# Pharmacological properties of NOACs relevant for bleeding

### Direct thrombin inhibitor dabigatran

Dabigatran etexilate is a prodrug with a bioavailabilty of 6.5% that is rapidly absorbed in the gastrointestinal tract. After oral absorption, dabigatran etexilate is rapidly and completely converted by plasma and liver esterases to dabigatran, a competitive and reversible thrombin inhibitor.1 The maximum anticoagulant effect of dabigatran occurs at the peak of drug plasma concentrations (i.e., after 2h) with the anticoagulant effect decreasing to ~50% of its maximum at 12h after its administration.2-5 Because dabigatran is eliminated mainly by the renal route, its plasma concentration and, in parallel, its anticoagulant effect depend very strongly on changes in renal function. The mean half-life increases to 18h with a creatinine clearance (CrCl) between 30 and 49 ml/min. As recommended by the ESC for all NOAC,6 dabigatran should not be used below a CrCl of <30 mL/min where the half life further increases to 27h. In case of an overdose, administration of oral activated charcoal (30-50g) may be helpful to reduce drug absorption following recent ingestion (<2h). Due to its low plasma protein binding, haemodialysis may be effective in removing dabigatran in cases of severe bleeding or in patients with renal impairment.7 In patients with end-stage renal disease, 68% of active dabigatran was removed after 4h of haemodialysis. While the settings of haemodialysis seems to be less important8, it remains unclear whether these data can be translated to unstable patients undergoing haemodiafiltration.As expected for an anticoagulant, major bleeding increased with the concentration of dabigatran in the RE-LY trial. Median trough concentrations were increased by 55% in patients with major bleeding compared to those without.9 Clinical characteristics associated with high plasma concentrations were age and reduced renal function, reemphasizing the importance of dose reduction in patients over 80 years of age and with moderately reduced renal function (CrCl < 50 ml/min). Close clinical surveillance is recommended in patients with weight < 50kg. Relevant drug interactions increasing dabigatran plasma concentrations have been observed with verapamil, requiring dose reduction, and with systemic ketoconazole, cyclosporine, itraconazole and dronedarone, contraindicating the combination of dabigatran with these agents.10

## Factor Xa inhibitors

Apixaban, edoxaban and rivaroxaban are oral selective, competitive and direct inhibitors of both free FXa and FXa associated with the prothrombinase complex, thus decreasing the conversion of prothrombin to thrombin.11-16 They decrease the generation of thrombin and thereby reduce thrombin-mediated activation of both coagulation and platelets, but allow residual thrombin to carry out its other important functions.17 FXa inhibitors have a much higher bioavailability than the prodrug dabigatran (*Table 1*). Their half-life of 5 to 14h is comparable to that of dabigatran. They are excreted by the kidney to a lesser extent than dabigatran (27-50%).

Rivaroxaban inhibits human FXa (inhibitory constant [Ki*]* 0.4 nM) with >10 000-fold greater selectivity than for related serine proteases.13, 18 In human plasma, it inhibits both clot-bound FXa (half maximal inhibitory concentration [*IC50*] 75 nM) and FXa associated with prothrombinase (IC50 2.1 nM),13, 17 and inhibits the generation of thrombin after activation of the extrinsic tissue factor pathway (IC50 25 nM).19 Rivaroxaban should be taken with food, increasing its bioavailability to almost 100%. It dose-dependently inhibits FXa activity by 20–60% after single- and multiple-dosing11-12, 20 and its plasma concentrations are closely correlated with FXa activity.11-12, 21-22 While having a similar half-life than other NOACs, it has been tested with a once daily (od) regimen for treatment of atrial fibrillation (AF) and venous thromboembolism (in the long-term phase). No dose reduction is required for elderly patients or low or high body weight.

Apixaban concentration-dependently inhibits human FXa (Ki 0.08 nM),23 whereas Ki values ≥ 3 000 nM were determined for other serine proteases.14, 23 It shows a moderate selectivity for clot-bound over free FXa (IC50 1.3 vs. 7.6 nM)24 and inhibits thrombin generation rate (IC50 35 nM). Dose reduction is recommend when at least two of the following characteristics exist: age ≥ 80 years, body weight ≤ 60 kg, or serum creatinine ≥ 1.5 mg/dL (133 µmol/L).

Activated charcoal can be administered if apixaban is taken within 3h or rivaroxaban within 2h. Rivaroxaban and apixaban cannot be removed by haemodialysis due to their high plasma protein binding. Rivaroxaban and apixaban should be used with caution in patients with severe renal impairment (CrCl 15–29 mL/min) and the dose should be reduced (rivaroxaban CrCl < 50 ml/min: 15mg od; apixaban CrCl <30 ml/min: 2.5mg twice daily, bid). Rivaroxaban and apixaban are contraindicated in patients with a CrCl of <15 ml/min or with any hepatic disease associated with coagulopathy, since drug exposure and bleeding risk may be increased (Summary of Product Characteristics of European Medicines Agency). Potent inhibitors of CYP3A4 and P-glycoprotein such as azole-antimycotics (e.g. ketoconazole, itraconazole, voriconazole and posaconazole) or HIV protease inhibitors (e.g. ritonavir) may cause a relevant increase in plasma concentrations of apixaban and rivaroxaban, thus increasing the risk of bleeding, and should therefore be avoided.

Edoxaban, the third FXa inhibitor just approved by the European Medicines Agency, dose-dependently inhibits free FXa (Ki 0.56 nM) and FXa bound to the prothrombinase complex (Ki 2.98 nM) and exhibited >10 000-fold selectivity for FXa relative to thrombin.25-27 The edoxaban dose should be halved (30mg od) if patients have a CrCl 15-50 mL/min, a body weight < 60 kg, or concomitant medication with potent P-glycoprotein interaction such as verapamil, quinidine, or dronedarone.28 These dose reduction criteria overcompensated for a potential increase of edoxaban plasma concentrations.29 Edoxaban is contraindicated in patients with a CrCl below 15 ml/min. In patients with end-stage renal disease, haemodialysis had minimal effects on the clearance of edoxaban.30 In the ENGAGE AF-TIMI 48 phase III trial, exodaban has been tested at doses of 15 to 60mg od. The mean trough plasma concentrations of edoxaban increased from 16.0 ng/ml with 15mg to 48.5 ng/ml with 60mg. The risk of major bleeding moderately increased (20-30%) between the 25th to 75th centiles of edoxaban plasma concentrations for each edoxaban dose.29

# Critical appraisal of surrogate endpoints for measurement of the reversal of NOAC effects with coagulation factor concentrates

As the effect of prohaemostatic agents on NOACs has not been tested in clinical trials so far, our evidence is based on surrogate endpoints such as normalization of coagulation tests, ex-vivo thrombus formation or animal bleeding models only (Supplemental *Table 3*). Global coagulation tests are used in clinical practice to monitor the effect of anticoagulation therapy. Specifically, calibrated laboratory tests measure the effect of NOACs, as recently summarized by Siegal et al..31 Thrombin generation (TG) tests such as the calibrated automated thrombogram and clotting tests may be even more sensitive for testing the reversal of OAC effects.32 The lag-time appears to be the most sensitive TG measurement for the effects of NOACs. However, divergent results have been reported for the effect of reversal agents on routine coagulation tests and TG tests,33 questioning the relevance of the information derived from routine laboratory testing. In the absence of ample clinical experience, animal models are a valuable alternative source of information about the effect on bleeding. Specific settings are developed to reflect the effect of reversal agents.34 These clinical models may be more sensitive in measuring the reversal of the effect of NOACs than routine coagulation assays.35 In particular, measurement of the bleeding time (BT) in these animal models may be more sensitive for the reversal effect than measurement of coagulation tests.36-37 However, these animal models may only partially mimic the complex situation of critically-ill patients with life-threatening bleeding

## Reversal of dabigatran effects

CFCs such as 4-factor PCC did not normalize global coagulation tests including thrombin time (TT) and activated partial thromboplastin time (aPTT) in individuals pretreated with dabigatran.38 However, CFCs improved TG in plasma from healthy individuals pretreated with dabigatran ex-vivo.33, 35, 39-40 The reversal effect was dependent on the concentration of dabigatran and CFCs.35 In animal models CFCs dose-dependently and rapidly decreased bleeding time.35, 37, 41 However, there were inconsistent results with regard to reduction of blood loss.42 In a murine ICH model, a 4-factor PCC but not rFVIIa reduced the expansion of ICH and 24h-mortality.41 Recent data from a lethal porcine polytrauma model suggested that high-dose (50 IU/kg) but not low-dose PCC43 reversed the effects of dabigatran on blood loss and survival to a similar extent than idarucizumab, a specific antibody against dabigatran.44-45 With regard to human data, a few case reports/series addressing very different clinical scenarios reported varying outcomes after reversal of dabigatran-induced bleeding with CFCs.46-50 In case of bleeding in patients with renal impairment, additional haemodialysis may be essential for elimination of dabigatran.50

## Reversal of factor Xa inhibitor effects

CFCs such as 3- and 4-factor PCC with 50 U/kg at least partially reverse prothrombin time (PT) in most studies in healthy individuals pretreated with rivaroxaban.38, 51 rFVIIa may have the most prominent reversal effect on PT.52 Moreover, activated PCC and rFVIIa with the most prominent effects but also high-dose 3- and 4-factor PCCs improved TG tests ex vivo using plasma from healthy donors pretreated with rivaroxaban.39, 51, 53-54 However, contrasting data without an effect of PCCs on PT and TG tests using rivaroxaban-anticoagulated blood samples from healthy volunteers are also published.53 With regard to experimental animal data, 4-factor PCC did not consistently reduce bleeding time and did not reduce blood loss.36, 55 Activated 4-factor PCC and rFVIIa, with an eventually more pronounced effect, reduced BT in animals pretreated with rivaroxaban.55 36 In a mouse model, a 4-factor PCC and rFVIIa prevented excess intracranial haematoma expansion with rivaroxaban.56 Despite correction of clinical bleeding in animal models, the PT remained prolonged, suggesting that procoagulants may attenuate bleeding, but fail to normalise global coagulation tests.36, 56

4-factor PCC, activated 4-factor PCC and rFVIIa improved TG after pretreatment with apixaban.57 In a rabbit model, rFVIIa reduced the BT to some extent but did not reduce blood loss with apixaban. In this setting, a 4-factor PCC did not affect clinical bleeding.58

Activated 4-factor PCC and rFVIIa improved anti-Xa activity in edoxaban-containing blood samples.59 4-factor PCC and, most potently, activated 4-factor PCC and rFVIIa dose-dependently reversed reduced TG by edoxaban.15-16, 59-61 In a recent double-blind, randomized, placebo-controlled, 2-way crossover study with 110 healthy individuals 4-factor PCC dose-dependently reversed the effect of edoxaban on TG and bleeding duration after a punch biopsy, with complete reversal at 50 IU/kg.62 No prothrombotic event was observed. With regard to animal data, high dose 4-factor PCC, activated 4-factor PCC and rFVIIa significantly reduced BT and/or blood loss60, 63 without increasing the risk of thrombosis.60

## Supplemental Table 1. Pharmacologic properties of NOACs

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Dabigatran** **(Pradaxa)**  | **Rivaroxaban** **(Xarelto)**  | **Apixaban** **(Eliquis)**  | **Edoxaban** **(Lixiana)**  |
| Company  | Boehringer Ingelheim  | Bayer  | BMS/Pfizer  | Daiichi Sankyo  |
| Direct inhibition of  | FIIa  | FXa  | FXa  | FXa  |
| Standard dosage for atrial fibrillation  | 150mg twice daily | 20mg once daily | 5mg twice daily | 60mg once daily |
| t max  | 1-3h  | 2-4h  | 1-3h  | 1-2h  |
| Half life (t ½)  | 12-17h  | 5-9h young11-13h elderly | 9-14h  | 10-14h |
| Bioavailability  | 6.5%  | 80%  | 60%  | 62%  |
| Renal elimination  | 80%  | 35%  | 25%  | 35%  |
| Dialysis? | yes | no | no | no |
| Drug interactions  |  |  |  |  |
|  P-glycoprotein involved  | yes | yes | yes | yes |
|  CYP3A4 involved | no | yes | yes | minimal |

## Supplemental Table 2 – Effects of NOACs in *ex vivo/in vitro* and *animal* studies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Dabigatran | Rivaroxaban | Apixaban | Edoxaban |
| Global coagulation tests  | - Prolonged5, 64 | - Prolonged13, 65 | - Prolonged (modest)23, 66 | - Prolonged25-27 |
| Thrombus formation | - Thrombus formation ↓67- Thrombin generation ↓5 | - Thrombus formation ↓68- Thrombin generation ↓19 | - Thrombus formation ↓23- Thrombin generation ↓23 | - Thrombus formation ↓25, 27, 69- Thrombin generation ↓26-27 |
| Platelet aggregation | - Thrombin- and TF-induced aggregation↓5- No effect on other platelet-stimulating mechanisms5, 70 | - Platelet-induced thrombin generation ↓71- Thrombin- and TF-induced aggregation↓70-73- No effect on other platelet-stimulating mechanisms5, 70, 73 | - TF-induced aggregation ↓- No effect on other platelet-stimulating mechanisms70 | - Weak inhibition of thrombin-induced aggregation25- No impact on other mechanisms25 |
| Animal data | - Thrombus formation ↓74-75- No prolonged bleeding time (with normal doses)76- No increased bleeding tendency74 | - Thrombus formation ↓13, 77- Modest increase in bleeding time76-77 | - Thrombus formation ↓78- Modest increase in bleeding time23, 76, 78 | - Thrombus formation ↓25- No prolonged bleeding time (with normal doses)25 |

## Supplemental Table 3. Experimental data about the reversal of NOAC effects with coagulation factor concentrates

## Table 3A. Reversal of routine laboratory tests

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | DABIGATRAN\* | RIVAROXABAN\*\*\* | APIXABAN\*\* | EDOXABAN\*\* |
| 3-factor PCC | No[35](#_ENREF_1) | - Partial reversal of PT ( no data on dose-dependency)[51](#_ENREF_2" \o "Levi, 2014 #124) - No effect on anti-FXa activity[51](#_ENREF_2)  | ND | ND |
| 4-factor PCC | No35, 38 | - Partial reversal of PT (dose-dependent effect; one negative study)[38, 51-52, 54](#_ENREF_2" \o "Levi, 2014 #124)- No effect on anti-FXa activity[51](#_ENREF_2) | ND | - Dose-dependent effect on bleeding time and blood loss (human data)62 |
| Activated 4-factor PCC | No[35](#_ENREF_1)  | - Partial reversal of PT (dose-dependent effect)[54](#_ENREF_4" \o "Perzborn, 2014 #100)- No data on anti-FXa activity | ND | - Reversal of PT (not dose-dependent)[59](#_ENREF_6" \o "Halim, 2014 #206)- Dose-dependent, modest reversal of FX activity59 |

\* aPTT, no data on TT or ECT \*\* Anti-FXa activity \*\*\* PT, anti-FXa activity ND, no data available; PCC prothrombin complex concentrate

## Table 3B. Reversal of thrombin generation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | DABIGATRAN | RIVAROXABAN | APIXABAN | EDOXABANa |
| 3-factor PCC | Yes (dose-dependent effect)[35](#_ENREF_1)  | Yes (dose-dependent effect)[51](#_ENREF_2) | ND\* | ND\* |
| 4-factor PCC | Yes (dose-dependent effect)35, 39  | Inconsistent data (dose-dependent effect)38-39, 51, 53-54 | Yes (no data on dose-dependency) [57](#_ENREF_8) | Yes (dose-dependent effect)62, [60](#_ENREF_9) |
| Activated 4-factor PCC | Yes (dose-dependent effect)35, 39  | Yes (dose-dependent effect)39, 54 | Yes (no data on dose-dependency)[57](#_ENREF_8) | Yes (dose-dependent effect)[60](#_ENREF_9) |
| rFVIIa | Yes35, 39  | Yes39, 54 | Yes (no data on dose-dependency)[57](#_ENREF_8) | Yes[60](#_ENREF_9)  |

Reversal was assumed to be “yes” if there was a consistent effect using at least one of the following tests: clotting time, thrombin generation (including lag time, maximum concentration, endogenous thrombin potential), prothrombin time, \*ND, no data available; PCC prothrombin complex concentrate

## Table 3C. Reversal of bleeding in animal models

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | DABIGATRAN | RIVAROXABAN | APIXABAN | EDOXABAN |
| 3-factor PCC | - Bleeding time ↓[35](#_ENREF_1)- No data on blood loss or ICH | ND\* | ND\* | ND\* |
| 4-factor PCC | - Inconsistent data regarding bleeding time35, 37, 41-42- Inconsistent data regarding blood loss[37, 41-42](#_ENREF_11)- ↓ Hematoma size in ICH model and improved survival (with high doses)33, 79 | - Inconsistent data regarding bleeding time36, 55- No reduced blood loss[55](#_ENREF_15)- ↓ Hematoma size in ICH model (dose-dependent)[56](#_ENREF_17) | - No effect on bleeding time[58](#_ENREF_14)- No reduced blood loss[58](#_ENREF_14)- No data on ICH | - Bleeding time ↓63- Blood loss ↓63- No data on ICH |
| Activated 4-factor PCC | - Bleeding time ↓35, 42- No reduced blood loss[42](#_ENREF_11)- No data on ICH | - Bleeding time ↓[36](#_ENREF_16)- No data on blood loss or ICH  | ND\* | - Bleeding time ↓[60](#_ENREF_9)- No data on blood loss or ICH |
| rFVIIa | - Inconsistent data regarding bleeding time35, 42- No reduced blood loss[42](#_ENREF_11)- No reduction of hematoma size in ICH model[41](#_ENREF_13) | - Bleeding time ↓36, 55- No reduced blood loss[55](#_ENREF_15)-↓ Hematoma size in ICH[56](#_ENREF_17) | - Bleeding time ↓(partially)[58](#_ENREF_14)- No reduced blood loss[58](#_ENREF_14)- No data on ICH | - Bleeding time ↓[60](#_ENREF_9)- No data on blood loss or ICH |

\*ND, no data available; PCC prothrombin complex concentrate; ICH intracranial haemorrhage

# References

1. van Ryn J, Goss A, Hauel N, Wienen W, Priepke H, Nar H, Clemens A. The discovery of dabigatran etexilate. *Front Pharmacol* 2013;**4**:12.

2. Stangier J, Rathgen K, Stahle H, Gansser D, Roth W. The pharmacokinetics, pharmacodynamics and tolerability of dabigatran etexilate, a new oral direct thrombin inhibitor, in healthy male subjects. *Br J Clin Pharmacol* 2007;**64**:292-303.

3. Stangier J, Stahle H, Rathgen K, Roth W, Shakeri-Nejad K. Pharmacokinetics and pharmacodynamics of dabigatran etexilate, an oral direct thrombin inhibitor, are not affected by moderate hepatic impairment. *J Clin Pharmacol* 2008;**48**:1411-9.

4. Stangier J, Stahle H, Rathgen K, Fuhr R. Pharmacokinetics and pharmacodynamics of the direct oral thrombin inhibitor dabigatran in healthy elderly subjects. *Clin Pharmacokinet* 2008;**47**:47-59.

5. Wienen W, Stassen JM, Priepke H, Ries UJ, Hauel N. In-vitro profile and ex-vivo anticoagulant activity of the direct thrombin inhibitor dabigatran and its orally active prodrug, dabigatran etexilate. *Thromb Haemost* 2007;**98**:155-62.

6. Camm AJ, Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH, Hindricks G, Kirchhof P. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation. Developed with the special contribution of the European Heart Rhythm Association. *European Heart Journal* 2012;**33**:2719-47.

7. De Caterina R, Husted S, Wallentin L, Agnelli G, Bachmann F, Baigent C, Jespersen J, Kristensen SD, Montalescot G, Siegbahn A, Verheugt FW, Weitz J. Anticoagulants in heart disease: current status and perspectives. *Eur Heart J* 2007;**28**:880-913.

8. Liesenfeld KH, Staab A, Hartter S, Formella S, Clemens A, Lehr T. Pharmacometric characterization of dabigatran hemodialysis. *Clin Pharmacokinet* 2013;**52**:453-62.

9. Reilly PA, Lehr T, Haertter S, Connolly SJ, Yusuf S, Eikelboom JW, Ezekowitz MD, Nehmiz G, Wang S, Wallentin L. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy). *J Am Coll Cardiol* 2014;**63**:321-8.

10. Heidbuchel H, Verhamme P, Alings M, Antz M, Hacke W, Oldgren J, Sinnaeve P, Camm AJ, Kirchhof P. EHRA practical guide on the use of new oral anticoagulants in patients with non-valvular atrial fibrillation: executive summary. *Eur Heart J* 2013;**34**:2094-106.

11. Kubitza D, Becka M, Voith B, Zuehlsdorf M, Wensing G. Safety, pharmacodynamics, and pharmacokinetics of single doses of BAY 59-7939, an oral, direct factor Xa inhibitor. *Clin Pharmacol Ther* 2005;**78**:412-21.

12. Kubitza D, Becka M, Wensing G, Voith B, Zuehlsdorf M. Safety, pharmacodynamics, and pharmacokinetics of BAY 59-7939--an oral, direct Factor Xa inhibitor--after multiple dosing in healthy male subjects. *Eur J Clin Pharmacol* 2005;**61**:873-80.

13. 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.

14. Samama MM, Guinet C. Laboratory assessment of new anticoagulants. *Clin Chem Lab Med* 2011;**49**:761-72.

15. Camm AJ, Bounameaux H. Edoxaban: a new oral direct factor xa inhibitor. *Drugs* 2011;**71**:1503-26.

16. Lip GY, Agnelli G. Edoxaban: a focused review of its clinical pharmacology. *Eur Heart J* 2014;**35**:1844-55.

17. Kubitza D, Perzborn E, Berkowitz SD. The discovery of rivaroxaban: translating preclinical assessments into clinical practice. *Front Pharmacol* 2013;**4**:145.

18. Perzborn E, Roehrig S, Straub A, Kubitza D, Mueck W, Laux V. Rivaroxaban: a new oral factor Xa inhibitor. *Arterioscler Thromb Vasc Biol* 2010;**30**:376-81.

19. Gerotziafas GT, Elalamy I, Depasse F, Perzborn E, Samama MM. In vitro inhibition of thrombin generation, after tissue factor pathway activation, by the oral, direct factor Xa inhibitor rivaroxaban. *J Thromb Haemost* 2007;**5**:886-8.

20. Heidbuchel H, Verhamme P, Alings M, Antz M, Hacke W, Oldgren J, Sinnaeve P, Camm AJ, Kirchhof P. European Heart Rhythm Association Practical Guide on the use of new oral anticoagulants in patients with non-valvular atrial fibrillation. *Europace* 2013;**15**:625-51.

21. Mueck W, Becka M, Kubitza D, Voith B, Zuehlsdorf M. Population model of the pharmacokinetics and pharmacodynamics of rivaroxaban--an oral, direct factor xa inhibitor--in healthy subjects. *Int J Clin Pharmacol Ther* 2007;**45**:335-44.

22. Mueck W, Eriksson BI, Bauer KA, Borris L, Dahl OE, Fisher WD, Gent M, Haas S, Huisman MV, Kakkar AK, Kalebo P, Kwong LM, Misselwitz F, Turpie AG. Population pharmacokinetics and pharmacodynamics of rivaroxaban--an oral, direct factor Xa inhibitor--in patients undergoing major orthopaedic surgery. *Clin Pharmacokinet* 2008;**47**:203-16.

23. 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.

24. 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.

25. Furugohri T, Isobe K, Honda Y, Kamisato-Matsumoto C, Sugiyama N, Nagahara T, Morishima Y, Shibano T. DU-176b, a potent and orally active factor Xa inhibitor: in vitro and in vivo pharmacological profiles. *J Thromb Haemost* 2008;**6**:1542-9.

26. Samama MM, Mendell J, Guinet C, Le Flem L, Kunitada S. In vitro study of the anticoagulant effects of edoxaban and its effect on thrombin generation in comparison to fondaparinux. *Thromb Res* 2012;**129**:e77-82.

27. Zafar MU, Vorchheimer DA, Gaztanaga J, Velez M, Yadegar D, Moreno PR, Kunitada S, Pagan J, Fuster V, Badimon JJ. Antithrombotic effects of factor Xa inhibition with DU-176b: Phase-I study of an oral, direct factor Xa inhibitor using an ex-vivo flow chamber. *Thromb Haemost* 2007;**98**:883-8.

28. Salazar DE, Mendell J, Kastrissios H, Green M, Carrothers TJ, Song S, Patel I, Bocanegra TS, Antman EM, Giugliano RP, Kunitada S, Dornseif B, Shi M, Tachibana M, Zhou S, Rohatagi S. Modelling and simulation of edoxaban exposure and response relationships in patients with atrial fibrillation. *Thromb Haemost* 2012;**107**:925-36.

29. Ruff CT, Giugliano RP, Braunwald E, Morrow DA, Murphy SA, Kuder JF, Deenadayalu N, Jarolim P, Betcher J, Shi M, Brown K, Patel I, Mercuri M, Antman EM. Association between edoxaban dose, concentration, anti-Factor Xa activity, and outcomes: an analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. *Lancet* 2015.

30. Parasrampuria DA, Marbury T, Matsushima N, Chen S, Wickremasingha PK, He L, Dishy V, Brown KS. Pharmacokinetics, safety, and tolerability of edoxaban in end-stage renal disease subjects undergoing haemodialysis. *Thromb Haemost* 2015;**113**:719-27.

31. Siegal DM, Crowther MA. Acute management of bleeding in patients on novel oral anticoagulants. *Eur Heart J* 2013;**34**:489-498b.

32. Gatt A, van Veen JJ, Woolley AM, Kitchen S, Cooper P, Makris M. Thrombin generation assays are superior to traditional tests in assessing anticoagulation reversal in vitro. *Thromb Haemost* 2008;**100**:350-5.

33. Grottke O, van Ryn J, Spronk HM, Rossaint R. Prothrombin complex concentrates and a specific antidote to dabigatran are effective ex-vivo in reversing the effects of dabigatran in an anticoagulation/liver trauma experimental model. *Crit Care* 2014;**18**:R27.

34. Monroe DM, Hoffman M. A mouse bleeding model to study oral anticoagulants. *Thromb Res* 2014;**133 Suppl 1**:S6-8.

35. van Ryn J, Schurer J, Kink-Eiband M, Clemens A. Reversal of dabigatran-induced bleeding by coagulation factor concentrates in a rat-tail bleeding model and lack of effect on assays of coagulation. *Anesthesiology* 2014;**120**:1429-40.

36. Perzborn E, Gruber A, Tinel H, Marzec UM, Buetehorn U, Buchmueller A, Heitmeier S, Laux V. Reversal of rivaroxaban anticoagulation by haemostatic agents in rats and primates. *Thromb Haemost* 2013;**110**:162-72.

37. Pragst I, Zeitler SH, Doerr B, Kaspereit FJ, Herzog E, Dickneite G, van Ryn J. Reversal of dabigatran anticoagulation by prothrombin complex concentrate (Beriplex P/N) in a rabbit model. *J Thromb Haemost* 2012;**10**:1841-8.

38. Eerenberg ES, Kamphuisen PW, Sijpkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. *Circulation* 2011;**124**:1573-9.

39. Marlu R, Hodaj E, Paris A, Albaladejo P, Cracowski JL, Pernod G. Effect of non-specific reversal agents on anticoagulant activity of dabigatran and rivaroxaban: a randomised crossover ex vivo study in healthy volunteers. *Thromb Haemost* 2012;**108**:217-24.

40. Khoo TL, Weatherburn C, Kershaw G, Reddel CJ, Curnow J, Dunkley S. The use of FEIBA(R) in the correction of coagulation abnormalities induced by dabigatran. *Int J Lab Hematol* 2013;**35**:222-4.

41. Zhou W, Schwarting S, Illanes S, Liesz A, Middelhoff M, Zorn M, Bendszus M, Heiland S, van Ryn J, Veltkamp R. Hemostatic therapy in experimental intracerebral hemorrhage associated with the direct thrombin inhibitor dabigatran. *Stroke* 2011;**42**:3594-9.

42. Lambourne MD, Eltringham-Smith LJ, Gataiance S, Arnold DM, Crowther MA, Sheffield WP. Prothrombin complex concentrates reduce blood loss in murine coagulopathy induced by warfarin, but not in that induced by dabigatran etexilate. *J Thromb Haemost* 2012;**10**:1830-40.

43. Honickel M, Maron B, van Ryn J, Braunschweig T, Ten Cate H, Spronk HM, Rossaint R, Grottke O. Therapy with activated prothrombin complex concentrate is effective in reducing dabigatran-associated blood loss in a porcine polytrauma model. *Thromb Haemost* 2015;**115**.

44. Honickel M, Treutler S, van Ryn J, Tillmann S, Rossaint R, Grottke O. Reversal of dabigatran anticoagulation ex vivo: Porcine study comparing prothrombin complex concentrates and idarucizumab. *Thromb Haemost* 2015;**113**.

45. Grottke O, Honickel M, Van Ryn J, Ten Cate H, Spronk H, Rossaint R. ABSTRACT: Dabigatran-induced anticoagulant and bleeding effects can be reversed with both prothrombin complex concentrates and a specific antidote (idarucizumab) in a lethal porcine polytrauma model. *European Heart Journal* 2014;**35**:863-863.

46. Lillo-Le Louet A, Wolf M, Soufir L, Galbois A, Dumenil AS, Offenstadt G, Samama MM. Life-threatening bleeding in four patients with an unusual excessive response to dabigatran: implications for emergency surgery and resuscitation. *Thromb Haemost* 2012;**108**:583-5.

47. Diaz MQ, Borobia AM, Nunez MA, Virto AM, Fabra S, Casado MS, Garcia-Erce JA, Samama CM. Use of prothrombin complex concentrates for urgent reversal of dabigatran in the Emergency Department. *Haematologica* 2013;**98**:e143-4.

48. Dager WE, Gosselin RC, Roberts AJ. Reversing dabigatran in life-threatening bleeding occurring during cardiac ablation with factor eight inhibitor bypassing activity. *Crit Care Med* 2013;**41**:e42-6.

49. Dumkow LE, Voss JR, Peters M, Jennings DL. Reversal of dabigatran-induced bleeding with a prothrombin complex concentrate and fresh frozen plasma. *Am J Health Syst Pharm* 2012;**69**:1646-50.

50. Warkentin TE, Margetts P, Connolly SJ, Lamy A, Ricci C, Eikelboom JW. Recombinant factor VIIa (rFVIIa) and hemodialysis to manage massive dabigatran-associated postcardiac surgery bleeding. *Blood* 2012;**119**:2172-4.

51. Levi M, Moore KT, Castillejos CF, Kubitza D, Berkowitz SD, Goldhaber SZ, Raghoebar M, Patel MR, Weitz JI, Levy JH. Comparison of three-factor and four-factor prothrombin complex concentrates regarding reversal of the anticoagulant effects of rivaroxaban in healthy volunteers. *J Thromb Haemost* 2014;**12**:1428-36.

52. Korber MK, Langer E, Ziemer S, Perzborn E, Gericke C, Heymann C. Measurement and reversal of prophylactic and therapeutic peak levels of rivaroxaban: an in vitro study. *Clin Appl Thromb Hemost* 2014;**20**:735-40.

53. Dinkelaar J, Molenaar PJ, Ninivaggi M, de Laat B, Brinkman HJ, Leyte A. In vitro assessment, using thrombin generation, of the applicability of prothrombin complex concentrate as an antidote for Rivaroxaban. *J Thromb Haemost* 2013;**11**:1111-8.

54. Perzborn E, Heitmeier S, Laux V, Buchmuller A. Reversal of rivaroxaban-induced anticoagulation with prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant activated factor VII in vitro. *Thromb Res* 2014;**133**:671-81.

55. Godier A, Miclot A, Le Bonniec B, Durand M, Fischer AM, Emmerich J, Marchand-Leroux C, Lecompte T, Samama CM. Evaluation of prothrombin complex concentrate and recombinant activated factor VII to reverse rivaroxaban in a rabbit model. *Anesthesiology* 2012;**116**:94-102.

56. Zhou W, Zorn M, Nawroth P, Butehorn U, Perzborn E, Heitmeier S, Veltkamp R. Hemostatic therapy in experimental intracerebral hemorrhage associated with rivaroxaban. *Stroke* 2013;**44**:771-8.

57. Escolar G, Fernandez-Gallego V, Arellano-Rodrigo E, Roquer J, Reverter JC, Sanz VV, Molina P, Lopez-Vilchez I, Diaz-Ricart M, Galan AM. Reversal of apixaban induced alterations in hemostasis by different coagulation factor concentrates: significance of studies in vitro with circulating human blood. *PLoS One* 2013;**8**:e78696.

58. Martin AC, Le Bonniec B, Fischer AM, Marchand-Leroux C, Gaussem P, Samama CM, Godier A. Evaluation of recombinant activated factor VII, prothrombin complex concentrate, and fibrinogen concentrate to reverse apixaban in a rabbit model of bleeding and thrombosis. *Int J Cardiol* 2013;**168**:4228-33.

59. Halim AB, Samama MM, Mendell J. Ex vivo reversal of the anticoagulant effects of edoxaban. *Thromb Res* 2014;**134**:909-13.

60. Fukuda T, Honda Y, Kamisato C, Morishima Y, Shibano T. Reversal of anticoagulant effects of edoxaban, an oral, direct factor Xa inhibitor, with haemostatic agents. *Thromb Haemost* 2012;**107**:253-9.

61. Halim AB, Li Y, Stein E, Mendell J. ABSTRACT: Low concentrations of rhFVIIa or FEIBA significantly and rapidly reverse the anticoagulant effects of supratherapeutic edoxaban. *Blood* 2011;**118**:560-560.

62. Zahir H, Brown KS, Vandell AG, Desai M, Maa JF, Dishy V, Lomeli B, Feussner A, Feng W, He L, Grosso MA, Lanz HJ, Antman EM. Edoxaban effects on bleeding following punch biopsy and reversal by a 4-factor prothrombin complex concentrate. *Circulation* 2015;**131**:82-90.

63. Herzog E, Kaspereit F, Krege W, Doerr B, Mueller-Cohrs J, Pragst I, Morishima Y, Dickneite G. Effective reversal of edoxaban-associated bleeding with four-factor prothrombin complex concentrate in a rabbit model of acute hemorrhage. *Anesthesiology* 2015;**122**:387-98.

64. Lindahl TL, Baghaei F, Blixter IF, Gustafsson KM, Stigendal L, Sten-Linder M, Strandberg K, Hillarp A. Effects of the oral, direct thrombin inhibitor dabigatran on five common coagulation assays. *Thromb Haemost* 2011;**105**:371-8.

65. Hillarp A, Baghaei F, Fagerberg Blixter I, Gustafsson KM, Stigendal L, Sten-Linder M, Strandberg K, Lindahl TL. Effects of the oral, direct factor Xa inhibitor rivaroxaban on commonly used coagulation assays. *J Thromb Haemost* 2011;**9**:133-9.

66. Hillarp A, Gustafsson KM, Faxalv L, Strandberg K, Baghaei F, Fagerberg Blixter I, Berndtsson M, Lindahl TL. Effects of the oral, direct factor Xa inhibitor apixaban on routine coagulation assays and anti-FXa assays. *J Thromb Haemost* 2014;**12**:1545-53.

67. Maegdefessel L, Linde T, Krapiec F, Hamilton K, Steinseifer U, van Ryn J, Raaz U, Buerke M, Werdan K, Schlitt A. In vitro comparison of dabigatran, unfractionated heparin, and low-molecular-weight heparin in preventing thrombus formation on mechanical heart valves. *Thromb Res* 2010;**126**:e196-200.

68. Wolzt M, Gouya G, Kapiotis S, Becka M, Mueck W, Kubitza D. Open-label, randomized study of the effect of rivaroxaban with or without acetylsalicylic acid on thrombus formation in a perfusion chamber. *Thromb Res* 2013;**132**:240-7.

69. Fukuda T, Tsuji N, Honda Y, Kamisato C, Morishima Y, Shibano T. Comparison of antithrombotic efficacy between edoxaban, a direct factor Xa inhibitor, and fondaparinux, an indirect factor Xa inhibitor under low and high shear rates. *Thromb Haemost* 2011;**106**:1062-8.

70. Wong PC, Jiang X. Apixaban, a direct factor Xa inhibitor, inhibits tissue-factor induced human platelet aggregation in vitro: comparison with direct inhibitors of factor VIIa, XIa and thrombin. *Thromb Haemost* 2010;**104**:302-10.

71. Graff J, von Hentig N, Misselwitz F, Kubitza D, Becka M, Breddin HK, Harder S. Effects of the oral, direct factor xa inhibitor rivaroxaban on platelet-induced thrombin generation and prothrombinase activity. *J Clin Pharmacol* 2007;**47**:1398-407.

72. Perzborn E, Heitmeier S, Laux V. Effects of Rivaroxaban on Platelet Activation and Platelet-Coagulation Pathway Interaction: In Vitro and In Vivo Studies. *J Cardiovasc Pharmacol Ther* 2015.

73. Ringwala SM, Dibattiste PM, Schneider DJ. Effects on platelet function of a direct acting antagonist of coagulation factor Xa. *J Thromb Thrombolysis* 2012;**34**:291-6.

74. 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.

75. 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.

76. 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.

77. Biemond BJ, Perzborn E, Friederich PW, Levi M, Buetehorn U, Buller 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.

78. Schumacher WA, Bostwick JS, Stewart AB, Steinbacher TE, Xin B, Wong PC. Effect of the direct factor Xa inhibitor apixaban in rat models of thrombosis and hemostasis. *J Cardiovasc Pharmacol* 2010;**55**:609-16.

79. Grottke O, Honickel M, Van Ryn J, Ten Cate H, Spronk H, Rossaint R. Dabigatran-induced anticoagulant and bleeding effects can be reversed with both prothrombin complex concentrates and a specific antidote (idarucizumab) in a lethal porcine polytrauma model. *European Heart Journal* 2014;**35**:863-863.