by 4.4-fold, which was still less than a 6.2-fold increase in BT by warfarin at an equivalent antithrombotic dose [4]. Together these results suggest that apixaban and rivaroxaban have a favorable efficacy/bleeding profile relative to dabigatran in rabbits. Similarly, favorable efficacy/bleeding profiles for apixaban and rivaroxaban were also observed in the treatment VT model. It is not clear why dabigatran has a higher effect on bleeding time than apixaban and rivaroxaban. A possible explanation is that FXa inhibitors, but not dabigatran, might not affect the existing level of thrombin. In addition, reversible FXa inhibitors might not completely suppress the production of thrombin. These small amounts of thrombin might be sufficient to activate high affinity platelet thrombin receptors to maintain hemostasis. Although several studies have shown that FXa inhibitors produce less increases in bleeding time than thrombin inhibitors in animals [20,23], the results of this study are still of interest because it compares the efficacy and safety of three drugs currently in phase III clinical trials.

We noted that apixaban increased cuticle BT, and this effect was statistically less than obtained with rivaroxaban. However, extrapolation of these rabbit BT results to humans requires caution. The cuticle BT may not apply to other vascular beds, because the regulation of hemostasis is known to be tissue specific [36]. Moreover, provoked BT measured in anesthetized healthy animals may not directly translate into spontaneous

© 2009 International Society on Thrombosis and Haemostasis

bleeding observed in elderly patients where additional complications of cardiovascular disease and polypharmacy are present. Indeed, clinical studies with apixaban and rivaroxaban have shown increased bleeding with increased dose [9,37]. Nevertheless, preclinical cuticle BT studies may still be useful in generating hypotheses for clinical investigation by ranking the antihemostatic profiles of test compounds to established agents such as warfarin [4].

The traditional laboratory tests for adjusting anticoagulant doses of heparin (aPTT) and warfarin (PT) have lacked sensitivity for more selective anticoagulants [38]. Indeed we noted that both aPTT and PT assays were insufficiently calibrated to monitor the antithrombotic activity of apixaban in the rabbit VT model, despite the sub-nanomolar affinity of apixaban for FXa [3,4]. Plasma protein binding and the excessive amounts of aPTT and PT reagents used in the standard clotting assays may contribute to this insensitivity to apixaban. Rivaroxaban was in comparison more potent than apixaban in prolonging both the aPTT and PT in rabbits. Mueck *et al*. [28] reported a strong correlation between rivaroxaban plasma concentration and PT in patients after hip-replacement surgery. It is currently unclear whether the more pronounced effect of rivaroxaban on the PT is compound specific or is related to the greater disruption of hemostasis observed with rivaroxaban at antithrombotic doses in rabbits. Clearly, the sensitivity range (EC_{2X}) of these assays in rabbits was above antithrombotic levels (EC_{50}) for both apixaban and rivaroxaban, demonstrating a limited utility for monitoring antithrombotic therapy.

With regard to dabigatran, the aPTT and PT were moderately sensitive and the TT overly sensitive to antithrombotic activity in the rabbit VT model. Wienen *et al*. [22] reported that the aPTT correlated well with the antithrombotic activity of dabigatran in a rabbit model of VT consisting of polidocanol-induced endothelial damage with blood flow reduction. We also found the aPTT more sensitive than PT for monitoring high level antithrombotic activity of dabigatran in rabbits. Of the assays studied, the mPT was the most sensitive for apixaban, rivaroxaban and dabigatran, and it also tracked well with the antithrombotic activity of each compound in rabbits. Although the mPT may have the potential to monitor both FXa and thrombin inhibitors, it may not be optimal. Preliminary data from a phase II study with apixaban show that the anti-FXa assay is more accurate and precise than the mPT [39].

In conclusion, apixaban was as efficacious as rivaroxaban and dabigatran in the prevention and treatment of VT in rabbits. At equivalent antithrombotic doses, apixaban and rivaroxaban preserved hemostasis better than dabigatran, suggesting a wider therapeutic index for FXa inhibition compared with direct thrombin inhibition in this model. However, caution should be applied in extrapolating these findings to humans. As the preclinical findings on the therapeutic windows of these agents still remain a hypothesis, head-to-head clinical studies are required to validate these results.

Acknowledgements

We thank J. Baumann for assistance with the animal studies, H. Ward for her editorial assistance, and W. Schumacher and D. Seiffert for providing comments on the manuscript.

Disclosure of Conflict of Interests

The authors are employees of Bristol-Myers Squibb.

References

1 Heit JA. The epidemiology of venous thromboembolism in the community. *Arterioscler Thromb Vasc Biol* 2008; **28**: 370–2.
2 Gross PL, Weitz JI. New anticoagulants for treatment of venous thromboembolism. *Arterioscler Thromb Vasc Biol* 2008; **28**: 380–6.
3 Pinto DJ, Orwat MJ, Koch S, Rossi KA, Alexander RS, Smallwood A, Wong PC, Rendina AR, Luettgen JM, Knabb RM, He K, Xin B, Wexler RR, Lam PY. Discovery of 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro- 1H-pyrazolo[3,4-c]pyridine-3-carboxamide (apixaban, BMS-562247), a highly potent, selective, efficacious, and orally bioavailable inhibitor of blood coagulation factor Xa. *J Med Chem* 2007; **50**: 5339–56.
4 Wong PC, Crain EJ, Xin B, Wexler RR, Lam PY, Pinto DJ, Luettgen JM, Knabb RM. Apixaban, an oral, direct and highly selective factor Xa inhibitor: in vitro, antithrombotic and antihemostatic studies. *J Thromb Haemost* 2008; **6**: 820–9.
5 Luettgen JM, Wang Z, Seiffert DA, Rendina AR, Knabb RM, Ogletree ML. Inhibition of measured thrombin generation in human plasma by apixaban: a predictive mathematical model based on experimentally determined rate constants. *J Thromb Haemost* 2007; **5** (Suppl. 2): PT633.
6 Jiang X, Crain EJ, Luettgen JM, Schumacher WA, Wong PC. Apixaban, an oral direct factor Xa inhibitor, inhibits human clot-bound factor Xa activity in vitro. *Thromb Haemost* 2009; **101**: 780–2.
7 Wong PC, Watson CA, Crain EJ. Arterial antithrombotic and bleeding time effects of apixaban, a direct factor Xa inhibitor, in combination with antiplatelet therapy in rabbits. *J Thromb Haemost* 2008; **6**: 1736–41.
8 Frost C, Yu Z, Moore K, Nepal S, Barrett Y, Mosqueda-Garcia R, Shenker A. Apixaban, an oral direct factor Xa inhibitor: multiple-dose safety, pharmacokinetics, and pharmacodynamics in healthy subjects. *J Thromb Haemost* 2007; **5** (Suppl. 2): PM664.
9 Lassen MR, Davidson BL, Gallus A, Pineo G, Ansell J, Deitchman D. The efficacy and safety of apixaban, an oral, direct factor Xa inhibitor, as thromboprophylaxis in patients following total knee replacement. *J Thromb Haemost* 2007; **5**: 2368–75.
10 Botticelli Investigators, Writing Committee, Büller H, Deitchman D, Prins M, Segers A. Efficacy and safety of the oral direct factor Xa inhibitor apixaban for symptomatic deep vein thrombosis. The Botticelli DVT dose-ranging study. *J Thromb Haemost* 2008; **6**: 1313–8.
11 Eriksson BI, Dahl OE, Rosencher N, Kurth AA, van Dijk CN, Frostick SP, Prins MH, Hettiarachchi R, Hantel S, Schnee J, Büller HR; RE-NOVATE Study Group. Dabigatran etexilate versus enoxaparin for prevention of venous thromboembolism after total hip replacement: a randomised, double-blind, non-inferiority trial. *Lancet* 2007; **370**: 949–56.
12 Eriksson BI, Dahl OE, Rosencher N, Kurth AA, van Dijk CN, Frostick SP, Kälebo P, Christiansen AV, Hantel S, Hettiarachchi R, Schnee J, Büller HR; RE-MODEL Study Group. Oral dabigatran etexilate vs. subcutaneous enoxaparin for the prevention of venous thromboembolism after total knee replacement: the RE-MODEL randomized trial. *J Thromb Haemost* 2007; **5**: 2178–85.

© 2009 International Society on Thrombosis and Haemostasis

13 Agnelli G, Gallus A, Goldhaber SZ, Haas S, Huisman MV, Hull RD, Kakkar AK, Misselwitz F, Schellong S; ODIXa-DVT Study Investigators. Treatment of proximal deep-vein thrombosis with the oral direct factor Xa inhibitor rivaroxaban (BAY 59-7939): the ODIXa-DVT (Oral Direct Factor Xa Inhibitor BAY 59-7939 in Patients With Acute Symptomatic Deep-Vein Thrombosis) study. *Circulation* 2007; **116**: 180–7.

14 Büller HR, Lensing AW, Prins MH, Agnelli G, Cohen A, Gallus AS, Misselwitz F, Raskob G, Schellong S, Segers A; Einstein-DVT Dose-Ranging Study investigators. A dose-ranging study evaluating once-daily oral administration of the factor Xa inhibitor rivaroxaban in the treatment of patients with acute symptomatic deep vein thrombosis: the Einstein-DVT Dose-Ranging Study. *Blood* 2008; **112**: 2242–7.

15 Eriksson BI, Borris LC, Friedman RJ, Haas S, Huisman MV, Kakkar AK, Bandel TJ, Beckmann H, Muehlhofer E, Misselwitz F, Geerts W; RECORD1 Study Group. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. *N Engl J Med* 2008; **358**: 2765–75.

16 Kakkar AK, Brenner B, Dahl OE, Eriksson BI, Mouret P, Muntz J, Soglian AG, Pap AF, Misselwitz F, Haas S; RECORD2 Investigators. Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty: a double-blind, randomised controlled trial. *Lancet* 2008; **372**: 31–9.

17 Lassen MR, Ageno W, Borris LC, Lieberman JR, Rosencher N, Bandel TJ, Misselwitz F, Turpie AG; RECORD3 Investigators. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. *N Engl J Med* 2008; **358**: 2776–86.

18 Hollenbach S, Sinha U, Lin PH, Needham K, Frey L, Hancock T, Wong A, Wolf D. A comparative study of prothrombinase and thrombin inhibitors in a novel rabbit model of non-occlusive deep vein thrombosis. *Thromb Haemost* 1994; **71**: 357–62.

19 Himber J, Kirchhofer D, Riederer M, Tschopp TB, Steiner B, Roux SP. Dissociation of antithrombotic effect and bleeding time prolongation in rabbits by inhibiting tissue factor function. *Thromb Haemost* 1997; **78**: 1142–9.

20 Wong PC, Crain EJ, Watson CA, Zaspel AM, Wright MR, Lam PY, Pinto DJP, Wexler RR, Knabb RM. Nonpeptide factor Xa inhibitors III: effects of DPC423, an orally-active pyrazole antithrombotic agent, on arterial thrombosis in rabbits. *J Pharmacol Exp Ther* 2002; **303**: 993–1000.

21 Perzborn E, Strassburger J, Wilmen A, Pohlmann J, Roehrig S, Schlemmer KH, Straub A. *In vitro* and *in vivo* studies of the novel antithrombotic agent BAY 59-7939–an oral, direct factor Xa inhibitor. *J Thromb Haemost* 2005; **3**: 514–21.

22 Wienen W, Stassen JM, Priepke H, Ries UJ, Hauel N. Antithrombotic and anticoagulant effects of the direct thrombin inhibitor dabigatran, and its oral prodrug, dabigatran etexilate, in a rabbit model of venous thrombosis. *J Thromb Haemost* 2007; **5**: 1237–42.

23 Sinha U, Ku P, Malinowski J, Zhu BY, Scarborough RM, Marlowe CK, Wong PW, Lin PH, Hollenbach SJ. Antithrombotic and hemostatic capacity of factor Xa versus thrombin inhibitors in models of venous and arteriovenous thrombosis. *Eur J Pharmacol* 2000; **395**: 51–9.

24 Biemond BJ, Perzborn E, Friederich PW, Levi M, Buetehorn U, Büller HR. Prevention and treatment of experimental thrombosis in rabbits with rivaroxaban (BAY 597939)–an oral, direct factor Xa inhibitor. *Thromb Haemost* 2007; **97**: 471–7.

25 Wienen W, Stassen JM, Priepke H, Ries UJ, Hauel N. Effects of the direct thrombin inhibitor dabigatran and its orally active prodrug, dabigatran etexilate, on thrombus formation and bleeding time in rats. *Thromb Haemost* 2007; **98**: 333–8.

26 Eriksson BI, Quinlan DJ, Weitz JI. Comparative pharmacodynamics and pharmacokinetics of oral direct thrombin and factor Xa inhibitors in development. *Clin Pharmacokinet* 2009; **48**: 1–22.

27 Stangier J. Clinical pharmacokinetics and pharmacodynamics of the oral direct thrombin inhibitor dabigatran etexilate. *Clin Pharmacokinet* 2008; **47**: 285–95.

28 Mueck W, Borris LC, Dahl OE, Haas S, Huisman MV, Kakkar AK, Kälebo P, Muelhofer E, Misselwitz F, Eriksson BI. Population pharmacokinetics and pharmacodynamics of once- and twice-daily rivaroxaban for the prevention of venous thromboembolism in patients undergoing total hip replacement. *Thromb Haemost* 2008; **100**: 453–61.

29 ClinicalTrials.gov. Efficacy and safety study of apixaban for the treatment of deep vein thrombosis or pulmonary embolism. Identifier NCT00643201 [online]. http://www.clinicaltrials.gov; Accessed 15 June 2009.

30 ClinicalTrials.gov. Study of apixaban for the prevention of thrombosis-related events following knee replacement surgery. Identifier NCT00371683 [online]. http://www.clinicaltrials.gov; Accessed 15 June 2009.

31 ClinicalTrials.gov. Regulation of coagulation in orthopedic surgery to prevent deep vein thromboembolism DVT and pulmonary embolism (PE); a study of BAY 59-7939 in the prevention of VTE in subjects undergoing elective total knee replacement (RECORD 4). Identifier NCT00362232 [online]. http://www.clinicaltrials.gov; Accessed 15 June 2009.

32 ClinicalTrials.gov. Oral direct factor Xa inhibitor rivaroxaban in patients with acute symptomatic pulmonary embolism with or without symptomatic deep-vein thrombosis: Einstein-PE evaluation. Identifier NCT00439777 [online]. http://www.clinicaltrials.gov; Accessed 15 June 2009.

33 ClinicalTrials.gov. Phase III study testing efficacy & saftety of oral dabigatran etexilate vs warfarin for 6 m treatment for acute symp VTE. Identifier NCT00680186 [online]. http://www.clinicaltrials.gov; Accessed 15 June 2009.

34 ClinicalTrials.gov. RE-MODEL dabigatran etexilate 150mg or 220mg o.d vs. enoxaparin 40mg o.d for prevention of thrombosis after knee surgery. Identifier NCT00168805 [online]. http://www.clinicaltrials.gov; Accessed 15 June 2009.

35 Prescribing information on Arixtra® [online]. http://www.accessdata.fda.gov/drugsatfda_docs/label/2005/021345s010lbl.pdf; Accessed 15 June 2009.

36 Mackman N. Tissue-specific hemostasis in mice. *Arterioscler Thromb Vasc Biol* 2005; **25**: 2273–81.

37 Fisher WD, Eriksson BI, Bauer KA, Borris L, Dahl OE, Gent M, Haas S, Homering M, Huisman MV, Kakkar AK, Kälebo P, Kwong LM, Misselwitz F, Turpie AG. Rivaroxaban for thromboprophylaxis after orthopaedic surgery: pooled analysis of two studies. *Thromb Haemost* 2007; **97**: 931–7.

38 Walenga JM, Hoppensteadt DA. Monitoring the new antithrombotic drugs. *Semin Thromb Hemost* 2004; **30**: 683–95.

39 Barrett YC, Wang J, Yu Z, Shenker A, Knabb R, Mohan P. Apixaban treatment decreases coagulation activity in patients with acute deep-vein thrombosis. *Blood* (ASH Annual Meeting Abstracts) 2008; **112**: Abstract 1982.

© 2009 International Society on Thrombosis and Haemostasis