

THE REVERSAL OF DIRECT ORAL ANTICOAGULANTS IN ANIMAL MODELS

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ABSTRACT—Several direct oral anticoagulants (DOACs), including direct thrombin and factor Xa inhibitors, have been approved as alternatives to vitamin K antagonist anticoagulants. As with any anticoagulant, DOAC use carries a risk of bleeding. In patients with major bleeding or needing urgent surgery, reversal of DOAC anticoagulation may be required, presenting a clinical challenge. The optimal strategy for DOAC reversal is being refined, and may include use of hemostatic agents such as prothrombin complex concentrates (PCCs; a source of concentrated clotting factors), or DOAC-specific antidotes (which bind their target DOAC to abrogate its activity). Though promising, most specific antidotes are still in development. Preclinical animal research is the key to establishing the efficacy and safety of potential reversal agents. Here, we summarize published preclinical animal studies on reversal of DOAC anticoagulation. These studies ($n=26$) were identified via a PubMed search, and used rodent, rabbit, pig, and non-human primate models. The larger of these animals have the advantages of similar blood volume/hemodynamics to humans, and can be used to model polytrauma. We find that in addition to varied species being used, there is variability in the models and assays used between studies; we suggest that blood loss (bleeding volume) is the most clinically relevant measure of DOAC anticoagulation-related bleeding and its reversal. The studies covered indicate that both PCCs and specific reversal agents have the potential to be used as part of a clinical strategy for DOAC reversal. For the future, we advocate the development and use of standardized, clinically, and pharmacologically relevant animal models to study novel DOAC reversal strategies.

KEYWORDS—Bleeding, NOAC, reversal, trauma

INTRODUCTION

For the treatment of thrombotic disorders, vitamin K antagonists (VKAs) have been successfully used as oral anticoagulants. However, this group of treatments carries several disadvantages, which include variable plasma concentrations (as a result of patient genotype, drug–drug interactions, and diet), delayed onset of anticoagulant effect, and a requirement for bridging with heparins (1, 2). These drawbacks may translate into cumbersome patient monitoring requirements and potentially put patients at risk of over- or under-dosing.

In response to the limitations of VKA therapy, direct oral anticoagulants (DOACs), inhibitors of thrombin, or factor (F) Xa (Fig. 1) have been developed. Compared with VKAs,

DOACs are characterized by more defined pharmacokinetic and pharmacodynamic profiles (3). Since the introduction of DOACs, the number of DOAC prescriptions has risen while VKA prescription numbers have fallen (4). Currently available DOACs comprise the direct thrombin (FIIa) inhibitor dabigatran (Pradaxa, Boehringer Ingelheim Pharma, Ingelheim, Germany) (5) and direct FXa inhibitors rivaroxaban (Xarelto, Bayer Pharma AG, Leverkusen, Germany), apixaban (Eliquis, Bristol-Myers Squibb/Pfizer, New York, NY) and edoxaban (Lixiana, Savaysa, Daiichi Sankyo, Tokyo, Japan) (5–8). DOAC efficacy compared with VKAs has been demonstrated in a number of large multicenter randomized controlled trials in atrial fibrillation (9–12), and in venous thromboembolism (13–15). Based on these data, DOACs have been licensed for indications including prophylaxis against venous thromboembolism in orthopedic surgery, as treatment in symptomatic venous or pulmonary thromboembolism, and stroke prevention in non-valvular atrial fibrillation (16–19).

In terms of safety, meta-analyses of randomized controlled trials suggest that DOACs are noninferior to VKAs for overall risk of bleeding complications (20, 21). In case of life-threatening bleeding events, or the need for emergency surgery, there are approved treatments for reversal of VKA anticoagulation (22), but strategies for reversal of DOAC anticoagulation in these situations are still evolving and remain a matter of investigation and debate. Based on expert recommendations for massive bleeding under DOAC treatment, several strategies have been proposed, as follows: immediate discontinuation of DOAC uptake, reduction of gastrointestinal uptake by active charcoal application or elevation of drug clearance by dialysis in case of recent dabigatran intake, and

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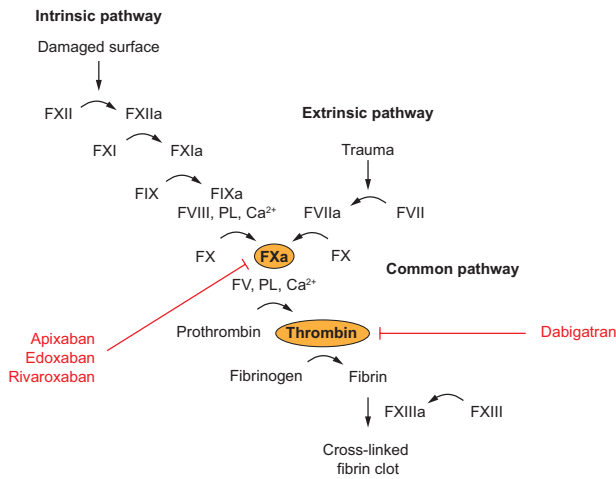


FIG. 1. **Mechanism of action of DOACs within the coagulation cascade.** DOAC indicates direct oral anticoagulant; F, factor; PL, phospholipid. DOACs specifically inhibit either thrombin (dabigatran) or FXa (apixaban, edoxaban, or rivaroxaban).

use of hemostatic agents such as activated and nonactivated prothrombin complex concentrates (PCCs) (23–26). PCCs comprise three-factor (3F)-PCCs (containing FII, FIX, and FX), four-factor (4F)-PCCs (which also include clinically relevant quantities of FVII), and activated (a)PCC, which also contains four factors, including activated FVII. Recombinant activated FVII (rFVIIa) is sometimes considered a treatment of last resort in DOAC-associated bleeding.

In addition to these reversal strategies, specific antidotes for DOACs are being developed (27). A humanized antibody fragment (fragment antigen-binding; Fab), idarucizumab (Praxbind, Boehringer Ingelheim Pharma), was licensed in 2015 as a specific reversal agent for dabigatran (28–30). Andexanet alfa, in development at the time of writing, is a recombinant FXa derivative, designed to be catalytically inactive and to reverse the anticoagulant activity of both direct and indirect FXa inhibitors by competitive binding (31, 32). Also in development, and currently in Phase 2 trials, is PER977 (aripazine, ciraparantag), a broad-spectrum reversal agent for anticoagulants, including low-molecular-weight heparin, unfractionated heparin, and DOACs (33, 34). A zymogen-like engineered FXa is showing promise in pre-clinical experiments as a universal DOAC reversal agent, demonstrating potential to reverse of the effects of FXa inhibitors and dabigatran (35). Finally, a trypsinized, site-mutated thrombin, γ T-S195A-IIa, is also in development as a dabigatran reversal agent, with development currently at a preclinical stage (36).

Here, we shall use “reversal” to mean “reversal of the anticoagulant effects of.” The mechanisms of action of hemostatic agents and specific antidotes in DOAC reversal are distinct. Specific antidotes (idarucizumab and andexanet alfa) abrogate the activity of DOACs, by competitive binding to their target DOAC with high affinity so that it is no longer available for target inhibition. PCCs, on the other hand, have an indirect effect. PCCs can accomplish “reversal” of the anticoagulant effects of thrombin (FIIa) inhibitors as they are a

source of FII (prothrombin), which is cleaved to thrombin, and also contain FIX, FX, and FVII, which augment thrombin generation. When there is a stoichiometric excess of thrombin over FIIa inhibitor, the inhibitory effect will be overcome. A similar logic applies for PCC reversal of FXa-inhibitor DOACs, since PCCs are a source of FX. In addition, the increased concentration of FII may also play a role, bypassing the FXa-inhibitor and increasing prothrombin cleavage. In either instance, PCCs provide a source of all vitamin K-dependent clotting factors, which can be activated in cases of bleeding, restoring coagulation.

Animal models provide a means to investigate and explore the reversal of DOAC anticoagulation via different strategies under standardized conditions. This review summarizes the current evidence for DOAC reversal, using all strategies, in preclinical animal studies.

LITERATURE SEARCH

We identified 26 original articles on DOAC reversal that used animal models. Literature search methodology is summarized in the Supplemental Information, <http://links.lww.com/SHK/A564>. Overall, 16, five, three, and two studies investigated the reversal of dabigatran, rivaroxaban, apixaban, and edoxaban, respectively (Tables 1–4). Below, we summarize key efficacy and safety findings from the totality of these studies, with particular attention to the model(s) used for each finding.

REVERSAL OF DABIGATRAN

In terms of reversal of anticoagulation, dabigatran is the most extensively studied of the DOACs. Various animal models have been used to investigate the potential of coagulation factor concentrates, the licensed specific antidote idarucizumab, and alternative strategies, for reversal of dabigatran anticoagulation (Table 1).

Reversal with coagulation factor concentrates

Small animal models—Tail vein bleeding time following transection, either in mice or in rats, is increased following dabigatran treatment, and is consistently reduced when these anticoagulated animals are treated with PCC. This has been demonstrated with 4F-PCC (Beriplex/Kcentra, CSL Behring, Marburg, Germany) 25 U/kg, 50 U/kg, or 100 U/kg in a dose-dependent manner (37), and with other PCCs or aPCCs administered at various doses (as detailed in Table 1) (38, 39). One study found significant reductions in bleeding time (with aPCC or with 4F-PCC+rFVIIa) but no accompanying reduction in bleeding volume (38). Notably, tail vein bleeding time can be more difficult to measure than total volume of blood loss, as transient cessation of bleeding may precede rebleeding (40). In addition, bleeding volume has been shown to be a more sensitive hemostatic measure (41).

Similar results have been obtained using a kidney incision model in rabbits, in which dabigatran treatment markedly increased blood loss. Dose-dependent reduction of bleeding time and bleeding volume was observed with a range of 4F-PCC (Beriplex) doses: 20 U/kg, 35 U/kg, and 50 U/kg, and a

TABLE 1. Dabigatran reversal studies

Reference	Species	Model	Dabigatran dose	Intervention	Bleeding endpoints	Surrogate endpoints
<i>Coagulation factor concentrate reversal</i> Zhou, 2011 (37)	Mouse	ICH	4.5 or 9 mg/kg, i.p.	4F-PCC (Beriplex) 25 U/kg, 50 U/kg, or 100 U/kg	Dose-dependent prevention of hematoma expansion Significant reduction in mortality (100 U/kg dose only) Dose-dependent reduction in bleeding time No effect on hematoma expansion	
Lambourne, 2012 (38)	Mouse	Tail bleeding	60 mg/kg, oral (1.5 mg)	rFVIIa (NovoSeven) 8 mg/kg 4F-PCC (Octaplex) 14.3 U/kg	No impact on mortality No effect on blood loss or bleeding time No effect on blood loss	No effect
van Ryn, 2014 (39)	Rat	Tail bleeding	30 mg/kg, oral	4F-PCC (Octaplex) 14.3 U/kg + rFVIIa (NiaStase) 3 mg/kg aPCC 100 U/kg rFVIIa (NiaStase) 3 mg/kg	Reduced bleeding time No effect on blood loss Reduced bleeding time No effect on blood loss or bleeding time Reduced bleeding time	Reduced aPTT Reduced TT Reduced aPTT
Sheffield, 2014 (36)	Mouse	Tail bleeding and carotid artery occlusion	13 mg/kg and 60 mg/kg	4F-PCC (Beriplex) 35 U/kg and 50 U/kg 4F-PCC (Octaplex) 40 U/kg 3F-PCC (Profilimine) 50 U/kg 3F-PCC (Bebulin) 60 U/kg aPCC 100 U/kg rFVIIa (NovoSeven) 90 µg/kg, 270 µg/kg and 500 µg/kg	Reduced bleeding time Reduced bleeding time Restored occlusive thrombus formation in FeCl ₃ -treated carotid arteries	Beriplex increased TG (CAT) Octaplex reduced TG (CAT) Increased TG (CAT) Increased TG (CAT) Reduced lag time, no effect on total TG (CAT)
Pragst, 2012 (42)	Rabbit	Standardized kidney incision	0.4 mg/kg, i.v.	4F-PCC (Beriplex) 20 U/kg, 35 U/kg, or 50 U/kg	Dose-dependent reduction in blood loss (normalized to baseline with 4F-PCC 50 U/kg) Reduced time to hemostasis	Dose-dependent shortening of PT Normalization of V _{max} for thrombus formation No change in aPTT
Herzog, 2014 (43)	Rabbit	Standardized kidney incision, various thrombosis models	75 µg/kg, 200 µg/kg, or 450 µg/kg i.v. boluses followed by continuous infusions	4F-PCC (Beriplex) 50 U/kg or 300 U/kg	Dose-dependent attenuation of blood loss Dose-dependent shortening of time to hemostasis (no hemostasis in dabigatran 450 µg/kg group)	Dose-dependent reversal of peak TG (over-correction with 4F-PCC 300 U/kg, CAT)
Grottke, 2014 (46)	Pig	Blunt liver trauma	30 mg/kg bid, 3 days, oral, then i.v. infusion to reach supratherapeutic levels	4F-PCC (Beriplex) 30 U/kg and 60 U/kg ex vivo		Reduced PT, CFT, and CT (thromboelastometry; EXTEM) No effect on aPTT or MCF

TABLE 1. (continued)

Reference	Species	Model	Dabigatran dose	Intervention	Bleeding endpoints	Surrogate endpoints
Honickel, 2015 (47)	Pig	Polytrauma (blunt liver trauma with bilateral femur fractures)	30 mg/kg bid, 3 days, oral, then i.v. infusion to reach 442 ± 138 ng/mL in plasma, given with or without TXA (Cyclocapron) 20 mg/kg plus FC (Hemocomplettan P) 80 mg/kg	aPCC 30 U/kg and 60 U/kg <i>ex vivo</i> rFVIIa (Novoseven) 90 µg/kg and 180 µg/kg <i>ex vivo</i> 4F-PCC (Beriplex, Cofact, Prothromplex, Octaplex) 30 U/kg and 60 U/kg <i>ex vivo</i> 3F-PCC (Bebulin Profiline) 30 U/kg and 60 U/kg <i>ex vivo</i>	Reduced blood loss and increased survival with PCC 50 U/kg or 100 U/kg No reduction in blood loss with PCC 25 U/kg	Reduced PT, CFT, and CT (thromboelastometry; EXTEM) No effect on aPTT or MCF No effect on PT, aPTT, CFT, CT, or MCF (thromboelastometry; EXTEM) Reduced PT, further reduced by FC/TXA Reduced TG lag time, increased ETP and peak TG (no effect of FC/TXA, CAT) No reduction in aPTT, some reduction with FC/TXA Reduced CT and CFT, increased V _{max} and MCF, all except CT enhanced with FC/TXA (thromboelastometry; EXTEM)
Honickel, 2015 (44)	Pig	Polytrauma (blunt liver trauma with bilateral femur fractures)	30 mg/kg bid, 3 days, oral, then i.v. infusion to reach 487 ± 161 ng/mL in plasma	4F-PCC (Beriplex) 25 U/kg, 50 U/kg, or 100 U/kg	Reduced blood loss and increased survival with PCC 50 U/kg or 100 U/kg No reduction in blood loss with PCC 25 U/kg	Normalization of all coagulation parameters with PCC 50 U/kg or 100 U/kg Normalization of all coagulation parameters except aPTT and ACT with PCC 25 U/kg
Honickel, 2016 (45)	Pig	Polytrauma (blunt liver trauma with bilateral femur fractures)	30 mg/kg bid, 3 days, oral, then i.v. infusion to reach supratherapeutic levels	aPCC 25 U/kg and 50 U/kg	Significant reduction in total blood loss and improved survival with aPCC 50 U/kg compared with aPCC 25 U/kg or saline	aPCC 50 U/kg: sustained improvement in PT, TG parameters (CAT), platelet aggregation, CT and CFT (thromboelastometry, EXTEM and INTEM [partial]). No change in aPTT or ACT aPCC 25 U/kg: transient partial correction of PT, CT, and CFT (thromboelastometry, EXTEM and INTEM), TG parameters (CAT), platelet aggregation
Specific antidote reversal Na, 2015 (49)	Mouse	ICH	2.25 mg/kg, 4.5 mg/kg, or 9 mg/kg, i.p.	2 mmol/kg, 4 mmol/kg, 8 mmol/kg, or 16 mmol/kg idarucizumab	Prevention of excess hematoma expansion, reduced mortality	Dose-dependent reversal of prolonged dTT and bleeding time γT-S195A-IIa returned dTT to baseline level
Sheffield, 2014 (36)	Mouse	Tail transection and occlusion of FeCl ₃ -treated carotid arteries by thrombus formation	13 mg/kg and 60 mg/kg	γT-S195A-IIa 6 mg/kg S195A-IIa 6 mg/kg FPR-IIa (dose not stated)	Bleeding time unaltered, γT-S195A-IIa restored occlusive thrombus formation	

TABLE 1. (continued)

Reference	Species	Model	Dabigatran dose	Intervention	Bleeding endpoints	Surrogate endpoints
Schiele, 2013 (48)	Rat	Tail bleeding	0.3 mmol/kg bolus + 0.1 mmol/kg/h over 50 min, i.v.	Idarucizumab 0.3 μ mol/kg single bolus injection		Reduction of TT and aPTT
Grottke, 2015 (50)	Pig	Blunt liver injury	30 mg/kg bid, 3 days, oral, then i.v. infusion to reach 1,161 \pm 372 ng/mL in plasma	Idarucizumab 30 mg/kg, 60 mg/kg, and 120 mg/kg	Dose-dependent reduction of total blood loss 100% survival in 60 mg/kg, and 120 mg/kg groups	Dose-dependent reduction in aPTT Dose-dependent increase in ETP (CAT) All coagulation measures returned to baseline with 120 mg/kg dose Neutralization of plasma concentrations of dabigatran Reduced PT, and aPTT further reduced by FC/TXA
Honickel, 2015 (47)	Pig	Polytrauma (blunt liver trauma with bilateral femur fractures)	30 mg/kg bid, 3 days, oral, then i.v. infusion to reach 442 \pm 138 ng/mL in plasma, given with or without TXA (Cycloacron) 20 mg/kg plus FC (Hemocompletan P) 80 mg/kg	Idarucizumab 30 mg/kg and 60 mg/kg <i>ex vivo</i>		Reduced TG lag time, increased ETP and peak TG (no effect of FC/TXA; smaller increases in TG than with PCC, CAT) Reduced CT and CFT, increased V_{max} and MCF, all except CT enhanced with FC/TXA (thromboelastometry; EXTEM)
Grottke, 2014 (46)	Pig	Blunt liver trauma	30 mg/kg bid, 3 days, oral, then i.v. infusion to reach supratherapeutic levels	Idarucizumab 30 mg/kg and 60 mg/kg, <i>ex vivo</i>		Reduced PT, aPTT CFT and CT (thromboelastometry; EXTEM) No effect on MCF
<i>Other means of reversal</i> Zhou, 2011 (37)	Mouse	ICH	4.5 mg/kg or 9 mg/kg, i.p.	Murine FFP 200 μ L	Prevention of excess hematoma expansion in dabigatran 4.5 mg/kg group (less effective than PCC) No impact on mortality No effect on bleeding time Lipid emulsion itself prolongs bleeding time	
Blum, 2013 (52)	Rat	Tail bleeding	15 mg/kg, oral	Lipid emulsion, 15 mL/kg by i.v. infusion over 7 min		
Levine, 2015 (55)	Rat	ACT measurement in withdrawn blood 24 h after drug administration	2 mg/kg, oral	Aminocaproic acid 820 mg/kg, s.c. Tranexamic acid 340 mg/kg, s.c.		No change in ACT No change in ACT
DeNino, 2015 (54)	Pig	Acute renal failure	20 mg/pig (approx. 70 kg)	Hemodialysis, ultrafiltration on cardiopulmonary bypass		No change in dabigatran level

ACT indicates activated clotting time; aPCC, activated PCC; aPTT, activated partial thromboplastin time; AT, antithrombin; bid, twice daily; CAT, calibrated automated thrombogram; CFT, clot formation time; CT, clotting time; dTT, diluted thrombin time; ETP, endogenous thrombin potential; FC, fibrinogen concentrate; FFP, fresh frozen plasma; FPA, fibrinopeptide A; FPR-IIa, FPR-chloromethyl ketone-treated thrombin; ICH, intracerebral hemorrhage; i.v., intravenously; MCF, maximum clot firmness; PCC, prothrombin complex concentrate; PT, prothrombin time; F, recombinant factor; ROTEM, rotational thromboelastometry; s.c., subcutaneously; TG, thrombin generation; TXA, tranexamic acid; V_{max} , maximal clot formation velocity.

TABLE 2. Rivaroxaban reversal studies

Reference	Species	Model	Rivaroxaban dose	Intervention	Bleeding endpoints	Surrogate endpoints
<i>Coagulation factor concentrate reversal</i> Zhou, 2013 (56)	Mouse	ICH	3 mg/kg, 10 mg/kg, or 30 mg/kg, oral	4F-PCC (Beriplex) 25 U/kg, 50 U/kg, or 100 U/kg	Dose-dependent prevention of excess hematoma expansion Improvement of neurological deficits	No significant reduction of prolonged PT Over-correction of factor deficiencies (2- to 8-fold increase in FII, FIX, and FX)
Godler, 2012 (57)	Rabbit	Bleeding (ear incision, hepatosplenic section) and arterial thrombosis	5 mg/kg, i.v.	rFVIIa (NovoSeven) 1 mg/kg 4F-PCC (Kaskadil) 40 U/mL	Prevention of excess hematoma expansion No reduction in blood loss	Significant reduction of prolonged PT Restoration of factor deficiencies (2-fold increase in FVII above normal) Normalized aPTT Partially corrected PT Moderate improvement in ETP (CAT) Normalized CFT in INTEM, reduced CFT and CT in EXTEM
Herzog, 2015 (58)	Rabbit	Standardized kidney incision	150 mg/kg, 300 mg/kg, and 450 mg/kg i.v. bolus	rFVIIa 150 µg/kg 4F-PCC (Beriplex) 25 U/kg, 50 U/kg, and 100 U/kg	No reduction in blood loss Decreased bleeding time Dose-dependent reduction in blood loss	Normalized aPTT Partially corrected PT Limited improvement in ETP, peak TG and lag time (CAT) Decreased CFT and increased MCF in INTEM; decreased CT in EXTEM Partial reversal of PT and WBCT but no reversal of aPTT
Perzborn, 2013 (59)	Rat	Mesenteric bleeding	2 mg/kg, i.v.	4F-PCC (Beriplex) 25 U/kg and 50 U/kg aPCC 50 U/kg and 100 U/kg rFVIIa 100 µg/kg, and 400 µg/kg	Dose-dependent reversal of time to hemostasis (both 150 µg/kg and 300 µg/kg rivaroxaban only) Significant reduction in bleeding time	Extrinsic TG: no change in peak TG and ETP (CAT) TG with PLs only: reversal of peak TG and ETP correlated with blood loss and time to hemostasis Partial reversal of prolonged PT, restoration of TAT levels with 50 U/kg dose
	Baboon	Standardized incision on volar surface of forearm	0.6 mg/kg i.v. bolus followed by continuous 0.6 mg/kg/h infusion	aPCC 50 U/kg rFVIIa 210 µg/kg	Dose-dependent reduction in bleeding time Significant reduction of bleeding time with 400 µg/kg Non-sustainable correction of bleeding time to the baseline Non-significant reduction of bleeding time	Partial restoration of TAT levels, dose-dependent partial reversal of prolonged PT Partial reduction of prolonged PT, no restoration of reduced TAT levels Sustained partial reduction in PT, increase of TAT levels to 3-fold over baseline Sustained partial reduction of prolonged PT, no restoration of reduced TAT levels
<i>Specific antidote reversal</i> Lu, 2013 (32)	Rat	Coagulation studies	0.25 mg/kg/h i.v. infusion over a 30-min period 50 mg/kg, oral 1 mg/kg, i.v. bolus	Andexanet alfa 4 mg i.v. bolus over 5 min plus infusion (4 mg/h) for up to 90 min Andexanet alfa bolus i.v. injection (0.96 mg/mouse) Andexanet alfa bolus injection (75 mg/rabbit)	Significant reduction in increased blood loss Reduced blood loss by >85%	Correction of whole blood INR, significant reduction in anti-FXa activity Significant reduction in plasma anti-FXa activity Decreased peak anti-FXa activity by 98% Decreased PT by 74% and aPTT by 66% Nearly zeroed non-protein-bound fraction of rivaroxaban in plasma

aPCC indicates activated PCC; aPTT, activated partial thromboplastin time; CAT, calibrated automated thrombogram; CFT, clot formation time; CT, clotting time; ETP, endogenous thrombin potential; FC, fibrinogen concentrate; ICH, intracerebral hemorrhage; INR, international normalized ratio; i.p., intraperitoneally; i.v., intravenously; MCF, maximum clot firmness; PCC, prothrombin complex concentrate; PL, phospholipid; PT, prothrombin time; rF, recombinant factor; ROTEM, rotational thromboelastometry; s.c., subcutaneously; TAT, thrombin-antithrombin; TG, thrombin generation; WBCT, whole blood clotting time.

supratherapeutic 300 U/kg dose (42, 43). At high dabigatran doses (450 $\mu\text{g}/\text{kg}$), hemostasis was not attained even with 4F-PCC 300 U/kg (43).

Survival in a mouse model of intracerebral hemorrhage (ICH) with dabigatran anticoagulation has also been studied. Dose-dependent prevention of hematoma expansion was observed with 4F-PCC (Beriplex, 25 U/kg, 50 U/kg, or 100 U/kg), with reduced mortality in the group receiving the 100 U/kg dose. rFVIIa (8 mg/kg) reduced neither hematoma expansion nor mortality (37).

In terms of coagulation assays performed on dabigatran-anticoagulated small animals, thrombin generation (TG) was generally corrected by coagulation factor concentrates, with the degree of correction being dependent on the dose of dabigatran and of coagulation factor concentrates (38, 39, 42). Effects of factor concentrates on dabigatran-mediated increases in thrombin time (TT), prothrombin time (PT), and activated partial thromboplastin time (aPTT), where measured, were variable (38, 42).

Large animal models—A standardized porcine polytrauma model has been used to demonstrate the efficacy of treatments for dabigatran reversal, with blood loss as the primary endpoint. 4F-PCC (Beriplex) 25 U/kg had limited effects in this model, whereas the 50 U/kg and 100 U/kg doses were sufficient to reduce blood loss and increase survival to 100%, though there were signs that the supratherapeutic 100 U/kg dose induced an overactivation of coagulation as indicated by high levels of D-dimers (44). Similar findings were observed using aPCC: a dose of 25 U/kg did not reverse dabigatran effects on bleeding but a 50 U/kg dose improved blood loss and increased survival (45). Effects of dabigatran on TG and on coagulation parameters (PT, aPTT, and clotting time [CT] and clot formation time [CFT] as measured by rotational thromboelastometry [ROTEM]) were abrogated by the two higher 4F-PCC doses tested and by aPCC 50 U/kg (44, 45).

To obtain more insight into the effects of PCCs on dabigatran anticoagulation, coagulation assays were performed following *ex vivo* addition of these agents to blood obtained from traumatized pigs (46, 47). In these studies, aPCC 30 U/kg and 60 U/kg, and a range of different 4F- and 3F-PCCs used at doses of 30 U/kg and 60 U/kg, were able to dose-dependently restore TG, PT, and ROTEM measures (CT, CFT, and maximum clot firmness [MCF]). Post-trauma treatment of aPCC/PCC-treated animals with tranexamic acid (TXA) and fibrinogen concentrate (FC) (which themselves had no influence on blood loss without aPCC or PCC) led to further improvements in coagulation parameters, including aPTT, but not TG and ROTEM CT (47). The relevance of these *in vitro* coagulation endpoints for assessing DOAC reversal will be discussed later.

Reversal with specific antidotes

Small animal models—While idarucizumab is now licensed for dabigatran reversal (30), based on the results of a clinical study in dabigatran-anticoagulated patients (28), much of our understanding of its effects on coagulation has been derived from work in animal models.

In a rat model of anticoagulation with dabigatran, a single bolus injection of idarucizumab (0.3 $\mu\text{mol}/\text{kg}$) rapidly reversed dabigatran anticoagulant activity, as shown by sustained and complete reversal of TT and aPTT (48). Effects on bleeding were demonstrated in a mouse model of ICH, in which hematoma expansion (primary endpoint) and mortality were increased with dabigatran treatment (49); all doses of idarucizumab tested prevented excess hematoma expansion and reduced mortality versus control. The authors also observed reversal of dabigatran prolongation of tail vein bleeding time (49).

Large animal models—The effects of idarucizumab on bleeding in dabigatran anticoagulation have also been demonstrated in a porcine liver injury model (50). Here, blood loss post-trauma was approximately doubled following treatment with dabigatran. Idarucizumab (30 mg/kg, 60 mg/kg, and 120 mg/kg) treatment led to a dose-dependent reduction in total blood loss, and 100% of the 60 mg/kg and 120 mg/kg groups survived. All coagulation parameters, including aPTT, were normalized, with the degree of normalization depending on the idarucizumab dose (50).

The *ex vivo* studies of blood obtained from traumatized pigs described above for coagulation factor concentrates have also been conducted with idarucizumab. Idarucizumab administration reduced the plasma concentration of dabigatran below the lower limit of detection (46, 47). The studies found that dabigatran effects on all coagulation parameters tested were reversed by idarucizumab, though MCF was only partially corrected. The lack of full reversal of the MCF was attributed to low levels of fibrinogen and platelets due to bleeding. Though PCCs did not affect aPTT in these models, this parameter was normalized by idarucizumab, although the clinical relevance of this finding remains to be established. TXA/FC treatment allowed for further correction of many parameters by idarucizumab, including clot strength (MCF) (46, 47). The utility of coagulation assays for predicting blood loss is discussed later in this article.

Other methods of reversal

FFP was used as a comparator in one of the above studies of ICH in dabigatran-anticoagulated mice (37). FFP 200 μL reduced hematoma expansion with dabigatran 4.5 mg/kg, but had no impact on hematoma expansion with the higher 9 mg/kg dose. In contrast with the results obtained for PCC, FFP 200 μL had no impact on mortality (37).

Administration of intravenous lipid emulsion (ILE) is an established method for elimination of lipophilic drugs (51). However, despite the lipophilic nature of dabigatran, infusion of ILE had no influence on bleeding time following tail incision in dabigatran-anticoagulated rats (52).

As the majority of dabigatran is not plasma protein bound (53), DeNino et al. (54) speculated that ultrafiltration might eliminate dabigatran. However, in their porcine model of acute renal failure, ultrafiltration during cardiopulmonary bypass achieved only 3% reduction in dabigatran levels (54).

Finally, neither aminocaproic acid nor tranexamic acid administered to dabigatran-anticoagulated rats made a

TABLE 3. Apixaban reversal studies

Reference	Species	Model	Apixaban dose	Intervention	Bleeding endpoints	Surrogate endpoints
<i>Coagulation factor concentrate reversal</i>						
Martin, 2013 (62)	Rabbit	Hepatosplenic section, Folts model	0.4 mg/kg i.v. bolus of apixaban + 0.6 mg/kg/h continuous perfusion	4F-PCC (Kanokad) 60 U/kg	No reduction in hepatosplenic blood loss No change in ear immersion bleeding time	No change in PT, aPTT substantially shortened Normalization of the CFT in INTEM, reduction of CT in EXTEM, correction of MCF in FIBTEM TG: lag time improved, ETP restored, no effect on peak height (CAT) No effect on PT or aPTT Increased MCF in INTEM, EXTEM and FIBTEM (FIBTEM showed 4-fold over-correction), prolongation of CT in EXTEM and INTEM TG: doubling of ETP, improvement in peak height (CAT) Normalization of PT, aPTT substantially shortened Correction of CT and CFT in EXTEM, INTEM and FIBTEM; incomplete correction of MCF in FIBTEM TG: Partial improvement in lag time and ETP; no effect on peak height (CAT)
				FC (Clottafact) 300 mg/kg	Increased hepatosplenic blood loss Prolongation of ear immersion bleeding time	Extrinsic TG: no restoration (CAT) Intrinsic TG: Significant increases in peak TG and ETP (all doses)
				rFVIIa (NovoSeven) 240 µg/kg	No reduction in hepatosplenic blood loss Partial correction of ear immersion bleeding time	Dose-dependent partial reversal of PT and WBCT. Slight reversal of aPTT
Herzog, 2015 (61)	Rabbit	Standardized kidney incision	1,200 µg/kg i.v. bolus of apixaban	4F-PCC (Beriplex) 6.25 U/kg, 12.5 U/kg, 25 U/kg, 50 U/kg, 75 U/kg, or 100 U/kg	Significant reductions in total blood loss (for 4F-PCC doses ≥ 12.5 U/kg) Significant reductions in time to hemostasis for all doses tested	Extrinsic TG: no restoration (CAT) Intrinsic TG: Significant increases in peak TG and ETP (all doses)
<i>Specific antidote reversal</i>						
Lu, 2013 (32)	Rat	Coagulation studies	0.5 mg/kg/h i.v. infusion over 30 min	6 mg i.v. bolus of andexanet alfa in 5 min + 6 mg/h infusion for 90 min		Correction of whole-blood INR Significant reduction in anti-FXa activity

aPTT indicates activated partial thromboplastin time; CAT, calibrated automated thrombogram; CT, clotting time; ETP, endogenous thrombin potential; F, factor; FC, fibrinogen concentrate; INR, international normalized ratio; MCF, maximum clot firmness; P, prothrombin time; rF, recombinant factor; TG, thrombin generation; WBCT, whole blood clotting time.

TABLE 4. Edoxaban reversal studies

Reference	Model	Study design	Edoxaban dose	Intervention	Bleeding endpoints	Surrogate endpoints
Coagulation factor concentrate reversal Fukuda, 2012 (63)	Rat	Planta template bleeding and venous thrombosis	0.3 mg/kg/h or 1 mg/kg/h i.v. infusion	4F-PCC (PPSB-HT)*	Reduced bleeding time at 100 U/kg dose Dose-dependent reduction in bleeding time	Dose-dependent reversal of prolonged PT (less pronounced than with aPCC or rFVIIa) Dose-dependent reversal of prolonged PT Dose-dependent reversal of prolonged PT
Herzog, 2015 (64)	Rabbit	Standardized kidney incision	1,200 µg/kg i.v. bolus	aPCC 50 U/kg and 100 U/kg rFVIIa (NovoSeven) 0.3 mg/kg, 1 mg/kg and 3 mg/kg 4F-PCC (Beriplex) 25 U/kg, 50 U/kg and 75 U/kg	Significant reduction in bleeding time and blood loss with 50 U/kg and 75 U/kg doses	Correction of TG: ETP, peak TG and lag time (CAT) Overcorrection of ETP for lower doses of edoxaban (50 ng/mL, 200 ng/mL) Reduced PT No change in aPTT

*Used *in vitro* in edoxaban-spiked pooled human plasma samples.

aPCC indicates activated PCC; aPTT, activated partial thromboplastin time; CAT, calibrated automated thrombogram; ETP, endogenous thrombin potential; i.v., intravenously; PCC, prothrombin complex concentrate; PT, prothrombin time; rF, recombinant factor; TG, thrombin generation.

difference to activated clotting time measured using blood drawn after 24 h (55).

Thromboembolic risk with dabigatran reversal agents

With any treatment designed to enhance coagulation, there is a potential for thromboembolic events (TEEs). TEEs have not been systematically studied in the setting of dabigatran reversal, though reports from clinical use suggest that they do occur (28).

A single preclinical study has addressed the potential for TEEs with PCC use in the reversal of dabigatran anticoagulation (43). In this study, arteriovenous shunt occlusion in 4F-PCC 50 U/kg or 300 U/kg-treated rabbits was delayed or fully abolished with dabigatran doses (above 75 µg/kg). Upon histological examination, grade 1 (minimal) pulmonary thrombi were found in all rabbits treated with a supratherapeutic 4F-PCC dose of 300 U/kg. The frequency of pulmonary thrombi declined progressively with increasing concomitant dabigatran doses. Together, these observations suggest that the protective anticoagulant effect of dabigatran is retained in the presence of supratherapeutic levels of PCC.

Some thrombotic safety observations have also been made in the porcine trauma models described above. With 4F-PCC 100 U/kg, there was an increase in markers related to coagulation activation and fibrinolysis, including thrombin–antithrombin (TAT) complexes, D-dimers, and fibrinopeptide A. Together, these were suggestive of coagulation factor activation and secondary hyperfibrinolysis, though no histologic evidence of thrombosis was found (44). Comparable experiments with aPCC also found increases in TAT complexes and D-dimers at higher aPCC doses, but neither histopathologic examination nor immunostaining gave evidence of thrombus formation (45). Similarly, on post-mortem examination, no evidence of thromboembolism was found in pigs treated with dabigatran and idarucizumab (30 mg/kg, 60 mg/kg, and 120 mg/kg) (50).

REVERSAL OF RIVAROXABAN

For FXa inhibitor anticoagulation reversal in preclinical models, rivaroxaban is the best-studied agent. The utilities of both coagulation factor concentrates and of the specific reversal agent andexanet alfa have been investigated using rodents, rabbits, and also baboons (Table 2).

Reversal with coagulation factor concentrates

Small animal models—In a mouse ICH model, rivaroxaban treatment substantially increased hematoma volume compared with nonanticoagulated mice; 4F-PCC (Beriplex) prevented excess hematoma expansion and improved associated neurological deficits in a dose-dependent manner. While there was over-correction of factor deficiencies, there was no significant effect of 4F-PCC on PT. In the same model, administration of rFVIIa prevented excess hematoma expansion and partially corrected PT (56).

The action of 4F-PCC as a rivaroxaban reversal agent has also been demonstrated in rabbits (57, 58). While 4F-PCC (Kaskadil 40 U/mL or Beriplex 25 U/kg, 50 U/kg, or 100 U/kg) did not reduce the increase in bleeding seen with administration

of rivaroxaban 5 mg/kg or 450 µg/kg in hepatosplenic and kidney incision bleeding models, Beriplex dose-dependently reduced blood loss and bleeding time at lower rivaroxaban doses in the kidney incision model. Interestingly, rFVIIa reduced bleeding time but not blood loss in the hepatosplenic bleeding model. While Godier et al. (57) observed correction of rivaroxaban effects on aPTT and ROTEM measurements with 4F-PCC, Herzog et al. (58) did not see effects on aPTT. Both groups noted partial effects of 4F-PCC on rivaroxaban-mediated PT prolongation. Herzog et al. found that TG assay results were highly dependent on the reagent used; a phospholipid-only reagent appeared more sensitive to 4F-PCC activity. Whole blood clotting time also correlated with *in vivo* endpoints.

Work in a rat model of mesenteric bleeding showed that 4F-PCC (Beriplex 50 U/kg), rFVIIa (400 µg/kg), or aPCC (50 U/kg or 100 U/kg) can reverse the effects of rivaroxaban (2 mg/kg) on blood loss, with 4F-PCC 50 U/kg reducing bleeding time the most (59). All three treatments reversed rivaroxaban-prolonged PT, but reversal was most pronounced in the presence of rFVIIa, and was weakest with the nonactivated 4F-PCC. Despite its strong effect on PT, the only agent with no effect on TG or TAT levels was rFVIIa.

Large animal models—Baboons have been used as a large primate model to study the effects of aPCC (50 U/kg) or rFVIIa (210 µg/kg) on bleeding time after rivaroxaban treatment (59). Of these two treatments, aPCC gave a greater reduction in bleeding time (to pre-rivaroxaban baseline values), though bleeding time increased again after the end of infusion. Two of the 11 animals experienced a substantial increase in bleeding time after the end of aPCC infusion, accompanied by increases in TAT, suggestive of a mild consumptive coagulopathy.

Reversal with specific antidotes

Small animal models—In a comprehensive evaluation of andexanet alfa in rivaroxaban-anticoagulated animals, Lu et al. (32) demonstrated that bolus injection of this antidote (4 mg) followed by sustained infusion (4 mg/h) reduced increases in blood loss observed after rivaroxaban treatment in a mouse tail vein bleeding model and in a rabbit liver laceration model. By anti-FXa assay, andexanet alfa significantly reduced FXa activity in mouse, rat, and rabbit (32). In rats, the plasma molar ratios of andexanet alfa to rivaroxaban were 2.1, 1.7, and 1.3 at 35, 60, and 90 min, respectively. A rivaroxaban-treated rat model was used to demonstrate international normalized ratio (INR) correction with andexanet alfa, and in the rivaroxaban-treated rabbit model, PT and aPTT were reduced by 74% and 66%, respectively, compared with rivaroxaban plus vehicle control (32). Andexanet alfa has not yet been studied in large animal models.

Thromboembolic risk with rivaroxaban reversal agents

In a modified Folts model (in which thrombosis is induced by stenosis and injury on the carotid artery and detected by cyclic flow reductions [CFRs] (60)) used in rivaroxaban-treated rabbits as mentioned earlier, neither 4F-PCC nor rFVIIa increased thrombotic risk; neither led to any CFRs in rabbits treated with rivaroxaban (57).

REVERSAL OF APIXABAN

Three preclinical studies have investigated apixaban reversal, all using small animal models (Table 3).

Reversal with coagulation factor concentrates

Small animal models—Two studies have used a rabbit model to evaluate factor concentrates for apixaban reversal. In both studies, administration of apixaban resulted in significant increases in bleeding parameters. While Herzog et al. (61) found significant reductions in blood loss and bleeding time (both primary endpoints) when dosing apixaban-anticoagulated rabbits with 4F-PCC (Beriplex; ≥ 12.5 U/kg), Martin et al. did not find reductions in blood loss in apixaban-anticoagulated rabbits dosed with 4F-PCC (Kanokad; 60 U/kg) or rFVIIa (240 µg/kg). Indeed, the latter group found increased blood loss when FC (300 mg/kg) was tested for anticoagulation reversal (62). However, apixaban only increased bleeding 1.4-fold in the study from Martin et al., which may have limited the scope to detect reversal (by contrast, Herzog et al. used a higher apixaban dose, which increased the bleeding volume approximately 11-fold, suggesting that injury type and severity may play a role in identifying treatment effects).

Effects on PT were variable: in the study by Martin et al., only rFVIIa normalized PT, while Herzog et al. found a dose-dependent reversal of apixaban effects on PT with 4F-PCC. Conversely, Herzog et al. did not observe alterations in aPTT with 4F-PCC, while Martin et al. noted substantial reductions in aPTT with both 4F-PCC and rFVIIa. Martin et al. (62) analyzed ROTEM parameters and generally found these were at least partially corrected by the factor concentrates they tested. MCF in FibTEM was very high (elevated 4-fold) with FC. Across the two studies, TG parameters (lag time, endogenous thrombin potential [ETP], peak height) were generally improved by the presence of coagulation factor concentrates; Herzog et al. (61) found that intrinsic TG was again most sensitive to PCC effects.

Reversal with specific antidotes

Small animal models—Though the study of andexanet alfa as a factor Xa reversal agent in rats primarily focussed on rivaroxaban reversal (described above), some investigation of effects on apixaban anticoagulation was also made. In apixaban-anticoagulated rats, andexanet alfa corrected the INR and caused a significant reduction in anti-FXa activity (32).

Thromboembolic risk with apixaban reversal agents

In the rabbit study by Martin et al. (62), neither aPCC, rFVIIa, nor FC, at the doses tested, led to CFRs in a Folts model, suggesting no induction of thrombosis with any of the evaluated products.

REVERSAL OF EDOXABAN

Only two published preclinical studies have investigated the reversal of edoxaban anticoagulation; both of which investigated PCCs (Table 4).

Reversal with coagulation factor concentrates

Small animal models—In a rat model of bleeding (63), aPCC 100 U/kg and all rFVIIa doses tested reversed edoxaban prolongation of bleeding time (4F-PCC was not tested in these experiments). *In vitro* analysis of edoxaban-spiked healthy human volunteer plasma showed that 4F-PCC, aPCC, and rFVIIa all reversed edoxaban prolongation of PT, in a dose-dependent manner, though the effects of 4F-PCC were less marked.

In the rabbit model of standardized kidney incision (64), edoxaban administration increased blood loss and time to hemostasis compared with control-treated animals (both primary endpoints); subsequent administration of 4F-PCC (Beriplex) significantly reduced these parameters. TG parameters (peak TG, lag time, and ETP) were corrected by 4F-PCC, with overcorrection of ETP observed at the lower edoxaban doses tested (≤ 200 ng/mL). As observed with PCC use for the reversal of other DOACs, aPTT was not altered by 4F-PCC.

Thromboembolic risk with edoxaban reversal agents

Venous thrombosis formation/potential was investigated for rFVIIa (though not for PCC or aPCC), and in the presence of edoxaban, rFVIIa-mediated thrombus formation was reduced (63).

The thrombogenicity of 4F-PCC (Beriplex 50 U/kg or 300 U/kg) was assessed in edoxaban-treated (300 μ g/kg or 600 μ g/kg) rabbits using a modified Wessler stasis model (65). No prothrombotic signal was observed in rabbits treated with a therapeutic dose of 4F-PCC (50 U/kg). Nonocclusive clots formed with the supratherapeutic dose of 4F-PCC (300 U/kg), but this was inhibited in the presence of edoxaban.

DISCUSSION

Use of animal models to evaluate DOAC reversal treatments

Unlike *in vitro* assay systems, animal models offer the opportunity to test anticoagulation reversal in the context of a complete coagulation system, both in terms of physiology, and in terms of pharmacokinetics and pharmacodynamics. Further, trauma and bleeding with hemorrhagic shock can only be assessed *in vivo*.

A range of bleeding or thrombosis models have been used to assess the potential of various agents for DOAC reversal. However, a comprehensive comparison of these datasets is virtually impossible given the differences in species, types of injuries applied, dosing regimens, and bleeding or laboratory endpoints tested. The largest amount of data comes from use of various PCC formulations for DOAC reversal, so it is valuable to consider these for comparison of bleeding models (Box 1).

In vivo considerations: species, model of bleeding, and dosing—The majority of studies have used small animal models with some form of minor vascular damage; only a few, more recent studies have used larger animals (pig or non-human primate [NHP]).

Rodent tail vein bleeding is probably the simplest model to use for assessment of bleeding time and volume, and is

therefore widely used (37–39). Valuable insights have also been gained from studies using other standardized injuries in small animals, including kidney incision, hepatosplenic bleeding, and mesenteric bleeding (57, 58, 61, 62, 64). Results from these models are broadly consistent, though it is important to be aware of the distinctions between individual studies. The advantages and disadvantages of small versus large animal models are shown in Table 5 (64, 66–70).

Species-specific differences in metabolism and drug clearance are intrinsically linked with dosing. For example, smaller animals have higher metabolic rates than larger animals. This is evident when comparing the results of a mouse ICH model with a rabbit kidney incision model, both investigating the effects of the same 4F-PCC for reversal of dabigatran anticoagulation (37, 42). A higher dose of dabigatran was required to induce coagulopathy in the mouse model (4.5 mg/kg) versus the rabbit model (0.4 mg/kg). In the mouse, a dose of 100 U/kg 4F-PCC was necessary to overcome the effects of dabigatran, whereas in the rabbit, 50 U/kg was sufficient to restore blood loss to levels within the range of nonanticoagulated animals.

It is important to consider the pharmacological relevance of the species in terms of their sensitivity to DOACs. Rabbit models have been suggested as appropriate models for assessment of FXa inhibitors, given the similar potencies of these agents in rabbit and human plasma (8, 71). In contrast, FXa inhibitors are less potent in rat and dog plasma compared with that from rabbits and humans (8, 71). For dabigatran, *in vitro* effects on thrombin inhibition were comparable in pigs and humans, but it was less potent in rats and mice (72).

Dosing can affect the outcomes observed in relatively similar models using the same species. For example, in reversal of rivaroxaban with 4F-PCC in rabbits, Godier et al. did not observe reversal of rivaroxaban effects on blood loss from ear or hepatosplenic incision, while Herzog et al. demonstrated reversal of rivaroxaban by a significant reduction in blood loss from kidney incision. While one might conclude that the site of injury has influenced results, the differences in dosing and formulation may have added to the differences observed. In Godier et al., heparin-containing 4F-PCC (Kaskadil; 40 U/kg) was not sufficient to reverse effects of rivaroxaban 5 mg/kg, while in Herzog et al., 4F-PCC (Beriplex; 100 U/kg), containing only trace amounts of heparin, could reverse the effects of rivaroxaban 300 μ g/kg (57, 58). Similarly, Herzog et al. found 4F-PCC (Beriplex; ≥ 12.5 U/kg) treatment reduced bleeding following kidney incision in apixaban-anticoagulated rabbits, while Martin et al. found no effect of 4F-PCC (Kanokad; 60 U/kg) on blood loss in rabbits following hepatosplenic injury (though rabbits in the latter study received a lower apixaban dose and had less evidence of coagulopathy) (61, 62).

Differences in formulation/factor content of PCCs, which are generally standardized based on their FIX content, may also contribute to differences in results between studies not using the same PCC, even if the stated dosing is similar—content of FII, FVII, and FX and anticoagulatory protein(s) varies (73, 74). It is also worth noting that PCC preparations contain human coagulation factors, which do not perform consistently across species. While a complex of murine FVIIa and murine tissue

Box 1 Key conclusions

- To understand and compare the effects of DOAC reversal treatments standardized animal models with standardized outcomes are essential
- While small animal models offer a relatively cost-effective and high throughput assay system, the advantage of larger trauma animal models is their closer resemblance to humans in terms of blood volume, hemorrhagic shock and alteration in hemodynamics
- An awareness that there are species-specific differences in response to a given dose of DOAC or reversal treatment is essential
- Blood loss (bleeding volume) is the ideal measure (i.e. preferred primary study end point) of DOAC anticoagulation and its reversal. The utility of laboratory assays is uncertain in this context

DOAC indicates direct oral anticoagulant

factor generates FXa effectively, a complex of human FVIIa and murine tissue factor generates very little FXa (75, 76). Therefore, use of mouse models may lead to an underestimation of the efficacy of PCCs in humans, and the most clinically and pharmacologically relevant animal models should be used to confirm results.

A difference in rivaroxaban binding to plasma FXa has been shown for rat (IC₅₀ 290 nM) versus human or rabbit (both IC₅₀ 21 nM), thus rat and rabbit experiments on rivaroxaban reversal cannot be directly compared. The IC₅₀ values translate into a greater dose of rivaroxaban needed to reduce thrombus formation in an arteriovenous shunt model in rat, compared with rabbit (7).

Taking these observations together, it appears that, though animal models can demonstrate the activities of DOACs and their reversal agents, optimal dosing in humans may not be obvious from animal studies, and clinical work will be required to define this.

In vitro considerations: choice of assay—Aside from bleeding volume and bleeding time, assays that may be used to assess reversal of DOAC anticoagulation include traditional

laboratory assays reflective of fibrin clot formation (PT, aPTT), TG, which demonstrates the overall function of the coagulation system, and thromboelastometry, a viscoelastic measurement of clot formation.

All of these assays show varied sensitivities to each DOAC, and to DOAC-mediated anticoagulation (77, 78), so, in turn, need to be interpreted carefully when reversal of this anticoagulation is being evaluated; results may not correlate with effects on bleeding. While some evidence suggests that whole blood clotting tests are useful for this purpose (58), other studies suggest that TG is likely the best correlate of bleeding outcomes in a DOAC anticoagulation setting (61); however, this theory is far from established.

A further complication is the diversity of reagents available for laboratory tests, which may give differing results depending on the experimental setting. For example, in our own laboratory, in the aPTT assay, we find all reagents equally sensitive to the effects of dabigatran in human plasma, but variably sensitive to the effects of dabigatran in porcine plasma (Oliver Grottke, personal communication). Other groups have also found that coagulation assay results vary according to the reagent used (58).

Early detection and treatment of coagulopathy is important for improving outcomes in critically ill patients, for example in trauma or perioperative settings (26, 79–81). In contrast to laboratory-based tests, ROTEM and thromboelastography allow rapid assessment of coagulation status at the point of care (80–83). However, these tests are not available in all hospitals, and are not calibrated to measure the anticoagulant effects of the DOACs or their reversal. Thus, there is an urgent need for the development of new point-of-care devices to guide reversal therapy for DOAC-induced anticoagulation. The pressing need for rapid reversal of coagulopathy, irrespective of the agent(s) originally administered, is underscored when considering the subset of critically ill patients who may have been

TABLE 5. Advantages and disadvantages of species for assessment of anticoagulation reversal

	Small animal (rodent, rabbit)	Large animal (pig, NHP)
Advantages	Standardization facilitated High numbers can be used for dose-selection and other high-throughput studies Reduced drug requirements due to lower body weight Animal welfare—use of lower order animal species (especially compared with NHP) Ease of sample collection (with rabbits) Ease of regional tissue assessment (with rabbits) Lower variability, resulting in smaller animal numbers required for hypothesis testing	Pig: organ size, blood volume and hemodynamic response comparable to humans (66, 67) NHP: closely analogous to human physiology, hemodynamics, pharmacokinetics, pharmacodynamics, immunology and genetics (68) Ease of sample collection Ease of regional tissue assessment Polytrauma can be inflicted
Disadvantages	Low blood volume Small size—less suitable for use in trauma studies Small tissues—surgery is technically challenging Species differences (compared with humans) in pharmacodynamics of DOACs/potential reversal agents (64, 69), and in coagulation (70) Risk of immunogenicity reactions to heterologous human proteins	Expensive Time-consuming and low throughput Standardization more difficult Ethical approvals may be harder to obtain, particularly for NHP Species differences (compared with humans) in pharmacodynamics of DOACs/potential reversal agents, and in coagulation (70) Risk of immunogenicity reactions to heterologous human proteins

NHP indicates non-human primate.

prescribed DOACs as well as antiplatelet therapies. Therefore, future studies of point-of-care devices that assess platelet function are also warranted.

CONCLUSIONS

Preclinical animal studies have shown that either PCCs, or, where available, specific antidotes, are likely to be effective means of reversing the anticoagulant effects of DOACs. Though PCCs are an established therapy for VKA reversal, understanding how to redeploy them in DOAC reversal is a challenge and animal models have shown their value here. At present, reversal of dabigatran and rivaroxaban have been most comprehensively evaluated of the DOACs, having been studied in both small and large animal models. Across the studies we reviewed, there were no unexpected safety findings (supratherapeutic PCC concentrations are associated with a potential prothrombotic state), though these were for the most part small studies and focussed on efficacy.

While small animal models have a clear place, given the ease of handling large numbers of rodents or rabbits, we believe that the most informative experiments for studying DOAC anticoagulation and its reversal are those conducted in large animals with relevant trauma and severe bleeding with hemorrhagic shock, and animal species with pharmacological relevance. Complex trauma or surgery, two of the situations where DOAC reversal would be required, is best reflected by larger animals with similar physiology to humans. Regardless of the model(s) used, our review found a diversity of experimental designs and outcomes used to test DOAC reversal. While this diversity may be reflective of the heterogeneous trauma patient population, and allow assessment of treatment effectiveness in different clinical settings, it may limit comparisons between studies. Though it is possible to take these studies together and reach a balanced conclusion—here, that both PCCs and specific antidotes appear promising for anticoagulation reversal—we urge investigators to consider using standard models, to allow for comparisons with previous work. Further, we suggest that there is a need for reliable preclinical animal models of clinical and pharmacological relevance with established coagulation assay profiles. In addition, none of the current studies have investigated the reversal of DOACs in the setting of other common clinical conditions or complications, such as sepsis or disseminated intravascular coagulation. Considering the characteristics of patients receiving DOACs (e.g., older age and high prevalence of comorbidities), which may place them at high risk of such complications (84), future studies should address these aspects.

In terms of the assessment of anticoagulation and its reversal, we are concerned by the use of bleeding time in preference to bleeding volume in some studies. We consider that bleeding volume should be regarded as the gold standard of outcomes, and have found that bleeding time can be less reliable (for example, both Godier et al. and Lambourne et al. saw effect on bleeding time without changes in overall blood loss (38, 57), and comparisons of bleeding volume versus bleeding time have found the former a more sensitive and reliable outcome (40, 41)). While laboratory assays may

provide useful ancillary information about the function of elements of the coagulation cascade, their variability in the DOAC anticoagulation setting means that their results should be interpreted with caution.

In the clinic, there is as yet no universally adopted strategy for emergency reversal of DOAC anticoagulation, and given the complexity of major bleeding-associated coagulopathy it is unlikely that such a single, universally applicable strategy will be established (26). As DOAC use becomes widespread (4), the challenge of emergency reversal becomes more pressing, and, used alongside clinical studies, animal models of anticoagulation reversal will be important in defining appropriate treatment strategies, timing and dosing of hemostatic interventions.

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