Effect of non-specific reversal agents on anticoagulant activity of dabigatran and rivaroxaban

A randomised crossover ex vivo study in healthy volunteers

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Summary
The new anticoagulants dabigatran and rivaroxaban can be responsible for haemorrhagic complications. As for any anticoagulant, bleeding management is challenging. We aimed to test the effect of all putative haemostatic agents on the anticoagulant activity of these new drugs using thrombin generation tests. In an ex vivo study, 10 healthy white male subjects were randomised to receive rivaroxaban (20 mg) or dabigatran (150 mg) in one oral administration. After a two weeks washout period, they received the other anticoagulant. Venous blood samples were collected just before drug administration (H0) and 2 hours thereafter. Reversal of anticoagulation was tested in vitro using prothrombin complex concentrate (PCC), rFVIIa or FEIBA at various concentrations. Rivaroxaban affects quantitative and kinetic parameters, including the endogenous thrombin potential (ETP-AUC and more pronoucedly the thrombin peak), the lag-time and time to peak. PCC strongly corrected ETP-AUC, whereas rFVIIa only modified the kinetic parameters. FEIBA corrected all parameters. Dabigatran specially affects the kinetics of thrombin generation with prolonged lag-time and time to peak. Although PCC increased ETP-AUC, only rFVIIa and FEIBA corrected the altered lag-time. For both anticoagulants, lower doses of FEIBA, corresponding to a quarter to half the dose usually used, have potential reversal profile of interest. In conclusion, some non-specific reversal agents appear to be able to reverse the anticoagulant activity of rivaroxaban or dabigatran. However, clinical evaluation is needed regarding haemorrhagic situations, and a meticulous risk-benefit evaluation regarding their use in this context is required.

Keywords
Anticoagulants, dabigatran, rivaroxaban, reversal, thrombin generation test

Introduction
New oral antithrombotic drugs that target either thrombin (dabigatran, Boehringer Ingelheim, Ingelheim, Germany) or factor (F)Xa (rivaroxaban, Bayer, Leverkusen, Germany) are under clinical development. Clinical phase III trials have been conducted with both drugs which were approved for the treatment of venous thromboembolic disease (VTE), and also for the prevention of systemic embolism in non-valvular atrial fibrillation (NVAF). Besides their efficacy, both drugs were responsible for haemorrhagic complications. In the treatment of NVAF, the yearly rate of major bleeding was 3.11% in the group receiving 150 mg of dabigatran twice daily, similar to the rate of major bleeding of 3.36% in the warfarin group (1). Likewise, major bleeding was recently reported in 5.6% of NVAF patients treated with rivaroxaban, again similar to the warfarin group, although intracranial haemorrhages were significantly reduced by factor two (2).

As promising drugs, it is expected that these treatments will be widely used in long-term indications such as AF. However, as for any anticoagulant, the management of any bleeding event will be challenging. Several haemostatic agents have been proposed to reverse the anticoagulant activity of these new drugs, although only spurious clinical data, based on some published case reports, are available (3–5). Therefore, our aim was to test the ability of all putative non-specific haemostatic agents currently available, at different dosages, to reverse the anticoagulant activity of both dabigatran and rivaroxaban, using thrombin generation tests (TGT) (6) in an ex vivo study in healthy volunteers.

Material and methods
Subject selection
Ten healthy white male subjects (age 18–45 years), with a body mass index between 18 and 30 kg/m2 were included in this study (ClinicalTrials.gov, number NCT01210755) between November
Reversal agents

The three reversal agents tested were recombinant factor VIIa (rFVIIa, Novoseven®, NovoNordisk, Copenhagen, Denmark), activated prothrombin complex concentrate (FEIBA®, Baxter AG, Vienna, Austria), and the four factor prothrombin complex concentrate (PCC) Kanokad® (LFB, Courtaboeuf, France). Since no data were available regarding the dose of haemostatic agent to use, each of these agents was tested at different dosages. Therefore, rFVIIa was tested at a final concentration of 3 µg/ml corresponding to a therapeutic dose of 120 µg/kg (8), and also at two lower doses, 0.5 and 1.5 µg/ml final concentration. FEIBA® was used at the final concentrations of 0.25, 0.5, 1 (corresponding to 80 U/kg) and 2 U/ml, and Kanokad® at the final concentration of 0.25, 0.5 (corresponding to 25 U/kg), and 1 U/ml.

Statistical analysis

Quantitative data were expressed as means ± standard deviations (SD).

Paired-t tests were used to analyse the reversal effect for each parameter expressed quantitatively. We checked the normality assumption with a Shapiro-Wilk’s test and the Wilcoxon signed-ranks test was used if the normality assumption was violated. Spearman’s coefficient of rank correlation (rho) was used to assess the correlation between the different parameters of the thrombin generation test. Two-sided significance tests were used throughout.

All statistical analyses were performed using Stata software (V.11, College Station, TX, USA). A p-value < 0.05 was considered as statistically significant.
Results

Anticoagulant effect of rivaroxaban and dabigatran

Classical haemostatic assays allowed us to detect the anticoagulant effect induced by rivaroxaban, which was responsible for significantly prolonging PT by 1.37-fold (p < 0.0001) and dabigatran, which was responsible for a significant increase of aPTT by 1.47-fold (p < 0.001).

Effect of reversal of rivaroxaban on TGT parameters

At 2 h after oral administration, the effect of 20 mg Rivaroxaban on thrombin generation was particularly marked with the thrombin peak reduced by a factor of 3, whereas there was only a modest reduction of 22% in the ETP-AUC (Table 1, Fig 1A). The initiation phase of thrombin generation was profoundly altered, as LT and TTP were more than doubled (Table 1, Fig 1A). This was followed by a pronounced inhibitory effect on the propagation phase of thrombin, as shown by the marked effect of rivaroxaban on the rate index of thrombin generation (16.6 ± 2.9 nM min⁻¹, vs. 104.6 ± 5.5 at H0, p < 0.0001). There was a good correlation between ETP-AUC and the Peak (rho = 0.68, p < 0.0001), but not for kinetic parameters. Also we found a strong correlation between LT and TTP (rho = 0.86, p < 0.0001).

At H2 (Fig. 1B), all reagents but rFVIIa were responsible for a dose-dependent increase in the ETP-AUC, by 37% for the lowest dose of PCC (0.25 U/ml, 1,147 ± 232 nM.min) and 50% for the lowest dose of FEIBA® (0.25 U/ml, 1,257 ± 240 nM.min) vs. H2 (p < 0.001, Fig 2A). However, only the lower doses of PCC (0.25 and 0.5 U/ml) and FEIBA® (0.25 U/ml) reversed the ETP-AUC to near baseline H0, values (1,313 ± 362 nM.min for PCC 0.5 U/ml, p = 0.07 vs. H0, Fig 2A). There was over-correction for all other concentrations of PCC and FEIBA®. There was also a significant dose-dependent correction of the thrombin peak using PCC (96.5 ± 29.5 nM for PCC 0.25 U/ml) or FEIBA® (127 ± 27.2 nM for FEIBA® 0.25 U/ml, p < 0.001) vs. H2. Besides the over correction of ETP-AUC, FEIBA® at doses 1 and 2 U/ml corrected the thrombin peak to close to its H0 value (175.9 ± 77.9 nM for FEIBA® 1 U/ml, p = 0.2) (Fig. 2B).

Regarding LT, PCC was responsible for a slight reduction in LT (15% for PCC 1 U/ml), compared to FEIBA® (47%) (p < 0.0001, Fig 1B, 2C). rFVIIa completely reverse the LT increase induced by rivaroxaban (2.24 s vs. 2.29 s at H0). The effect was similar for the different doses of rFVIIa or FEIBA®. However, only the use of rFVIIa corrected LT to near baseline H0 (p = 0.6, Fig. 2C). PCC was without effect on TTP, although rFVIIa and FEIBA® significantly decreased TTP by 44% and 30%, respectively, vs. H2 (6.32 ± 1.28 s for rFVIIa; 7.9 ± 1.54 s for FEIBA®, p < 0.001), without

Table 1: TGT parameters measured at baseline (H0) and 2 hours (H2) after oral intake of 20 mg rivaroxaban or 150 mg dabigatran. ETP-AUC denotes endogenous thrombin potential (nM.min); Peak is the maximum concentration of thrombin (nM); LT is lag time (s); TTP is the time to reach the maximum concentration of thrombin (s). Data were expressed as means ± SD (n = 10).

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<th>ETP</th>
<th>Peak</th>
<th>LT</th>
<th>TTP</th>
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<td>H0</td>
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<td>2.29 (0.45)</td>
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<td>H2</td>
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<td>5.59 (1.03)</td>
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<th>Peak</th>
<th>LT</th>
<th>TTP</th>
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<tr>
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<td>1191 (247)</td>
<td>227.1 (40.2)</td>
<td>2.16 (0.31)</td>
<td>4.23 (0.62)</td>
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<td>H2</td>
<td>953 (182)</td>
<td>220.5 (49.2)</td>
<td>3.78 (1.21)</td>
<td>5.4 (1.25)</td>
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<td>p</td>
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Figure 1: Effect of non-specific reversal agents on rivaroxaban anticoagulated plasma. TGT curves were obtained from one representative individual volunteer. A) Anticoagulant effect of rivaroxaban. H0 denotes baseline; H2 denotes 2 hours after 20 mg oral rivaroxaban. B) TGT curves 2 hours after 20 mg oral rivaroxaban, and after ex vivo addition of non-specific reversal agents at the highest concentration tested.
reaching baseline H0. None of the different doses of PCC modified the altered velocity induced by rivaroxaban. In contrast, rFVIIa and FEIBA increased the rate of thrombin formation to above 25 nM min⁻¹, whatever the tested dose, but without reaching the baseline rate (max observed for FEIBA 2 U/ml, 47.7 ± 7.7 nM.min⁻¹).

Effect of reversal of dabigatran on TGT parameters

At 2 h after receiving 150 mg of dabigatran, the ETP-AUC was modestly reduced by 20%, but the thrombin peak was unchanged (Table 1, Fig. 3A). The initiation phase of thrombin was significantly altered, as shown by the LT (Table 1, Fig. 3A). The index rate of thrombin generation was not inhibited (138.5 ± 10.8 nM s⁻¹ at H2, vs. 111.9 ± 6.8 at H0). There was very good correlation between the ETP-AUC and the Peak (rho = 0.93, p < 0.0001), and between LT and TTP (rho = 0.94, p < 0.0001).

At H2, PCC and FEIBA increased the ETP-AUC of dabigatran-anticoagulated plasma (Fig. 3B) in a concentration-dependent manner (Fig. 4A). Low doses of PCC or FEIBA reversed ETP-AUC results close to baseline H0 (p = 0.02), while there is a dramatic increase in thrombin generated when PCC or FEIBA were used at regular or half doses. Both the highest dose of rFVIIa (3 U/ml) and the three highest doses of FEIBA (range 0.5 to 2 U/ml) reduced the LT (Fig. 3B, Fig. 4B), with a more pronounced effect of rFVIIa (2.52 ± 1 s, ratio 0.66 ± 0.13 s vs. H2 values, p < 0.001) (Fig. 4B). This observed reduction in LT was close to H0 (p = 0.2). For the TTP, similar results were observed for rFVIIa and FEIBA, with, in this case a significant effect of the lowest dose of FEIBA (0.25 U/ml, 4.85 ± 0.52 s, ratio 0.83 ± 0.15 vs. H2, p = 0.03). Again the observed reversal was close to H0 (p = 0.08). All agents except rFVIIa were responsible for an increase in the thrombin peak above the baseline (H0) value (296.2 ± 57.3 nM for PCC 0.25U/ml, 361 ± 28 nM for FEIBA 0.25 U/ml, p < 0.001 vs. H2), with a maximum observed for PCC at 1 U/ml (462.1 ± 81.9 nM) and FEIBA at 2 U/ml (551.7 ± 99.5 nM) (data not shown).

Discussion

Due to their ease of use, the novel orally active anticoagulant drugs will probably be widely used in the near future, including in patients at higher risk of bleeding. Although these treatments have a

![Figure 2: Effect of non-specific reversal agents on rivaroxaban anticoagulated plasma.](https://www.thrombosis-online.com/figure2.jpg)
favourable risk-benefit profile compared to warfarin, major haemorrhages, including intracerebral haemorrhages, have been described in clinical trials evaluating both dabigatran and rivaroxaban (1, 2, 13). Among reversal agents, PCC has been already extensively evaluated to reverse VKA, although rFVIIa was reported against antiXa-anticoagulant as efficient drug (14). Also FEIBA was reported to be efficient to reverse VKA (15). These several haemostatic agents have been proposed to reverse the anticoagulant effect of these new drugs, although the level of evidence is poor in the absence of available clinical data (3–5). To improve our knowledge, we tested ex vivo the ability of these non-specific haemostatic drugs to reverse the anticoagulant activity of the new antithrombotics.

Rivaroxaban reversal

In the present study, rivaroxaban significantly affected both the quantitative and kinetic TGT parameters. As previously described, the effect of rivaroxaban on the ETP-Peak was more pronounced than on ETP-AUC (12). As expected, PCC strongly corrected ETP-AUC (16) (and more modestly the ETP-Peak), above the baseline values for the highest doses, whereas rFVIIa only had a relevant effect on kinetic parameters. However, evaluation of potential reversal of rivaroxaban anticoagulant effect should probably not be based uniquely on analysis of ETP-AUC, since, in a recently published rabbit haemorrhagic model, PCC did not reverse rivaroxaban-induced bleeding, although PCC corrects ETP-AUC by a factor of 2 (17). Rivaroxaban also altered the initiation (LT) and propagation phases of thrombin generation. These two parameters have been described as being potentially relevant for determining the antithrombotic activity of rivaroxaban, or its reversion (18). However, in the present study, the effect of PCC was non-significant on these kinetic parameters. In contrast, rFVIIa, even at its lowest concentration, showed complete reversion of the rivaroxaban-induced prolonged LT to the baseline level at H0. These results are very close to those already described using rFVIIa to reverse the in vitro anticoagulant effect of the indirect selective FXa inhibitors, fondaparinux (19), and idraparinux (20). Unfortunately, although rFVIIa had a pronounced effect on lag-time, it did not reverse rivaroxaban-induced haemorrhage in a rabbit model (17). Interestingly, FEIBA®, which contains FVII, mainly in the activated form, and FII, FIX and FX, mainly in non-activated forms, combines the effect of both rFVIIa and PCC, and corrected all TGT parameters compared to H2, even at the lowest dose. The observed correction never exceeds baseline (H0) values for the ETP-Peak, although for kinetics parameters it persisted for longer than at baseline. Furthermore, only FEIBA® corrected the markedly affected thrombin propagation phase by > 20%, theoretically compatible with a non-bleeding phenotype (21). Finally, FEIBA® at a dose of 50–100 U/kg was reported to reduce mesenteric bleeding time in a rat haemorrhagic model (22), and also bleeding time in baboons (23).

Dabigatran reversal

Dabigatran has been described as inhibiting thrombin generation in a concentration-dependent manner (24). The overall generation of thrombin assessed by ETP-AUC has been described as being moderately decreased. Dabigatran, as a thrombin retardant, isolated induced alteration of LT, without affecting the velocity of thrombin generation. Our results are in close agreement with these previous results. PCC increased ETP-AUC, but had no effect on dabigatran-modified TGT kinetic parameters, as previously described (16). However, PCC has been described as able to reverse the prolonged bleeding time and blood loss after dabigatran administration (25), and also to prevent intra-cerebral haematoma expansion in a murine model (26). LT (but not ETP-AUC or Peak) was described as correlated with circulating concentrations of da-
bigatran (10). In the present study, rFVIIa at the higher dose, and FEIBA\textsuperscript{®} corrected the altered LT to nearly baseline value. Nevertheless, previous data regarding the effect of rFVIIa on thrombin inhibitors are inconsistent. A single dose of rFVIIa did not reverse the anticoagulant effect of melagatran in healthy volunteers (27), although it did in another study (28). In a rat model, the addition of rFVIIa significantly reduced both tail bleeding time and aPTT after the administration of a high dose of dabigatran (29). Again, in animal models, bleeding caused by a high dose of dabigatran was reduced following the use of FEIBA\textsuperscript{®} (29). 

Clinical implications

If immediate reversal of the new anticoagulants is required, i.e. in the event of intra-cerebral haemorrhage, there is currently no clear evidence that any non-specific reversal agent can counteract the anticoagulant effect of either rivaroxaban or dabigatran.

FEIBA\textsuperscript{®} has the theoretical advantage of combining the effect of FVIIa and that of PCC. Therefore, a dose of 50 U/kg FEIBA corresponds to 37 µg/kg of FVIIa together with 50 U/kg PCC (30). In the case of rivaroxaban, FEIBA combines the correcting effect on lag time of rFVIIa and the correcting effect on peak value of PCC. Higher doses of FEIBA were responsible for an increase in total thrombin generation, as shown by the increase in ETP-AUC and Peak. This raises the problem of safety of such a product regarding thrombotic risk. For this purpose, the incidence of thrombotic adverse events was reported as low, four per 100,000 FEIBA\textsuperscript{®} infusions, and a dose-dependent effect on the incidence of thrombosis was reported (31). This underlines the need to avoid over-reversion, and to better define the potential minimum dose of reversal agent to use. Regarding TGT, quantitative parameters such as a Peak higher than 400 to 600 nM were correlated with thrombotic risk (32, 33). In our study for rivaroxaban, such an increase in thrombin generation above baseline H0 was not observed with FEIBA\textsuperscript{®}. Moreover, it seems that the lower doses of FEIBA\textsuperscript{®}, corresponding to a quarter and a half of the dose usually used, have
What is known about this topic?

- New anticoagulants such as dabigatran and rivaroxaban can be responsible for haemorrhagic complications.
- As promising drugs, it is expected that these treatments will be widely used in long-term indications such as atrial fibrillation.
- The management of any bleeding event or emergency surgery, as for any anticoagulant, is challenging.

What does this paper add?

- This study tested ex vivo, the ability of all putative non-specific haemostatic agents currently available, at different dosages, to reverse the anticoagulant activity of both dabigatran and rivaroxaban.
- For both drugs, PCC corrected the ETP-AUC, whereas rFVIIa only modified kinetic parameters. Higher doses of PCC and FEIBA were responsible for an over correction of ETP-AUC and could be harmful. Lower doses of FEIBA, corresponding to a quarter to half the dose usually used, have potential reversal profile of interest.
- Prospective validation in clinical trials is urgently needed.

Life of product such as rFVIIa, and we did not measure the duration of the response. Third, the doses of anticoagulant tested, 150 mg for dabigatran and 20 mg for rivaroxaban in one oral administration are not really comparable, as in clinical practice dabigatran is usually prescribed twice a day, and is potentially accumulated at steady state. Moreover, we did not test overdose conditions, such as the high concentrations potentially observed in patients who have kidney failure. Fourth, we performed TGT in platelet-poor plasma, at standard concentration of tissue factor. Therefore, the effect of drugs such as rFVIIa is likely to be different in vivo when there may be massive exposure of TF. Fifth, we did not use a haemorrhagic model, and our conclusions remain a theoretical extrapolation from in vitro to in vivo. Finally, Dabigatran increased aPTT, TT and ECT (35), and prolongation of TT or ECT was proposed to reflect a high concentration of circulating dabigatran and to help monitor surgery. Rivaroxaban significantly increased PT, as well as diluted PT (36), and anti-FXa activity was suggested as a better indicator of anti-FXa drugs plasma concentration (37). Unfortunately, we did not explore, for the different concentration of haemostatic agents, these tests, available in clinical practice, to judge the potential reversal effect.

Study limitations

They are several limitations in our study. First, our study was an ex vivo study, by contrast to already published studies in which the reversing agent has been administered and in vivo observations were made (16). However, in the absence of clear available data regarding the better choice to reverse anticoagulant effect, this was the only way to test several agents at several doses. Second, the ex vivo design has the disadvantage to not take into account the short half-life of product such as rFVIIa, and we did not measure the duration of the response. Third, the doses of anticoagulant tested, 150 mg for dabigatran and 20 mg for rivaroxaban in one oral administration are not really comparable, as in clinical practice dabigatran is usually prescribed twice a day, and is potentially accumulated at steady state. Moreover, we did not test overdose conditions, such as the high concentrations potentially observed in patients who have kidney failure. Fourth, we performed TGT in platelet-poor plasma, at standard concentration of tissue factor. Therefore, the effect of drugs such as rFVIIa is likely to be different in vivo when there may be massive exposure of TF. Fifth, we did not use a haemorrhagic model, and our conclusions remain a theoretical extrapolation from in vitro to in vivo. Finally, Dabigatran increased aPTT, TT and ECT (35), and prolongation of TT or ECT was proposed to reflect a high concentration of circulating dabigatran and to help monitor surgery. Rivaroxaban significantly increased PT, as well as diluted PT (36), and anti-FXa activity was suggested as a better indicator of anti-FXa drugs plasma concentration (37). Unfortunately, we did not explore, for the different concentration of haemostatic agents, these tests, available in clinical practice, to judge the potential reversal effect.

Conclusion

In case of severe haemorrhage, i.e. intracerebral haemorrhage needing rapid reversal of anticoagulant and, in the absence of specific antidotes, alternatives such as one of the non-specific haemostatic agents must be considered. However, clinical evaluation in haemorrhagic situations and a meticulous risk-benefit appraisal of the use of these treatments is urgently needed.
Acknowledgments
This study was sponsored by the Clinical Research and Innovation Department (Délegation à la Recherche Clinique et Innovation) of Grenoble University Hospital. This study was also supported by a grant from Diagnostica STAGO, France. All reagents for in vitro analysis were graciously given by Diagnostica-STAGO, Asnières, France. Novaseven®, FEIBA® and Kanokad® were graciously given by NovoNordisk, Baxter and Laboratoire Français des Biotechnologies, respectively. The authors thank Alison Foote (Grenoble Clinical Research Center) for help in preparing the English version of the manuscript.

Conflicts of interest
G. Pernod was investigator for the EINSTEIN program using Rivaroxaban for venous thromboembolic disease. None of the other authors declares any conflict of interest.

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Thrombosis and Haemostasis 108.2/2012
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