

Reversing the new oral anticoagulants with prothrombin complex concentrates (PCCs): what is the evidence?

Gerhard Dickneite¹; Maureane Hoffman²

¹CSL Behring, Preclinical R&D, Marburg, Germany; ²Department of Pathology, Duke University Medical Center, Durham, North Carolina, USA

Summary

Newer oral anticoagulants offer several advantages over traditional agents (e.g. warfarin), but they are still associated with a bleeding risk and currently there is no validated reversal treatment for them. While there is little support for the use of fresh frozen plasma, and limited data available on the effects of activated recombinant factor VII, pre-clinical data suggest that prothrombin complex concentrates (PCCs) may have potential in this setting. PCCs are currently used to successfully reverse warfarin-induced anticoagulation; however, clinical evidence for their use with new oral anticoagulants is lacking, with most of the available data coming from preclinical animal studies. Furthermore, there appears to be variation in the ability of different PCCs to reverse the coagulopathy induced by the new anticoagulants, and a lack of correlation between the reversal of laboratory test results and

the reversal of anticoagulant-induced bleeding. Although there have been encouraging results, care must be taken in generalising findings from animal models and nonbleeding human subjects to the situation in bleeding patients. Ultimately, more evidence supporting anticoagulation reversal for new anticoagulants is needed, particularly regarding the treatment of bleeding in human patients in a clinical setting. According to the current evidence, use of PCCs may be considered a reasonable approach in dire clinical situations; however, a consensus has not yet been reached regarding PCC use or dosing, due to lack of clinical data.

Keywords

Prothrombin complex concentrate, direct thrombin inhibitors, FXa inhibitors, new oral anticoagulants, anticoagulant reversal

Correspondence to:

Prof. Dr. Gerhard Dickneite
Preclinical R&D, CSL Behring
PO Box 1230, 35002 Marburg, Germany
Tel.: +49 6421 39 2306, Fax: +49 6421 39 4663
E-mail: Gerhard.Dickneite@cslbehring.com

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Introduction

Until recently, vitamin K antagonists (VKAs), such as warfarin, have been the only oral option for anticoagulation therapy, but these agents have a delayed onset of action, interact with a number of foods and drugs and require routine monitoring (1). In an attempt to address these shortcomings and improve on the bleeding-related safety profile of traditional agents, new oral anticoagulants have been developed. While VKAs act by inhibiting the post-translational modification of vitamin K-dependent coagulation factors (II, VII, IX and X), the new anticoagulants are each a direct inhibitor of a single factor in the coagulation cascade: factor IIa (FIIa, thrombin; e.g. dabigatran etexilate [Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany]) or FXa (rivaroxaban [Bayer Pharma AG, Berlin, Germany], apixaban [Bristol-Myers Squibb/Pfizer EEIG, Uxbridge, UK] and edoxaban [Daiichi Sankyo Company Ltd, Tokyo, Japan]) (2, 3). These direct inhibitors have demonstrated favourable risk/benefit profiles in large clinical trials when compared with VKAs (4, 5). The new agents are associated with low potential for food and drug interactions, have a rapid and reliable onset of action and a relatively short half-life. This allows for a predictable anticoagulant effect which obviates the need for routine laboratory monitoring (6).

Although the new oral anticoagulants have been demonstrated to be at least as efficacious as traditional anticoagulants in a clinical

trial setting (2), bleeding still remains an important complication (► Table 1). While it appears that relatively few patients will require a reversal treatment, viable options to reverse the anticoagulant effects of the new agents are still required (7).

Prothrombin complex concentrates (PCCs) are the treatment of choice for VKA reversal. PCCs contain significant quantities of vitamin K-dependent factors II, IX and X, with either no (or very little) FVII (3-factor [3F]-PCC) or sufficient amounts of FVII (4-factor [4F]-PCC) (8). PCCs have been shown to reverse VKA-related coagulopathy more rapidly and completely than other interventions, such as fresh-frozen plasma (FFP) (9–14). Co-administration of PCCs and vitamin K is now typically recommended in many countries as the optimal approach for urgent VKA reversal (15–19), though FFP remains the standard treatment in the USA.

Reversal strategies for the new oral anticoagulants have not been established; however, a number of strategies have been proposed. There is little rationale for the use of FFP, and limited data on the effects of activated recombinant FVII (rFVIIa) (20). There is more extensive literature on the reversal of the new anticoagulants by PCCs. However, the studies use different models and the results are not entirely consistent. As mentioned above, 3F-PCCs contain high concentrations of coagulation factors II, IX and X and low and/or variable amounts of FVII, whereas 4F-PCCs additionally contain high levels of FVII. Raising the levels of these factors can enhance thrombin generation in *in vitro* models. Thus,

there is a rationale for hypothesising that they could overcome the anticoagulant effects of FIIa and FXa inhibitors (21). This use of PCCs would be mechanistically different from their use in reversing the effects of VKAs because administration of PCCs to patients on these direct inhibitors is an attempt to overcome or 'bypass' the effect of the inhibitor by raising the levels of vitamin K-dependent factors to supranormal levels. Thus, the situation is more complicated than simply replacing factors that are deficient. The effectiveness of this strategy and the appropriate doses of PCCs required still need to be established.

The aim of this article is to review the currently available data on the use of PCCs to reverse the effects of the new oral anticoagulants. All articles addressing the topic of this review were identified jointly by the authors by independent PubMed searches and by consultation with other experts in the field.

Overview of reversal data

FIIa inhibitors

Dabigatran

Reversal with PCCs: human studies

The impact of PCCs on the reversal of dabigatran-induced coagulation abnormalities has been assessed in a number of studies in humans: a phase I study in healthy male volunteers (21), an *ex vivo* study using blood samples from 10 healthy male subjects (22) and an *in vitro* cell-based model (23). In the phase I double-blind study, 12 healthy male volunteers received dabigatran 150 mg or placebo. Dabigatran significantly prolonged activated partial thromboplastin time (aPTT; 59.4 vs 33.6 seconds [sec]; $p < 0.05$), ecarin clotting time (ECT; 69 vs 33 sec; $p = 0.002$) and thrombin

Table 1: Efficacy and bleeding risks of new oral anticoagulants in key phase III trials. *combined endpoint of major and clinically relevant non-major bleeding. AF = atrial fibrillation; CNS = central nervous system; DVT = deep-vein thrombosis; GI = gastrointestinal; PE = pulmonary embolism; VTE = venous thromboembolism.

Drug	Com-parator	Endpoint	Efficacy vs comparator	Bleeding-related safety vs comparator			
				Major bleeding	Gastrointestinal bleeding	Intracranial haemorrhage	Fatal/life-threatening bleeding
Direct FIIa inhibitors							
Dabigatran (38)	Warfarin	Prevention of stroke of systemic embolism in patients with AF	Superior 1.11% vs 1.69%	3.11% vs 3.36%	1.51% vs 1.02%	0.30% vs 0.74%	1.45% vs 1.80%
Dabigatran (41)	Warfarin	Prevention of recurrent VTE in patients with acute VTE	Non-inferior 2.4% vs 2.1%	1.6% vs 1.9%	4.2% vs 2.8%	0.0% vs 0.2%	0.08% vs 0.08%
Direct FXa inhibitors							
Rivaroxaban (40)	Warfarin	Prevention of stroke and non-CNS systemic embolism in patients with AF	Non-inferior 1.7% vs 2.2%	3.6% vs 3.4%	No data available	0.5% vs 0.7%	0.2% vs 0.5%
Rivaroxaban (59)	Warfarin	Prevention of stroke and non-CNS systemic embolism in patients with AF	Superior 1.26% vs 2.61%	3.00% vs 3.59%	0.9% vs 1.9% (major GI bleeding)	0.8% vs 1.6%	0.16% vs 0.47% (fatal)
Rivaroxaban (60)	Enoxaparin	Prevention of recurrent VTE in patients with pulmonary embolism	Non-inferior 2.1% vs 1.8%	1.1% vs 2.2%	No data available	<0.1% vs 0.4% (non-fatal)	<0.1% vs 0.1%
Apixaban (61, 62)	Warfarin	Prevention of stroke or systemic embolism in patients with AF	Superior 1.27% vs 1.60%	2.13% vs 3.09%	0.76% vs 0.86%	0.33% vs 0.80%	No data available
Apixaban (63)	Enoxaparin	Prevention of DVT and PE (knee surgery patients)	Did not meet non-inferiority criteria (9.0% vs 8.8%)	0.7% vs 1.4%	<0.1% vs 0.4%	0.0% vs <0.1%	0.0% vs <0.1%
Apixaban (64)	Enoxaparin	Prevention of DVT and PE (hip surgery patients)	Superior 1.4% vs 3.9%	0.8% vs 0.7%	0.1% vs 0.0%	No data available	0.0% vs 0.0%
Edoxaban (39)	Enoxaparin	Prevention of VTE (hip surgery patients)	Superior 2.4% vs 6.9%	2.6% vs 3.7%*	No data available	No data available	No data available
		Prevention of VTE (knee surgery patients)	Superior 7.4% vs 13.9%	6.2% vs 3.7%*			

time (TT; >120 sec, upper limit for TT), compared with baseline. Administration of a 4F-PCC (Cofact[®]; Sanquin; Amsterdam, the Netherlands) was unable to correct any of these coagulation parameters; its effects on actual bleeding were not assessed.

In the *ex vivo* study, administration of a single therapeutic dose of dabigatran (150 mg) to healthy volunteers resulted in a modest alteration in the kinetics of thrombin generation in calibration automated thrombinography (CAT) assays (22). There was a 20% reduction from baseline in the area under the curve (AUC) of the endogenous thrombin potential (ETP) with no significant change in the peak thrombin level attained. The lag time was significantly increased from 2.16 to 3.78 minutes (min) and the time to peak from 4.23 to 5.40 min (22). Blood samples were treated with the 4F-PCC Kanokad[®] (LFB, Courtaboef, France) dosed at 0.25, 0.5 or 1 U/ml (corresponding to administered doses of 12.5–50 U/kg), as well as the activated PCC FEIBA[®] (Factor VIII Inhibitor Bypassing Activity, Baxter Healthcare Corp, Westlake Village, CA, USA) dosed at 0.25, 0.5, 1 or 2 U/ml (corresponding to doses of 20–160 U/kg). The authors also tested rFVIIa (NovoSeven[®], Novo Nordisk, Copenhagen, Denmark). Both Kanokad[®] and FEIBA[®] increased the ETP and peak thrombin level in a dose-dependent manner. The lowest concentrations normalised the ETP, while higher concentrations increased thrombin generation to values up to 2.5 times baseline. Addition of Kanokad[®] did not shorten the lag time, while treatment with FEIBA[®] was also able to shorten the lag time back to baseline in this model. rFVIIa shortened the lag time without altering the peak or ETP, thus normalising the thrombin generation tests in this particular model, in accordance with previous results obtained with PCC/rFVIIa and VKAs (24).

The effects of a different 4F-PCC (Beriplex[®], CSL Behring, Marburg, Germany) on parameters of thrombin generation were investigated using a cell-based model, which allowed for testing of multiple levels of dabigatran and PCC in the presence of freshly isolated platelets (23). In this study, increasing concentrations of dabigatran (0.4–2 μ M) progressively decreased the rate and peak level of thrombin generation, as well as the AUC, while increasing the lag time before onset of thrombin generation. The lowest concentration of dabigatran tested in this study (0.4 μ M) was chosen to approximate the peak therapeutic level reached *in vivo*, while the two higher doses would correspond to supratherapeutic levels. Beriplex[®] (1 U/ml) was able to normalise most parameters of thrombin generation (rate, peak and AUC), but did not shorten the lag time. At the lowest level of dabigatran, Beriplex[®] increased the thrombin peak and AUC to levels significantly above baseline.

The common coagulation assays (PT, aPTT) primarily measure the lag before onset of clot formation. Thus, the failure to correct the lag time, even though thrombin generation was enhanced, may explain why a PCC could improve haemostasis in the presence of dabigatran, but not correct the coagulation assays to a similar degree (23).

Reversal with PCCs: animal studies

At least six animal studies have investigated the effect of PCCs on dabigatran-induced bleeding parameters, two studies each in

mice, rats and rabbits (25–30). In one of the murine studies, the effects of a 4F-PCC (Octaplex[®], Octapharma, Vienna, Austria) on bleeding time were investigated in mice pre-treated with warfarin or dabigatran (26). While administration of a low dose of Octaplex[®] (14.3 IU/kg) was efficacious in normalising warfarin-induced blood loss and bleeding time, it failed to significantly reduce bleeding time in mice treated with dabigatran. Moreover, administration of Octaplex[®] did not significantly reduce blood loss in mice treated with dabigatran prior to tail transection compared with control animals treated with saline. Octaplex[®] was also unable to normalise dabigatran-induced changes in TT or aPTT (26). It is possible that this study did not reveal a beneficial effect of Octaplex[®] in reversing dabigatran-induced anticoagulation because the dose used was too low. This dose of a PCC would be expected to reverse the effect of a VKA by replacing the deficient factors; however, other studies suggest that a higher dose of PCC is required to “bypass” the effect of a direct thrombin inhibitor (25, 27–29).

The other murine study evaluated the volume of intracerebral haemorrhage induced by collagenase injection in animals given dabigatran (4.5 or 9.0 mg/kg) (29). Thirty minutes after induction of haemorrhage, mice were injected with saline, Beriplex[®] (25, 50 or 100 U/kg), murine FFP or human rFVIIa. Haematoma expansion was monitored by serial magnetic resonance imaging, and the final volume of intracerebral haemorrhage was assessed by histopathology. Dabigatran increased haematoma expansion in a dose-dependent manner. Beriplex[®] at 50 and 100 U/kg prevented the excess haematoma growth associated with dabigatran administration (both doses). FFP reduced haematoma expansion at the low, but not the higher dose of dabigatran. Human rFVIIa was ineffective at reversing the effects induced by either dose of dabigatran. Dabigatran also prolonged the tail vein bleeding time, and only the 100 U/kg dose of Beriplex[®] was able to reduce the bleeding time to values close to baseline (29).

In an *in vivo* study in rats, administration of dabigatran resulted in a three-fold increase in the time to haemostasis (495 vs 171 sec for control) in a tail-bleeding model, and a three- to five-fold increase in coagulation parameters (aPTT, TT and ECT) (28). Treatment with 4F-PCCs (Beriplex[®] 35 U/kg or Octaplex[®] 40 U/kg) completely reversed the dabigatran-induced prolonged bleeding time within 5 min, and this effect was sustained over the 2-hour (h) assessment period. Notably, the reversal of the prolongation of bleeding time by these 4F-PCCs did not correlate with any reversal of the prolonged clotting tests (TT, aPTT and ECT), suggesting that these clinical systemic coagulation parameters may not predict reversal of bleeding (28). It is interesting that a different dose of PCC was found to be effective in normalising the tail bleeding time in this rat study compared with the previous mouse study (29).

While several publications have reported the ability of 4F-PCCs to reverse dabigatran-induced bleeding, there are fewer reports on the effects of 3F-PCCs. One *in vivo* rat study (30) tested the ability of 3F-PCCs to reverse bleeding following administration of dabigatran. Dabigatran administration (30 mg/kg) resulted in a 2.8-fold increase in bleeding time (470 vs 165 sec for control),

which was normalised within 5 min of treatment with 3F-PCC (Profilnine[®], Grifols Biologicals Inc., Los Angeles, CA, USA or Bebulin[®], Baxter AG, Vienna, Austria). However, normalisation of the bleeding time was only sustained for 30 min, with 2-h values not differing between the treatment and control groups (30). This is in contrast with the results obtained in the previous study using 4F-PCCs (28), in which bleeding was prevented up to 2 h post-wounding. As demonstrated with 4F-PCCs (28), the reversal of bleeding time with 3F-PCC was not associated with improvements in laboratory tests (aPTT, PT and ECT). These two studies suggest that the levels of FVII may play a role in the reversal of bleeding by PCCs.

Finally, two *in vivo* studies in rabbits evaluated whether a PCC could effectively reverse the effects of dabigatran (25, 27). In the first of these studies (27), intravenous administration of dabigatran (0.4 mg/kg active metabolite) significantly increased time to haemostasis, and blood loss during the 30min observation period after a kidney incision. Administration of Beriplex[®] (20, 35 or 50 IU/kg) reduced the excess bleeding and shortened the time to haemostasis in a dose-dependent manner. The 50 IU/kg dose of Beriplex[®] reduced blood loss to the range observed for control animals. Companion studies on thrombin generation were performed by spiking different levels of PCC into blood samples obtained from animals that had been given different doses of dabigatran (0.1, 0.2, 0.3, 0.4 and 0.5 mg/kg). Peak thrombin generation was dose-dependently reduced by dabigatran. Addition of Beriplex[®] restored peak thrombin to the normal range at all except the highest dabigatran dose. However, the concentration of Beriplex[®] required to do so increased as the dabigatran dose was increased, and higher concentrations of Beriplex[®] raised the peak thrombin to supranormal levels. At the highest dabigatran dose, none of the concentrations of Beriplex[®] tested were able to significantly improve the peak thrombin level. Other parameters of thrombin generation were not reported in this study. While the peak thrombin level in thrombin generation assays correlated well with PCC administration, the PT remained prolonged in some of the treated animals, even at the highest PCC dose. Furthermore, the aPTT was unaffected by administration of PCC, providing further evidence that these standard coagulation assays do not reflect the PCC-related improvements in haemostasis.

The second of these studies evaluated both the efficacy and thrombotic safety of Beriplex[®] during reversal of dabigatran-induced coagulopathy in a rabbit model combining the assessment of arterial-venous (AV) shunt occlusion and acute kidney injury (25). Administration of dabigatran (75, 200 and 450 µg/kg; plasma levels were kept elevated by continuous intravenous infusion of dabigatran) reduced the incidence of thrombotic occlusion, prolonged the thrombotic occlusion time and reduced thrombus wet weight following AV shunting. Furthermore, dabigatran 200 and 450 µg/kg resulted in a marked bleeding signal following standardised kidney injury. Treatment with Beriplex[®] (50 and 300 U/kg) was able to significantly reduce bleeding signals in a dose-dependent manner for each of the tested dabigatran doses, but was unable to fully reverse the anticoagulation effects of dabigatran doses ≥ 200 µg/kg. Histopathological analysis did not reveal any evidence

of thrombosis in the animals given Beriplex[®] to reverse the effects of dabigatran.

FXa inhibitors

Rivaroxaban

Reversal with PCCs: human studies

The phase I and *ex vivo* studies with dabigatran described earlier also included assessments of the effects of PCCs on rivaroxaban-induced anticoagulation (21, 22). In the randomised, double-blind, placebo-controlled, phase I study in healthy male volunteers, rivaroxaban significantly prolonged PT (15.8 vs 12.3 sec; $p < 0.001$) and inhibited ETP (51% vs 92%; $p = 0.002$) compared with baseline (21). Administration of Cofact[®] fully normalised both PT prolongation and ETP inhibition. This is in contrast to the lack of reversal of dabigatran-induced effects on clotting tests observed in the same study.

In the *ex vivo* study using blood samples from 10 healthy male subjects, administration of rivaroxaban resulted in a 22% reduction in ETP and a 3-fold reduction in thrombin peak, and more than doubled the lag time and time to peak compared with baseline (22). The 4F-PCC Kanokad[®] (0.25, 0.5 or 1 U/ml; corresponding to administered doses of 12.5–50 U/kg) increased the ETP and the thrombin peak in a dose-dependent manner. The two lowest concentrations were able to normalise the ETP, while the highest concentration (1 U/ml) increased the ETP to a level approximately double that observed at baseline. Addition of Kanokad[®] did not significantly decrease the lag time or time to peak (22). The activated PCC, FEIBA[®] (0.25, 0.5, 1 or 2 U/ml), increased the ETP in the presence of rivaroxaban even more substantially than Kanokad[®], with 0.5, 1 and 2 U/ml concentrations all increasing the ETP to a level significantly above baseline. Both Kanokad[®] and FEIBA[®] also increased the peak; however, only the two highest FEIBA[®] concentrations were able to restore the peak to baseline levels. Kanokad[®] did not significantly shorten the lag time. FEIBA[®] did shorten the lag time, but did not restore it to baseline at any of the concentrations tested (22). Addition of rFVIIa (NovoSeven[®]), also tested in this study, had no effect on the ETP or the peak of thrombin generation but significantly decreased the lag time (although not to baseline levels) (22).

Reversal of rivaroxaban-induced anticoagulation was also evaluated in an *in vitro* study using plasma and whole blood samples from healthy volunteers. In this study, administration of rivaroxaban prolonged the lag time and decreased the peak and AUC of thrombin generation in two different commercial thrombin generation assays (31). In both assays, addition of 4F-PCC (Cofact[®]) was unable to correct the effects of rivaroxaban on lag time, but did increase thrombin generation AUC in a dose-dependent manner. The extent of the correction of rivaroxaban-induced decrease in AUC was not only dependent on the 4F-PCC dose used but also on the tissue factor concentration used to initiate the assay and on whether the assay was conducted in plasma or whole blood. Indeed, normalisation, and even overcorrection, of AUC was observed in whole blood, but not in plateletpoor plasma

(31). This study clearly demonstrates that the parameters of the thrombin generation assay strongly influence the results, and that the addition of cellular components will strongly increase the PCC response.

Reversal with PCCs: animal studies

Three animal studies have investigated the effects of PCCs on rivaroxaban anticoagulation, one each in mice, rats and rabbits. A mouse intracerebral haemorrhage model, similar to the one used to assess the impact of PCC on dabigatran anticoagulation (29), was used to investigate the effectiveness of different haemostatic factors for the prevention of haematoma expansion (32). Administration of rivaroxaban (30 mg/kg) was found to significantly increase haematoma volume induced by intrastriatal collagenase injection compared with non-anticoagulated control animals. Beriplex® 25, 50 or 100 U/kg reduced haematoma expansion in a dose-dependent manner, with the two highest doses significantly reversing the anticoagulant effects of rivaroxaban ($p < 0.05$). Although the lowest dose of Beriplex® (25 U/kg) did not significantly reduce the haematoma volume, it did significantly improve neurological deficits resulting from intracranial haemorrhage in the rivaroxaban-treated animals.

An *in vivo* study in rats assessed whether the anticoagulant effects of high-dose rivaroxaban (2 mg/kg) could be neutralised by Beriplex® (25 and 50 U/kg), FEIBA® (50 and 100 U/kg) or rFVIIa (100 and 400 µg/kg, NovoSeven®) (33). Relative to baseline, rivaroxaban significantly increased bleeding time (5.4-fold), prolonged PT (6.4-fold) and decreased levels of thrombin-antithrombin complexes (TAT). The highest doses of the three reversal agents all significantly reduced mesenteric bleeding time compared with rivaroxaban alone. In addition, Beriplex® (50 U/kg), FEIBA® (both doses) and rFVIIa (400 µg/kg) all partially reversed rivaroxaban-induced PT prolongation. Beriplex® (50 U/kg) completely reversed rivaroxaban inhibition of thrombin formation as measured by TAT. However, FEIBA® was only able to partially reverse the inhibition of thrombin generation and rFVIIa had no effect on this parameter (33).

This same study further examined the effectiveness of FEIBA® infusion (50 U/kg over 25 min) or a bolus injection of FVIIa (210 µg/kg) in reversing the effects of high-dose rivaroxaban in baboons (33). Non-activated PCC was not tested in this model. Both agents shortened, but did not normalise, the PT. FEIBA® normalised the bleeding time, but this effect did not persist after the end of the infusion despite the sustained PT reduction. rFVIIa partially reversed the rivaroxaban-induced prolongation of the bleeding time, and the effect persisted for at least 30 min after the injection. Since only one dose of each agent was tested, it is difficult to directly compare the effectiveness of rFVIIa and FEIBA®. However, both agents seem to have the potential to improve haemostasis in the presence of rivaroxaban.

The effects of PCC on bleeding and thrombosis induced by rivaroxaban were also investigated in an *in vivo* study in rabbits (34). The dose of rivaroxaban was titrated to a level (5 mg/kg) that significantly increased blood loss compared with control rabbits in a hepato-splenic bleeding model (25.1 vs 11.5 g). At this dose, the

animal also had an increase in ear immersion bleeding time (100 vs 75.5 sec), PT (3-fold) and aPTT (1.7-fold). Neither administration of a 4F-PCC, Kaskadil® (LFB, Les Ullis, France) nor that of rFVIIa (NovoSeven®) normalised the amount of hepato-splenic bleeding. Treatment with rFVIIa, but not Kaskadil®, significantly shortened the bleeding time. Both Kaskadil® and rFVIIa normalised the aPTT and partially normalised PT. Both Kaskadil® and rFVIIa improved the ETP in thrombin generation assays, but did not increase the thrombin peak, and rFVIIa shortened the lag time while Kaskadil® did not. This study again highlights the discrepancy between effects on bleeding and effects on conventional coagulation tests. In addition, the effects of the treatments on thrombin generation assays are also less pronounced than in other studies.

Edoxaban

Reversal with PCCs: *in vitro* studies

Reversal of edoxaban-induced anticoagulation using PCCs was evaluated in an *in vitro* study using human plasma (35). This study showed that edoxaban administration significantly prolonged PT (31 and 43 sec for 150 and 300 ng/ml concentration, respectively) compared with baseline (18 sec). Administration of 4F-PCC (PPSB®-HT, Nihon Pharmaceutical Co., Ltd, Tokyo, Japan) was able to significantly shorten the prolonged PT ($p < 0.001$ vs edoxaban alone for doses between 0.15 and 1.5 U/ml).

Discussion

Newer anticoagulants offer simpler dosing regimens, more predictable pharmacodynamic and pharmacokinetic profiles, and have few or no laboratory monitoring requirements; traits that make them more attractive than traditional VKAs (1). Despite the clear advantages of the new agents, management of bleeding remains a significant concern (36–42), due to the lack of validated reversal strategies (43).

The current recommended pathways for the management of new oral anticoagulant-induced bleeding include: discontinuation of treatment, identification of the bleeding source, local/surgical haemostatic control and blood volume replacement. In patients with life-threatening bleeding, the use of haemostatic agents such as rFVIIa, activated and non-activated PCCs may be considered (44–48). Because the prothrombotic potential of activated PCCs and rFVIIa may be higher than that of non-activated PCCs, some guidelines recommend the use of non-activated PCCs as the first choice for reversal of new oral anticoagulant effects (47). In addition, haemodialysis may be considered for emergency reversal of dabigatran-induced anticoagulation (due to its low plasma protein binding), although it may be difficult to perform this procedure in a haemodynamically unstable patient (44–46).

Selective reversal agents, such as antibody fragments to bind FIIa inhibitors, or a truncated form of enzymatically inactive FXa to bind FXa inhibitors, are currently in development for the reversal of new oral anticoagulants (49–51). Dabigatran antibody frag-

ments have been shown to selectively reverse dabigatran anticoagulant activity in *in vitro* and *ex vivo* models of haemostasis, and to reverse bleeding induced by a 30 mg/kg dose of dabigatran to baseline levels in a rat *in vivo* model of blood loss (51). Similarly, the specific FXa inhibitor antidote PRT064445 (Portola Pharmaceuticals, South San Francisco, CA, USA) has been studied in a number of *in vitro*, *ex vivo* and *in vivo* models of haemostasis (50). Results obtained from *in vitro* and *ex vivo* models have suggested a complete and dose-dependent reversal of rivaroxaban inhibitory activity and of rivaroxaban-induced PT prolongation, while administration of the antidote alone was not associated with any pro-coagulant or anticoagulant activity in an *ex vivo* clotting assay in human plasma. It seems likely that these specific reversal agents will be available in the foreseeable future. However, a class of general “bypassing” agents for the new oral anticoagulants still has clinical utility. Specific reversal agents are not yet available, and the length of the approval process is unknown. In addition, specific and selective reversal agents would not be universal reversal agents, meaning that hospitals would have to stock multiple products to ensure availability of the most appropriate treatment de-

pending on the oral anticoagulant used. Finally, patients may not know or not be able to articulate which agent they have been taking. Thus, the availability of a general reversal agent is likely to be useful.

In preclinical and phase I settings, administration of PCCs has demonstrated encouraging results for the reversal of some new oral anticoagulants (► Table 2). However, there appears to be some variation in the ability of different PCCs to reverse the coagulopathy induced by the new anticoagulants and more data are needed to explore these differences further. For example, some 4F-PCCs, such as Cofact[®], are able to reverse the effects of rivaroxaban but not dabigatran (21), while others, such as Kaskadil[®], are able to reverse changes in coagulation parameters but have not been shown to affect the bleeding induced by administration of an FXa inhibitor such as rivaroxaban (34). Data from studies investigating the effects of Beriplex[®] are promising, with Beriplex[®] shown to decrease bleeding time and prevent haematoma growth induced by both dabigatran (27-29) and rivaroxaban (32, 52). Limited data are available to show the effectiveness of 3F-PCCs and some 3F-PCCs, such as Prothrombinex[®]-VF (a PCC

Table 2: Summary of new oral anticoagulants reversal data with prothrombin complex concentrates. ↑ = increased; ↓ = decreased; INTEM assay assesses intrinsic pathway using ellagic acid as activator; EXTEM assay assesses extrinsic pathway using tissue factor as activator. aPTT = activated partial thromboplastin time; AUC = area under the curve; dPT = diluted prothrombin time; CAT = calibrated automated thrombography; ECT = ecarin clotting time; ETP = endogenous thrombin potential; PCC = prothrombin complex concentrates; TAT = thrombin-antithrombin complex; TG = thrombin generation; TGA = thrombin generation assay; TT = thrombin time; TTP = time to peak concentration of thrombin; V_{max} = maximum velocity.

Study drug	Brand of PCC	Model	Results	
			Coagulation parameters	Bleeding
Dabigatran				
Non-activated PCCs				
Zhou et al., 2011 (29)	Beriplex [®]	Mouse intracerebral haemorrhage model	No data	↓
Pragst et al., 2012 (27)	Beriplex [®]	Kidney bleeding / coagulation rabbit	↓ PT and normalisation of V _{max} for thrombus formation but no change in aPTT	↓
Hoffman et al., 2012 (23)	Beriplex [®]	Cellular <i>in vitro</i> coagulation (human)	↑ rate, peak and total amount of thrombin generation	No data
van Ryn et al., 2011 (28)	Beriplex [®] /Octaplex [®]	Tail cut model / coagulation rat	No change in TT, aPTT or ECT, but PT reversed to baseline	↓
Herzog et al., 2013 (65)	Beriplex [®]	Arterial venous shunt / coagulation rabbit	↓ thrombin generation, but no change in PT or dPT	↓
Lambourne et al., 2012 (26)	Octaplex [®]	Tail clip bleeding / coagulation mouse	No change in TT or aPTT	No change
Eerenberg et al., 2011 (21)	Cofact [®]	Human volunteers / coagulation	No change in aPTT, ETP lag time, ECT or TT	No data
Marlu et al., 2012 (22)	Kanokad [®]	<i>Ex vivo</i> coagulation model (human)	↑ in EPT-AUC and thrombin peak, no change in lag time	No data
van Ryn et al., 2012 (30)	Profilin [®] /Bebulin [®]	Tail cut model / coagulation rat	No change in aPTT, PT or ECT	↓
Activated PCC				
Marlu et al., 2012 (22)	FEIBA [®]	<i>Ex vivo</i> coagulation model (human)	↑ in EPT-AUC and thrombin peak, ↓ lag time	No data

Table 2: Continued

Study drug	Brand of PCC	Model	Results	
			Coagulation parameters	Bleeding
Rivaroxaban				
Non-activated PCCs				
Perzborn et al., 2013 (33)	Beriplex®	Mesenteric bleeding / coagulation, rats	↑ TAT; ↓ PT, but reversal was partial	↓
Zhou et al., 2013 (32)	Beriplex®	Mouse intracerebral haemorrhage model	No change in PT, but ↑ in plasma activity of FII, FIX, FX and proteins C and S	↓ (haematoma volume)
Godier et al., 2012 (34)	Kaskadil®	Hepatosplenic bleeding / coagulation, rabbits	↓ clot formation time (INTEM) and clotting time (EXTEM); moderate improvement in thrombin potential in TGA	No change
Eerenberg et al., 2012 (21)	Cofact®	Human volunteers / coagulation	↓ PT and EPT, both to normal levels	No data
Dinkelaar et al., 2013 (31)	Cofact®	<i>In vitro</i> coagulation model (human plasma and whole blood)	↓ PT (partial reversal), ↑ ETP-AUC and TG AUC (CAT plasma assay), normalisation of AUC (CAT whole blood assay), no change in lag time	No data
Marlu et al., 2012 (22)	Kanokad®	<i>Ex vivo</i> coagulation model (human)	↑ ETP-AUC and thrombin peak, slight ↓ in lag time, no change in TTP	No data
Activated PCC				
Marlu et al., 2012 (22)	FEIBA®	<i>Ex vivo</i> coagulation model (human)	↑ in EPT-AUC and thrombin peak, ↓ lag time and TTP	No data
Perzborn et al., 2013 (33)	FEIBA®	Mesenteric bleeding / coagulation, rats	↑ TAT and ↓ PT, but reversal was partial in both cases	↓
Perzborn et al., 2013 (33)	FEIBA®	Mesenteric bleeding / coagulation, baboons	↓ PT, but reversal was partial	↓ (not sustained post-infusion end)
Edoxaban				
Fukuda et al., 2012 (35)	PPSP®-HT	<i>In vitro</i> coagulation model (human plasma)	↓ PT	No data

commonly available in Australia and New Zealand, ► Table 3), have not yet been studied for the reversal of new oral anticoagulants. The dabigatran reversal study by van Ryn et al. demonstrated that two different 3F-PCCs were able to normalise the increased bleeding time following dabigatran administration (30). However, the effect of 3F-PCC is short lasting in comparison to 4F-PCC (28). The most likely explanation is that the small amount of FVII in the 3F-PCCs (see ► Table 3) is sufficient to prevent bleeding at early time points but, because of the short half-life of FVII, plasma levels quickly decrease below a critical level.

There is a wide variation in the amount of factors II, VII, IX and X, and antithrombotic proteins (proteins C and S) contained in the different PCCs (53). As PCCs are dosed based on FIX, the actual administered doses of the other components can vary considerably among different PCCs (► Table 3). PCCs also contain different amounts of anticoagulants, such as heparin and anti-thrombin. In light of this, results obtained for one PCC might not be directly applicable to another PCC. At the current time it is not known which components of PCCs are most important to their

apparent ability to improve haemostasis in the presence of the new oral anticoagulants.

While various PCCs have been used in reversal studies, no studies specifically designed as head-to-head comparisons have been published yet. Due to the heterogeneity of the studies conducted so far, including differences in study designs, animal models used, measured endpoints and doses of both PCCs and oral anticoagulants investigated, conclusions about the relative effectiveness of different PCCs would be inappropriate. However, it seems likely that the different PCCs will not be entirely equivalent in their reversal effects.

Despite preclinical evidence demonstrating reversal of anticoagulant-induced changes in some laboratory parameters, the correlation between the reversal of these parameters and the reversal of bleeding has not been established. In particular, the standard coagulation assays appear not to be predictive of the ability of reversal agents to decrease bleeding related to new anticoagulant treatments (27, 28). This may be due to the relative insensitivity of some of these assays to the presence of the anticoagulant (54). At the current time, thrombin generation assays seem to have the

Table 3: Actual dose (IU) of factor II, VII and X and proteins C and S based on an administered FIX dose of 100 IU. 4F-PCCs: calculations of actual dose of coagulation factors were scaled-up based on a 100 IU dose of FIX and the mean clotting activity values (IU/ml) levels reported in Kalina et al. 2008 (53); 3F-PCC: ^avalues derived from Goodnough and Shander 2011 (57); ^bvalues based on Prothrombinex[®]-VF Product Information (58); 3F-PCC = 3 factor prothrombin complex concentrate; 4F-PCC = 4 factor prothrombin complex concentrate; N/A = not available.

PCC component	4F-PCCs					3F-PCCs		
	Beriplex [®]	Octaplex [®]	PPSB [®]	Kaskadil [®]	Cofact [®]	Bebulin ^{®a}	Profilnine ^{®a}	Prothrombinex [®] -VF ^b
FII	107	141	139	162	132	100	≤150	100
FVII	56	72	62	123	58	<21	≤35	N/A
FIX	100	100	100	100	100	100	100	100
FX	143	109	110	148	115	100	≤100	100
Protein C	94	108	91	65	N/A	N/A	N/A	N/A
Protein S	62	54	19	51	17	N/A	N/A	N/A

most promise for assessing the degree of the haemostatic defect due to the novel anticoagulants. However, it appears that reversal agents do not have to return all parameters of thrombin generation to “normal” in order to have a significant haemostatic effect. Thus, the correlation between results of thrombin generation assays and haemostatic function in this setting requires further study.

Another concern raised by the studies conducted to date is the possibility that over-correction of coagulation parameters following high doses of PCCs could translate into an increased risk of thromboembolic events. Historically, the association of (activated) PCCs with thromboembolic events has been a concern; however, two recent comprehensive reviews have shown that the risk of thromboembolic events in patients treated with non-activated PCCs for VKA reversal is low and that underlying disease and dosing may be important factors in increasing risk (55, 56). The presence of coagulation inhibitors (antithrombin, proteins C and S) in modern formulations of PCCs has improved the safety profile of modern PCCs relative to those available in the 1970s and 1980s (56), and may play a role in moderating this risk. Indeed, pharmacovigilance data collected on some of the currently available PCCs, such as Beriplex[®] and Cofact[®], which have been administered to thousands of patients, have demonstrated that there have been no proven cases of treatment-related thromboembolism (56). Ultimately, the risk of thrombosis with a PCC must be weighed against the risk of continued bleeding in the patient.

In spite of the promising data on the ability of PCCs to reverse the effects of thrombin and FXa inhibitors, it is important to note that we have no high-quality data in human patients with clinically relevant bleeding. As the pathophysiology of events may only be partially reflected by experimental models, these agents need to be evaluated further in the clinical setting (32).

Conclusions

In summary, we can draw the following conclusions on the reversal of the effects of the new oral anticoagulants from the existing literature:

- PCCs (including activated PCCs) show promise for reversing the anticoagulant effects of the new oral anticoagulants.
- Conventional laboratory assays do not correlate well with bleeding or reversal of anticoagulation in this setting; thrombin generation assays appear to have the best predictive value. However, it should be noted that there are significant differences in the methods for conducting such assays, which can complicate comparisons between studies.
- Both activated (e.g. FEIBA[®]) and non-activated PCCs correct most parameters of thrombin generation assays (initial rate, peak and ETP) *in vitro*. However, non-activated PCCs seem to lack the ability to correct the lag time before onset of thrombin generation while FEIBA[®] partially corrects the lag time.
- It is possible to “overshoot” and enhance parameters of thrombin generation to supranormal levels. This effect is more prominent with FEIBA[®] than non-activated PCCs. Thus, while FEIBA[®] might be more haemostatically effective than non-activated PCC, it may also pose a greater risk of thrombosis.
- The dosing and effectiveness of a strategy for reversal of the new oral anticoagulants probably depends on the level of the anticoagulant present.
- No studies have yet examined the effectiveness of any reversal strategy in bleeding human patients.

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Conflicts of interest

Prof. Dr Gerhard Dickneite is an employee of CSL Behring and owns CSL stock. Prof. Dr Maureane Hoffman received research funding from CSL Behring, Novo Nordisk and Boehringer Ingelheim and served as consultant for CSL Behring, Novo Nordisk, The Medicines Company, Baxter and Bristol-Myers Squibb.

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