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Antidotes for reversal of direct oral anticoagulants

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ABSTRACT

The main advantage of the direct oral anticoagulants over vitamin K antagonists is reduced rates of major bleeding, especially intracranial hemorrhage. While use of different clotting factor supplements have been used in patients with direct oral anticoagulant induced major bleeding or when there is need for urgent surgery, the lack of preclinical and clinical data are concerning. Idarucizumab is a specific antibody developed with a 350-fold greater affinity for dabigatran than its pharmacologic target thrombin. Andexanet is a modified factor Xa molecule that binds the direct and indirect Xa inhibitors without being enzymatically active. Ciraparantag, has potential to reverse the anticoagulant activity of multiple agents. The pharmacology, preclinical, and clinical data that have developed these specific antidotes are reviewed in this manuscript.

Contents

Abbreviations: ACT, activated clotting time; aPTT, activated partial thromboplastin time; AUC, area under the curve; AT, antithrombin; Cmax, peak concentration; DOACs, direct oral anticoagulants; dTT, diluted thrombin time; ECT, Ecarin clotting time; ETP, exogenous thrombin potential; Fab, antigen binding fragment; FDA, Food and Drug Administration; GLA, γ carboxyglutamic acid; INR, international normalized ratio; IV, intravenous; Ki, inhibitor constant; LMWH, low molecular weight heparin; NVAF, nonvalvular atrial fibrillation; PCC, prothrombin complex concentrate; TEG, thromboelastography; TFPI, tissue factor pathway inhibitor; TT, thrombin time; UFH, unfractionated heparin; VKA, vitamin K antagonist; VTE, venous thromboembolism; WBCT, whole blood clotting test.

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1. Introduction

The direct oral anticoagulants (DOACs), which include dabigatran, rivaroxaban, apixaban, edoxaban, and betrixaban, are increasingly used in the management of thromboembolic disorders. Depending on the agent and jurisdiction, DOACs are licensed for reducing the risk of stroke in patients with nonvalvular atrial fibrillation (NVAF), prevention and treatment of venous thromboembolism (VTE), and specifically rivaroxaban in preventing arterial events in patients with coronary artery disease and/or peripheral arterial disease. A substantial body of scientific evidence demonstrates that DOACs are at least as effective as vitamin K antagonists (VKAs), with an improved safety profile (Lip et al., 2018; Kearon et al., 2016). Furthermore, some clinical trials demonstrated a lower incidence of major bleeding, but life-threatening bleeding, particularly intracranial hemorrhage, was consistently significantly lower with DOACs compared to VKAs (Lip et al., 2018; Kearon et al., 2016). Although the safety profile with DOACs is improved compared to VKAs, they still can produce significant bleeding. In addition to an overall lower frequency of bleeding compared to VKAs, when it does occur the outcomes of DOAC major bleeding are similar to those with VKAs, even in the absence of specific reversal agents (Eikelboom & Merli, 2016). The availability of a specific antidote for each class of DOAC would be useful to further improve their safety, or for rapid reversal of anticoagulation prior to urgent interventions. Two antidotes have achieved regulatory approval, with additional agents still under investigation (Shaw & Siegal, 2018).

1.1. Direct oral anticoagulants overview

Thrombin (factor IIa) and factor Xa have been the primary targets in the development of alternatives to VKAs for oral anticoagulation due to their key roles in the coagulation cascade (Fig. 1). Thrombin is a potent platelet agonist, and catalyzes the final step in the coagulation cascade, converting fibrinogen to fibrin and facilitating the generation of a stable clot. Factor Xa sits at an important juncture, facilitating the activation of the final common pathway in the coagulation cascade and mediating the conversion of prothrombin to thrombin. Inhibition of either target has translated into meaningful tempering of coagulation cascade activity and improvement in clinical outcomes for patients with thromboembolic diseases (Caterina et al., 2013).

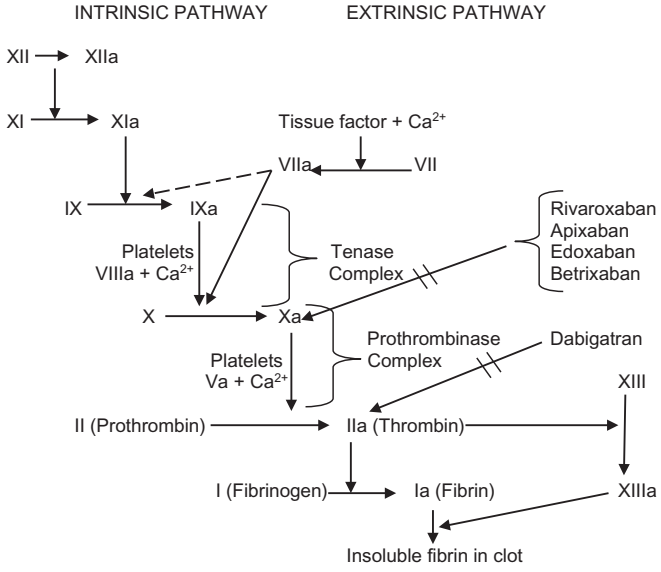

Fig. 1. Clotting cascade with targets of the direct oral anticoagulants.

Dabigatran (Pradaxa®) is the only oral direct thrombin inhibitor that exerts its anticoagulant effect by blocking the generation of fibrin through thrombin inhibition. Similar to other direct thrombin inhibitors, this also leads to inhibition of platelet aggregation, and dampening the activity of Factors V, VIII, and XI (Fig. 1). Dabigatran is approved for reducing the risk of stroke in patients with NVAF, treatment and secondary prevention of VTE, as well as VTE prophylaxis after total hip arthroplasties (Table 1). Key pharmacokinetic and pharmacodynamic properties of dabigatran are listed in Table 2 (Dobesh & Stacy, 2018).

Rivaroxaban (Xarelto®), apixaban (Eliquis®), edoxaban (Savaysa®), and betrixaban (Bevyxxa®) are all oral direct factor Xa inhibitors. All of the agents, except betrixaban, are approved for reducing the risk of stroke in patients with NVAF, as well as treatment and secondary prevention of VTE. Rivaroxaban and apixaban are approved for VTE prophylaxis after total hip and knee arthroplasties. Rivaroxaban is the only agent approved for prevention of cardiovascular events in patients with coronary artery disease and/or peripheral arterial disease. Betrixaban is the only agent approved for extended VTE prophylaxis in medically ill patients. (Table 1). Important pharmacokinetic and pharmacodynamic information for each of these agents are listed in Table 2 (Dobesh & Stacy, 2018).

1.2. Overview of bleeding with DOACs in phase 3 clinical trials

While the DOACs have demonstrated a favorable safety profile compared to VKAs in randomized phase 3 clinical trials, they are not free of major bleeding events. Rates of DOAC-associated major bleeding in patients treated for VTE range from 0.6% to 1.6% (Dobesh & Fanikos, 2014). Rates of DOAC-associated major bleeding in patients receiving stroke prevention therapy for NVAF range from 2.1% to 3.6%, with rates of major gastrointestinal bleeding ranging from 0.8% to 3.2% and intracranial hemorrhage ranging from 0.3% to 0.5% (Dobesh & Fanikos, 2016). Cross trial comparisons, or comparisons between different DOACs is challenging given different patient populations randomized, as well as different definitions of major bleeding used in each clinical trial (Dobesh & Fanikos, 2016). However, the overall message of equivalent efficacy and improved safety for DOACs compared to VKAs is present. Importantly the risk of intracranial hemorrhage is reduced by approximately 50% with DOACs compared to VKAs (Siegal & Crowther, 2013). As DOACs are implemented in real world practice patients will experience bleeding which can sometimes be severe or fatal. The availability of a rapid, safe, and specific method to reverse anticoagulation when major bleeding occurs is desirable for healthcare clinicians.

1.3. Management of DOAC-induced bleeding prior to availability of drug-specific antidotes

Prior to the advent of drug-specific antidotes the management of bleeding with DOACs consisted of general supportive measures plus administration of either coagulation factor replacement or prohemostatic agents. General supportive measures include administration of fluids and blood products as needed in addition to appropriate management of the bleeding site. Small studies in animal models suggest that administration of four-factor prothrombin complex concentrates (PCCs) can normalize coagulation tests with oral direct factor Xa inhibitors (Smythe, Trujillo, & Fanikos, 2016; Piran, Khatib, Schulman, et al., 2019). Administration of prohemostatic agents such as recombinant factor VIIa or factor eight inhibitor bypassing activity (FEIBA®) can rapidly correct coagulation tests with both oral factor Xa inhibitors and direct thrombin inhibitors. Clinical experience with any of these agents is limited to case series or cohort studies where results have been generally favorable. However, none have been evaluated in a randomized controlled trial during DOAC anticoagulation, and all carry the risk of thromboembolism due to the exposure of supraphysiologic

Table 1
FDA–approved indications and doses for the direct oral anticoagulants.

	Stroke prevention in non--valvular atrial fibrillation	VTE prophylaxis	VTE Treatment and secondary prevention	Prevention of cardiovascular events in patients with CAD and/or PAD
Dabigatran	CrCl >30 mL/min: 150 mg twice daily CrCl 15--30 mL/min: 75 mg twice daily CrCL <15 ml/min: not studied, avoid use	Total hip/knee replacement: CrCl >30 mL/min: 110 mg day 1, then 220 mg daily CrCL <30 ml/min: not studied, avoid use	CrCl >30 mL/min: 150 mg twice daily CrCl <30 mL/min: not studied, avoid use	N/A
Rivaroxaban	CrCl >50 mL/min: 20 mg daily CrCl ≤50 mL/min: 15 mg daily	Total hip/knee replacement: CrCl >30 mL/min: 10 mg daily CrCL <30 ml/min: not studied, avoid use	CrCl ≥30 mL/min: 15 mg twice daily x 21 days, then 20 mg daily x 6 months, then 10 mg daily thereafter CrCl <30 mL/min: not studied, avoid use	2.5 mg twice daily with aspirin ≤100 mg daily CrCl ≤15 mL/min: not studied, avoid use
Apixaban	5 mg twice daily; 2.5 mg twice daily if 2 of 3 criteria met: Cr ≥ 1.5 mg/dL, age ≥ 80 years, or weight ≤ 60 kg	Total hip/knee replacement: CrCL >25 ml/min: 2.5 mg twice daily CrCl <25 mL/min: not studied, avoid use	CrCL >25 ml/min: 10 mg twice daily for 7 days, then 5 mg twice daily x 6 months, then 2.5 mg twice daily thereafter CrCl <25 mL/min: not studied, avoid use	N/A
Edoxaban	CrCL >95 ml/min: avoid use CrCL 50–95 ml/min: 60 mg daily CrCL 15–50 ml/min: 30 mg daily CrCL <15 ml/min: not studied, avoid use	N/A	CrCL >50 ml/min: 60 mg daily CrCL 15–50 ml/min: 30 mg daily CrCL <15 ml/min: not studied, avoid use	N/A
Betrixaban	N/A	Acute medical illness: CrCL >30 ml/min: 160 mg load, 80 mg daily for 35–42 days CrCL 15–30 ml/min: 80 mg load, 40 mg daily for 35–42 days CrCL <15 ml/min: not studied, avoid use	N/A	N/A

FDA = Food and Drug Administration; VTE = venous thromboembolism; CAD = coronary artery disease; PAD = peripheral arterial disease; CrCl = creatinine clearance; N/A = not approved for this indication.

concentrations of clotting factors. The development and availability of specific reversal agents that can rapidly reverse anticoagulation with DOACs without increasing the risk of thromboembolism beyond baseline risk is clinically desirable (Tomaselli et al., 2017).

2. Idarucizumab

2.1. Pharmacology

Idarucizumab is a humanized monoclonal antibody fragment that rapidly binds to and neutralizes the anticoagulant effect of the oral direct thrombin inhibitor dabigatran. The development of idarucizumab began by immunizing mice with dabigatran-derived haptens coupled to carrier proteins to produce antibodies against dabigatran (Schiele et al., 2013). Monoclonal antibodies produced from mice with titers exhibiting the highest affinity for dabigatran were selected, and the antigen binding fragments (Fab) from these antibodies were isolated. Murine protein sequences were then replaced with human sequences thus creating a humanized Fab, reducing the potential for immunologic reactions (Fig. 2) (Eikelboom, Quinlan, van Ryn, & Weitz, 2015).

Idarucizumab binding to dabigatran is mediated by hydrophobic interactions, a salt bridge and H-bonding, resulting in a high affinity for dabigatran binding which is approximately 350 times greater than dabigatran's affinity for thrombin (Reilly, van Ryn, Grotte, Glund, & Stangier, 2016). This high affinity for dabigatran binding corresponds with a rapid onset of reversal activity coupled with a slow offset, resulting in almost irreversible binding of idarucizumab to dabigatran. Idarucizumab binds both unbound dabigatran as well as dabigatran bound to thrombin (Fig. 3).

Idarucizumab is also structurally similar to thrombin, however its specificity is for dabigatran only as it does not bind to thrombin or its substrates Factor V, VIII, XIII, protease-activated receptor-1, protein C

Table 2
Pharmacokinetic and Pharmacodynamic properties of direct oral Anticoagulants (Dobesh and Stacy, 2018.).

Property	Dabigatran	Rivaroxaban	Apixaban	Edoxaban	Betrixaban
MOA	Direct IIa inhibitor	Factor Xa inhibitor	Factor Xa inhibitor	Factor Xa inhibitor	Factor Xa inhibitor
Bioavailability	3–7%	66% without food 80–100% with food	50%	62%	34%
Onset of activity	1.5 h	2–4 h	1–3 h	1–2 h	3–4 h
Half-life	12–17 h	9–13 h	8–15 h	9–11 h	19–25 h
Hepatic metabolism	None	CYP3A4/5 and CYP2J2	CYP3A4/5	Minimal (4% CYP3A4/5)	Hydrolysis (no CYP)
Drug interactions	P-gp	CYP3A4/P-gp	CYP3A4/P-gp	P-gp	P-gp
Protein binding	35%	90%	87%	55%	60%
Renal elimination	80%	35%	25%	50%	5–11%
Dialyzable	Yes	No	No	No	No
Renal dosing	Yes	Yes	Yes?	Yes	Yes

CYP = cytochrome P450 enzyme; P-pg = P-glycoprotein.

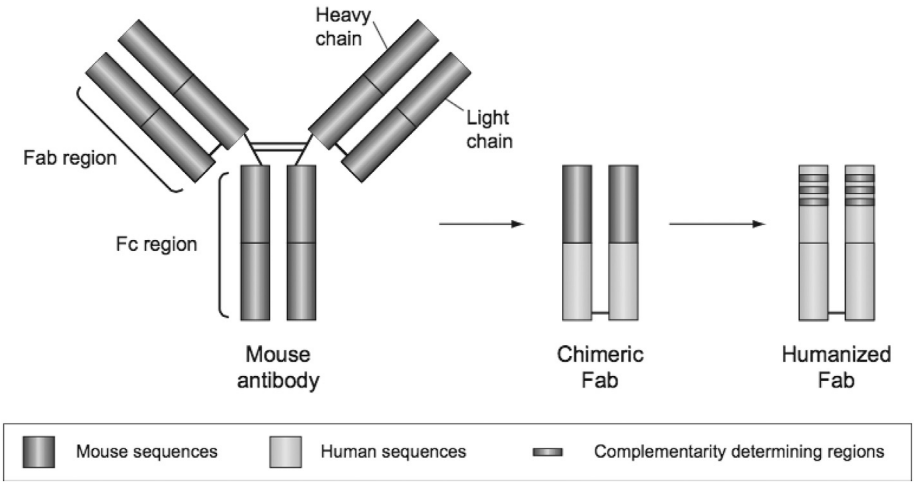

Fig. 2. Development and structure of idarucizumab.(Eikelboom et al., 2015). From: Eikelboom JW, Quinlan DJ, van Ryn, J and Weitz J. The antidote for reversal of dabigatran. Circulation 2015; 132:2412–2422. The fragment antigen-binding (Fab) region is composed of a light and heavy chain and contains the part of the antibody that binds to dabigatran. It also contains a constant region, which, when murine sequences are replaced with human ones, is called a chimeric Fab. The fragment constant (FC) region interacts directly with the immune system; however, such nonspecific binding is avoided by removal of the Fc region.

or von Willebrand factor. It does not activate platelets or convert fibrinogen to fibrin, nor does it exhibit thrombin-like activity (Schiele et al., 2013). Idarucizumab added to plasma in vitro did not affect clotting as measured by the dilute thrombin time (dTT) assay, and did not shorten the clotting time of prothrombin-depleted plasma. Measurements of fibrinopeptide A were not increased. This demonstrates that idarucizumab does not convert fibrinogen to fibrin, and thrombin generation was not increased when idarucizumab was added to plasma in thrombin generation assays. These data demonstrated that idarucizumab administration is unlikely to exhibit procoagulant effects.

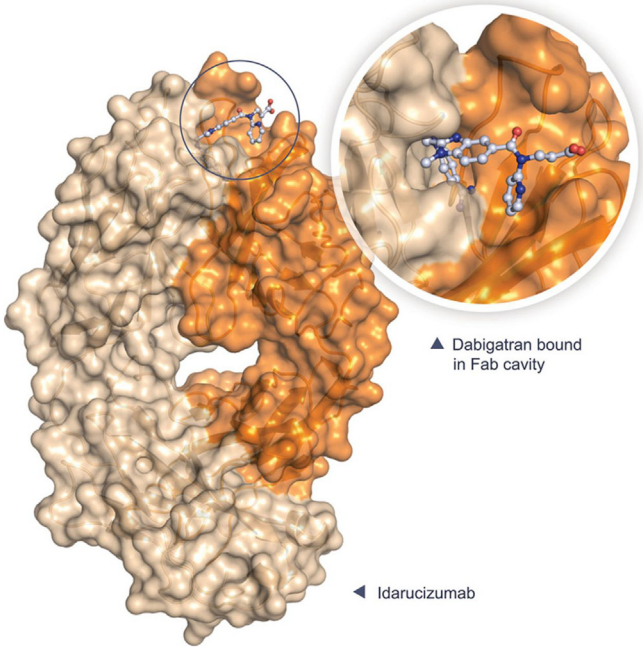

Fig. 3. Idarucizumab bound to Dabigatran. (Eikelboom et al., 2015). Eikelboom JW, Quinlan DJ, van Ryn, J and Weitz J. The antidote for reversal of dabigatran. Circulation 2015; 132:2412–2422. X-ray crystallography surface representation of dabigatran (stick representation) bound to idarucizumab. Insert, A zoom into the cavity formed by the interface of the light (orange) and heavy chains of the fragment antigen-binding into which the benzamidine moiety of dabigatran fits. When dabigatran is bound in this cavity, only ≈ 20% of the molecule is exposed, and does not protrude from the binding site. Fab indicates fragment antigen-binding.

Idarucizumab achieves peak plasma concentrations approximately five minutes after the end of infusion suggesting that it is immediately available in plasma for binding to dabigatran. It has a volume of distribution of approximately 0.06 L/kg, which is similar to the blood volume, and idarucizumab predominantly circulates in plasma. This is in contrast to dabigatran, which has a volume of distribution of approximately 1 L/kg, allowing dabigatran to move freely between the blood and extravascular compartments (Bleech, Ebner, Ludwig-Schwellinger, Stangier, & Roth, 2008; Hauel et al., 2002). Binding of dabigatran to idarucizumab in plasma occurs via 1:1 stoichiometric complexes and results in decreasing unbound dabigatran concentrations in plasma and subsequent redistribution of dabigatran from the extravascular compartment into the plasma. This process continues as long as there is unbound idarucizumab available in plasma, terminating only when all dabigatran is bound or until all the idarucizumab becomes saturated with dabigatran (Reilly et al., 2016).

In healthy volunteers with normal renal function, idarucizumab plasma concentrations decline in a biphasic manner with a mean initial half-life of approximately 45 min and a geometric mean initial half-life of 4.5–8.1 h. Less than 5% of peak concentration remaining after 4 h when idarucizumab doses were ≥600 mg (Glund, Stangier et al., 2015). Idarucizumab elimination is predominantly due to renal elimination, with the majority of excretion occurring within the first four hours of administration (Glund, Moschetti et al., 2015). The main route of idarucizumab renal elimination is thought to be renal catabolism.

Moderate renal impairment (CrCl 30–60 mL/min) increases the half-life to approximately 70 min and increases the overall idarucizumab area under the curve (AUC) by 83.5% (Glund et al., 2017). The effects of renal impairment or age did not impact the reversal of dabigatran anticoagulant activity when compared with healthy volunteers (Glund et al., 2017). Renal impairment can result in increases in dabigatran concentrations, thus the increase in idarucizumab concentrations may be advantageous, although definitive clinical data in this population is currently lacking.

Idarucizumab reversal of the anticoagulant effects of dabigatran has been assessed by coagulation laboratory tests such as the activated partial thromboplastin time (aPTT), thrombin time (TT), dTT, and ecarin clotting time (ECT). Studies conducted in patients exposed to

dabigatran demonstrated that the anticoagulation effect of dabigatran as measured by these laboratory parameters declined rapidly after idarucizumab administration in a dose dependent manner when given as either a bolus or a 5-min infusion (Glund, Moschetti et al., 2015; Glund, Stangier et al., 2015).

2.2. Animal data

The anticoagulant reversal effects of idarucizumab have been studied in rats, and in a variety of pig models. In rats, a single dose of intravenous (IV) idarucizumab completely reversed the dabigatran anticoagulant activity of 200 ng/mL within one minute when measured by both aPTT and TT (Schiele et al., 2013). In a lethal porcine trauma model, pigs were given high doses of dabigatran (30 mg/kg for 3 days) followed by blunt liver injury (Grottke et al., 2015). Fifteen minutes after injury, pigs received either a saline infusion or various doses of idarucizumab doses (30, 60 or 120 mg/kg). Blood loss and coagulation parameters were recorded for 6 h post-trauma, or until death. Compared with saline, idarucizumab improved survival up to 100% and dramatically reduced blood loss in a dose dependent manner. Coagulation parameters such as the aPTT, dTT, ECT and thrombin generation were all restored to baseline in the pigs receiving idarucizumab.

Idarucizumab was compared with 4-factor PCC in 45 pigs in a blunt liver injury and femoral fracture model. Pigs were given dabigatran 30 mg/kg twice daily for 7 doses, which would be expected to provide dabigatran concentrations higher than those seen in humans in the prevention of stroke for atrial fibrillation or the treatment of venous thromboembolism.. Two hours after the final dose, pigs were subjected to both blunt liver injury and bilateral femur fractures. After injury, pigs were randomized to receive one of five treatments: tranexamic acid 20 mg/kg plus human fibrinogen concentrate 80 mg/kg, 4-factor PCC at either 25 units/kg or 50 units/kg plus tranexamic acid 20 mg/kg plus human fibrinogen concentrate 80 mg/kg, idarucizumab 60 mg/kg plus tranexamic acid 20 mg/kg plus human fibrinogen concentrate 80 mg/kg, or placebo. Twelve minutes after injury and after the onset of hemorrhagic shock, animals were resuscitated with Ringer's solution and study treatments were administered. Compared with the other treatment groups, animals in the idarucizumab and 50 unit/kg 4-factor PCC groups all survived, whereas the mortality rates ranged from 42 to 100% in the rest of the treatment groups. Blood loss in these two groups were also substantially lower (61–73%) compared with the other groups (Honickel et al., 2017). Thrombin generation, exogenous thrombin potential (ETP) and thrombin-antithombin levels were significantly higher in the 4-factor PCC group compared with the idarucizumab group, leading the authors to suggest that idarucizumab may be preferred over 4-factor PCC as initial therapy because it is not associated with markers of increased thrombosis risk. The use of tranexamic acid and human fibrinogen concentrate may have also contributed to the reduction in blood loss.

2.3. Phase I and 2 studies

The safety and tolerability of idarucizumab was evaluated in a randomized, placebo-controlled, double blind, two-part phase 1 study of healthy males (Glund, Moschetti et al., 2015; Glund, Stangier et al., 2015). Part 1 was a single ascending dose study, designed to assess the safety, tolerability, and pharmacokinetics of idarucizumab and its impact on coagulation parameters, whereas part 2 was designed to test dabigatran anticoagulant reversal with various doses of idarucizumab. In part 1, 110 patients were randomized in a 3:1 ratio to receive 1 of 10 different doses of idarucizumab or placebo. Idarucizumab did not exhibit procoagulant effects, as there was no detectable binding to thrombin or its targets. No anticoagulant effects were observed, as there were no effects when measuring the activated clotting time (ACT), dTT, ECT, or aPTT. Drug-related adverse events were generally mild and occurred in 3 subjects receiving idarucizumab (headache, erythema), and infusion site reactions (redness, swelling, pain, erythema) were only observed in the subjects who received idarucizumab as a 1-h infusion.

In part 2, all patients received dabigatran 220 mg twice daily for three days with a final dose administered on day 4. Approximately two hours following the final dose, patients were randomized to receive one of four different doses of idarucizumab (1 g, 2 g or 4 g infused over 5 min, or 5 g plus 2.5 g in two 5 min infusions given one hour apart), or placebo. The objectives of the study were to evaluate tolerability, along with reversal of dabigatran anticoagulation effect measured by the TT, dTT, ECT and aPTT. While at least one adverse event was reported in 31 (66%) subjects, only seven were deemed to be drug related. Four of these seven events were reported while receiving dabigatran and prior to receiving either idarucizumab or placebo (3 participants reported hematuria and 1 reported epistaxis). One patient receiving the 1 g infusion of idarucizumab reported infusion site erythema and hot flashes, one patient in the 5 mg plus 2.5 mg idarucizumab group reported epistaxis and one patient in the placebo group experienced infusion site hematoma. All of these events were mild in intensity. With regards to coagulation parameters, prior to idarucizumab infusion, prolongation of the dTT (1.82-fold), TT (11-fold), ECT (2.94-fold) and aPTT (1.63-fold) were observed, but were immediately and completely normalized post infusion in patients receiving the idarucizumab doses of 2 g, 4 g and 5 g plus 2.5 g. Exogenous thrombin potential was restored to pre-dabigatran baseline levels 30 min post infusion and persisted for 4 h with idarucizumab doses >2 g. Fibrinopeptide A concentrations in shed blood from a created incision of 5 mm long and 1 mm deep made on the forearm parallel to the antecubital crease all increased in a dose-dependent fashion with mean fibrinopeptide A concentrations returning to 95% of baseline values in the idarucizumab 4 g and 5 g plus 2.5 g group.

These data established that a 5 g dose of idarucizumab would completely reverse all available dabigatran up to the 99th percentile of dabigatran plasma concentrations measured in the pivotal Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial (Eikelboom et al., 2015; Reilly et al., 2014) which demonstrated the efficacy of dabigatran for reducing the risk of stroke in patients with NVAF.

2.4. Phase 3 studies

The Reversal Effects of Idarucizumab on Active Dabigatran (REVERSE AD) study was a multicenter, prospective, single cohort study designed to evaluate the capacity of idarucizumab's reversal of dabigatran anticoagulation in two distinct patient groups requiring urgent reversal. Group A included patients with life-threating bleeding, while group B included patients requiring an urgent procedure without active bleeding (Pollack et al., 2017). A total of 503 patients were enrolled, 301 patients in group A and 202 in group B. Within group A, 137 patients (45.5%) had gastrointestinal bleeding, 98 patients (32.6%) had intracranial hemorrhage, and 78 patients (25.9%) had trauma as the cause of bleeding. Bleeding was adjudicated as life-threatening in 265 patients (88%) in group A. Of the 202 patients in group B, 197 (97.5%) underwent the intended procedure or surgery, with a median time of 16 h from the first idarucizumab infusion. All patients received idarucizumab 5 g intravenously as two 50-mL bolus infusions, each containing 2.5 g of idarucizumab, given no >15-min apart. The primary efficacy endpoint of the REVERSE-AD study was the maximum percent reversal of dabigatran anticoagulation effect as measured by either the dTT or ECT within 4 h after idarucizumab administration.

At study entry 91.7% of patients (n = 461) had a prolonged dTT or ECT and were included in the primary endpoint analysis. The median

maximum percentage reversal of dabigatran within 4 h was 100% assessed by either the dTT or ECT. Reversal was rapid (within 15 min), as observed in between the 2.5 g infusions and sustained thereafter. Reversal occurred regardless of age, renal function, sex or dabigatran concentrations at baseline. Similarly, unbound dabigatran concentrations decreased rapidly and correlated with the dTT and ECT results observed. Time to bleeding cessation within 24 h was confirmed in 67% of patients in group A with a median time of cessation of 2.5 h after idarucizumab administration. In group B, periprocedural hemostasis was normal in 93.4% (184 out of 197 patients), mildly abnormal in 10 patients (5.1%) and moderately normal in 3 patients. Bleeding cessation could not be assessed in 98 patients with intracranial hemorrhage because this would require frequent head CT imaging, which is not practical. The 30-day mortality rate was 13.5% in group A and 12.6% in group B, and the number of deaths within 5 days was 19 (6.3%) in group A and 16 (7.9%) in group B.

While the results of the REVERSE-AD study are encouraging, important clinical questions regarding the use and efficacy of idarucizumab still remain. Within the REVERSE-AD study, a slight rebound in dabigatran concentrations above 20 ng/mL was noted at 12 h in 114 (23%) patients, and this was associated with recurrent or continued bleeding in 10 patients in group A, but no patients in group B. Of these 10 patients, 3 received an additional dose of idarucizumab. Thus while the 5 g dose of idarucizumab was expected to reverse the 99th percentile of dabigatran levels, there may be situations where the 5 g dose may not sufficiently reverse dabigatran. This rebound effect has also been demonstrated in case reports of use in real world practice (Rottenstreich, Jahshan, & Kalish, 2016; Simon et al., 2017). These data suggest that the duration of anticoagulation reversal from a single dose of idarucizumab may not successfully reverse dabigatran anticoagulation in all patients, especially those with significantly excessive dabigatran concentrations that are much higher than those observed in the RE-LY trial.

The potential for idarucizumab immunogenicity is also a concern. Pre-existing antibodies to idarucizumab were also observed in approximately 11% of patients in a phase 1 study (Glund, Moschetti et al., 2015). These antibodies were directed against the C-terminus of the idarucizumab Fab and not the dabigatran binding site. Anti-idarucizumab antibodies were detected in 28 patients (5.6%) in REVERSE-AD. Of these patients, 19 tested positive for pre-existing antibodies that cross-react to idarucizumab, whereas an additional 9 patients developed antibodies during treatment (Pollack et al., 2017). While the antibody titers were low and had no detectable influence on idarucizumab activity, additional clinical data is needed to determine the true impact of these antibodies on the potential for immunogenicity, decreased reversal effect, and hypersensitivity.

3. Andexanet alfa

3.1. Pharmacology

Andexanet alfa is a modified recombinant protein derived from human coagulation factor Xa (Lu et al., 2013). Structural changes were made to maintain the active site binding pocket to allow the binding of the direct Xa inhibitors, but not be enzymatically active to cause proteolytic cleavage of prothrombin, and consequently thrombin generation. To prevent thrombin generation the catalytic triad was altered by substituting an alanine for serine residue (S419A). Therefore, andexanet functions as a decoy for binding of the direct Xa inhibitors and reverses their anticoagulant effect (Fig. 4). The active site binding pocket of andexanet can also compete with natural factor Xa for binding of antithrombin (AT), and consequently reverse the anticoagulant effect of AT dependent agents such as low molecular weight heparin (LMWH) and fondaparinux. Andexanet would not be expected to have an effect on the thrombin inhibition produced by LMWH.

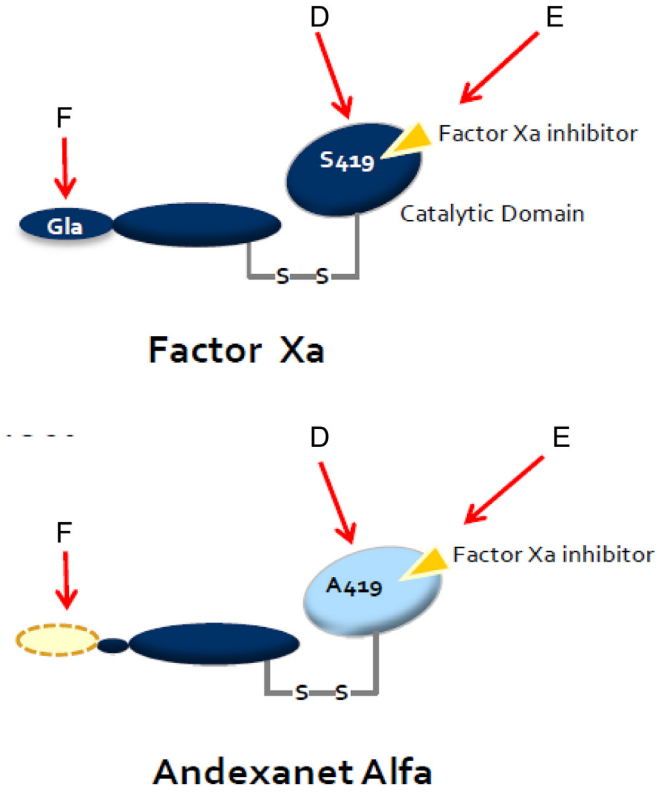

Fig. 4. Native Factor Xa Compared to Andexanet Alfa. Letter A represents the active site for Factor Xa where prothrombin is converted to thrombin. Letter B represents the binding site for factor Xa inhibitors. Andexanet alfa has an amino acid changed at location 491 from serine to alanine. This amino acid renders andexanet alfa unable to convert prothrombin into thrombin, but can still bind factor Xa inhibitors. Letter C represents the ɣ-carboxyglutamic acid (GLA) domain that is responsible for Factor Xa binding to phospholipid membranes such as the prothrombinase complex on the platelet surface. Removal of this in andexanet alfa prevents the inactive molecule from incorporation into the prothrombinase complex and prevents an anticoagulant effect.

With only the active site mutation, the modified protein would still be able to bind to factor Va and compete for incorporation with functional factor Xa into the prothrombinase complex on the platelet surface. Since the modified protein would be unable to convert prothrombin into thrombin, this would create an anticoagulant effect and potentially counter the use of the antidote. Therefore, the membrane-binding ɣ-carboxyglutamic acid (GLA) domain was deleted, preventing its interaction with the phospholipid membrane of the platelet (Fig. 4) (Lu et al., 2013). The modified recombinant protein, andexanet, is expressed in Chinese hamster ovary cells in its active form (Lu et al., 2009). The two-step purification procedure produces purified proteins of the expected molecular weight for the light (~11 kDa) and heavy (~28 kDa) chains. Andexanet does retain an N-terminal amino acid sequence of the native protein, which has the potential for immunogenicity (Lu et al., 2013).

Despite the protein modification in the creation of andexanet, it still has the ability to bind to tissue factor pathway inhibitor (TFPI) similar to endogenous factor Xa (Lu, Lin, Coffey, Curnutte, & Conley, 2015). Since TFPI is a natural anticoagulant, binding to andexanet could theoretically reduce circulating TFPI and create a prothrombotic state. This has been suggested as the mechanism of elevated D-dimer and/or thrombin-antithrombin complexes seen in porcine and monkey models, as well as in healthy human subjects (Grottke, Akman, Conley, & Honickel, 2017; Lu, Hollenbach et al., 2017; Lu et al., 2018). Despite these elevations, no clinical or subclinical thrombosis has been identified in these studies. This may be explained by differences in the Xa-TFPI complex compared to the andexanet-TFPI complex. Unlike the Xa-TFPI complex, the andexanet-TFPI complex does not inhibit fVIIa/TF enzymatic

cleavage of factor Xa (Lu, Lin, Coffey et al., 2015). This is likely due to andexanet's lack of the GLA domain and inability to bind to phospholipid membranes. These transient elevations in coagulation biomarkers without thrombosis may also be explained by the fact that while the andexanet-TFPI complex may impair natural TF-TFPI activity in the initiation phase of coagulation, it has been found to have minimal effect on the later phase of reactions and the overall thrombin formation potential (Lu et al., 2018). It has also been demonstrated that the andexanet-TFPI interaction is significantly reduced in the setting of factor Xa inhibition. This is likely due to the competition of the anticoagulant to bind to andexanet compared to TFPI, thereby limiting the andexanet-TFPI interaction.

The terminal half-life of andexanet is about 6–7 h, but its pharmacodynamic half-life is approximately 1 h (Yeh, Fredenburgh, & Weitz, 2013). The volume of distribution is 5 L, which is approximately the same to total blood volume. Similar to endogenous factor Xa, andexanet is metabolized by protease enzymes and has no measurable renal elimination. Factor Xa inhibitors do not alter the pharmacokinetics of andexanet and andexanet does not alter the clearance of factor Xa inhibitors (Leeds et al., 2016). Upon release from andexanet, the Xa inhibitor is able to return to the central compartment, and therefore, is not cleared bound to andexanet.

3.2. Studies in human plasma

To evaluate the potency (K_d) of andexanet binding to the available direct Xa inhibitors, studies were conducted with human plasma using a peptidyl substrate in a purified enzyme system. Andexanet reversed the inhibitory activity of the direct Xa inhibitors in a dose-dependent fashion, and the relative potency of the binding was in the same order of magnitude as the inhibitor constants (K_i) for the direct Xa inhibitors against endogenous factor Xa (Table 3) (Lu et al., 2013). In plasma samples without direct Xa inhibitors added, andexanet produced no change in the rate of peptidyl substrate cleavage by factor Xa, supporting the lack of catalytic activity of andexanet.

Andexanet has also demonstrated to ability to completely reverse the anticoagulation of rivaroxaban or edoxaban in human plasma as measured by anti-Xa activity. Correction of ex vivo elevations of the prothrombin time (PT) produced by peak therapeutic concentrations of rivaroxaban (1 μM) was achieved with approximately equal molar concentrations of andexanet (Lu et al., 2013). Similar results on normalization of the PT induced by edoxaban have also been reported (Crowther, Levy, Lu, Leeds et al., 2014). Again, even the highest doses of andexanet in the absence a Xa inhibitor did not produce any change in the PT, reinforcing the lack of anticoagulant activity of the antidote.

To further evaluate the potential procoagulant or anticoagulant activity of andexanet, an analysis was conducted using a thrombin generation assay (Lu et al., 2013). This assay tests factor Xa activity in the presence of factor Va and phospholipids in the prothrombinase complex. Andexanet, which has the GLA domain removed, would not be expected to compete with endogenous factor Xa for assembly into the prothrombinase complex, and should not alter thrombin generation (through this prothrombinase system) in the setting of endogenous factor Xa (no anticoagulant effect). Measurement of prothrombinase activity by thrombin generation of andexanet was compared to the thrombin generation of a factor Xa protein without the active catalytic site (similar to andexanet), but with the GLA domain still intact (EGR-Xa). Thrombin generation in human plasma remained unchanged up to the highest concentration of andexanet evaluated (3.3 μM). In contrast, EGR-Xa demonstrated potent inhibition of thrombin generation at much lower concentrations (half-maximal inhibitory concentration of 26 nM). These results confirm the lack of prothrombinase involvement, and therefore anticoagulant activity, of andexanet.

Due to andexanet's binding site having the ability to also bind to AT, reversal of enoxaparin and fondaparinux has also been evaluated (Lu et al., 2010 Circulation). When human plasma was incubated with therapeutic amounts of enoxaparin or fondaparinux the anti-Xa activity was effectively reversed with the addition of andexanet. In the absence of the indirect Xa anticoagulant, andexanet had minimal effect on the ability of AT to inhibit endogenous factor Xa. Andexanet was also found to have enhanced binding of fondaparinux-AT complex compared to AT alone. Therefore, andexanet's ability to bind to AT alone is limited and only measurable when AT is altered with binding to an anticoagulant.

The ability of andexanet to reverse the anticoagulant effect of unfractionated heparin (UFH) was studied in an enzymatic assay in buffered solution (Lu, Lin, Curnutte, & Conley, 2015 Blood). Interestingly, andexanet was able to reverse not only the anti-Xa activity of UFH, but also the anti-IIa activity. In this same analysis, the direct Xa inhibitor rivaroxaban blocked the anti-IIa reversal of andexanet. These results suggest that the UFH-AT binding to andexanet competes for binding of the complex to thrombin (factor IIa), but does not directly interfere with UFH-AT-IIa binding.

3.3. Animal data

3.3.1. Direct Xa inhibitors

Andexanet has been evaluated in a number of animal models to assess its ability to reverse the anticoagulant effect of the direct Xa inhibitors (anti-Xa activity, PT, aPTT). Models have also been used to evaluate the ability of andexanet to limit and reduce blood loss. These models have included evaluation of the direct Xa inhibitors as well as the AT-dependent inhibitors enoxaparin and fondaparinux.

An in vivo rat model was used to evaluate the ability andexanet to reverse the anticoagulant activity, as measured by the INR, of rivaroxaban, betrixaban, and apixaban (Lu et al., 2013). An IV dose of the direct Xa inhibitors produced a 2-fold increase in the INR. Rats were then treated with andexanet or vehicle as bolus plus infusion. Rats treated with vehicle demonstrated a gradual reduction in the INR consistent with expected clearance of the direct Xa inhibitor. Rats receiving andexanet demonstrated a rapid, complete, and sustained reversal of the INR to baseline values. There was also substantial increase in the total plasma concentrations of the direct Xa inhibitors after andexanet dosing. Peak total plasma concentrations for rivaroxaban, betrixaban, and apixaban were 1.4 ± 0.4 μM, 0.2 ± 0.4 μM, and 1.4 ± 0.3 μM, respectively, and increased to 1.9 ± 0.09 μM, 2.0 ± 0.4 μM, and 4.2 ± 0.7 μM, respectively after administration of andexanet, effectively demonstrating the redistribution of the direct Xa inhibitors from the extravascular compartments. Despite these increases in total plasma concentration, the percent of unbound drug (active drug) still decreased from 2.2%, 40%, and 1.5%, respectively before andexanet to 0%, 0.3%, and 0.05%, respectively after andexanet. The reduction in INR achieved with andexanet correlated with the decreased free fraction of the direct Xa inhibitor.

While andexanet's ability to limit blood loss has been evaluated in both mouse and rat tail transection models, most studies have used the rabbit liver laceration model to evaluate blood loss as it represents bleeding in the setting of significant internal trauma. In one study with edoxaban, anticoagulated rabbits demonstrated a 2.4-fold increase in mean blood loss compared to nonanticoagulated rabbits (22.2 ± 8.9 g vs. 9.3 ± 3.0 g; $p = 0.0003$) (Lu et al., 2018). Rabbits receiving edoxaban and andexanet (75 mg) reduced mean blood loss by 80% to amount

Table 3
Affinity of andexanet for the oral direct Xa inhibitors (Lu et al., 2013, 2018).

Inhibitor	Andexanet Kd (nM)	factor Xa Ki (nM)
Apixaban	0.58	0.100
Betrixaban	0.53	0.117
Edoxaban	0.95	0.122
Rivaroxaban	1.53	0.400

similar to nonanticoagulated rabbits (11.9 ± 3.7 g). Edoxaban total plasma concentrations increased over 5-fold after administration of edoxaban, but mean unbound concentrations still deceased by 82% (99 ± 10 ng/mL to 21 ± 6 ng/mL). There was also an 82% reduction in anti-Xa levels after administration of andexanet. The reduction in blood loss with andexanet correlated with the reduction in anti-Xa ($r = 0.6993$, $p < 0.0001$) and unbound edoxaban ($r = 0.5951$; $p = 0.0035$).

Similar results were seen in a study with betrixaban (Conley et al., 2017). Betrixaban produced approximately a 2-fold increase in blood loss compared to nonanticoagulated rabbits. Doses of 75 mg and 125 mg of andexanet reduced blood loss by >90% compared to rabbits receiving vehicle, with total blood loss of 11.0 g and 9.54 g, respectively ($p < 0.002$ for both). Andexanet had no impact when given to nonanticoagulated rabbits (10.3 g). Total betrixaban plasma concentrations increased 7-fold after andexanet administration, but anti-Xa levels were still decreased by over 97% with the two doses of andexanet.

There have been a number of rabbit liver laceration blood loss model studies conducted with the direct Xa inhibitor rivaroxaban. Rivaroxaban increased mean blood loss by 2–3 fold compared to nonanticoagulated rabbits, and represented approximately 10% of total blood volume of the rabbits (Lu, Pine et al., 2017). Administration of 75 mg of andexanet produced over an 85% reduction in blood loss, 98% reduction in peak anti-Xa activity, a 75% reduction in PT, and a 66% reduction in aPTT. Andexanet had no impact on any of these parameters when given to nonanticoagulated rabbits. Rivaroxaban had no impact on plasma concentrations of andexanet (568 ± 88 μg/mL with rivaroxaban and 502 ± 101 μg/mL without rivaroxaban). As with other direct Xa inhibitors, andexanet produced a 6-fold increase in total rivaroxaban plasma concentrations (297 ± 105 ng/mL to 1865 ± 371 ng/mL), but also a reduction (99.9%) in the percent of unbound active rivaroxaban. There was a high level of correlation between the reduction in anti-Xa activity and the unbound rivaroxaban concentration ($r^2 = 0.992$).

Andexanet has been compared to other reversal strategies using the rabbit liver laceration blood loss model with rivaroxaban. Compared to reductions in blood loss and coagulation parameters with andexanet previously mentioned, recombinant factor VIIa, at a therapeutic dose of 150 μg/kg, demonstrate an 85% reduction in the PT and 54% reduction in the aPTT, but had no impact on blood loss (Hollenbach, Lu, Tan et al., 2012). Andexanet was also compared to a 3-factor and 4-factor PCC in this model (Lu, Pine et al., 2017). Andexanet produced a 90% reduction in rivaroxaban anti-Xa activity within 5 min that correlated with reduced blood loss. Neither 3-factor nor 4-factor PCC had an impact on anti-Xa activity, PT, aPTT, or blood loss induced by rivaroxaban.

Andexanet's ability to reverse apixaban was evaluated in a porcine polytrauma model, which includes blunt liver injury and bilateral femur fracture (Grottke et al., 2017). Animals received apixaban 20 mg once daily for 3 days ($n = 21$) or placebo ($n = 7$). Twelve minutes after injury, animals were randomized to andexanet 1000 mg IV bolus, andexanet 1000 mg IV bolus plus infusion of 1200 mg over 2 h, or vehicle ($n = 7$ per group). Apixaban levels prior to injury were considered to be therapeutic at 192 ± 22 ng/mL. Two hours after injury, apixaban levels were 107 ± 38 ng/mL with bolus only and 16 ± 5 ng/mL with bolus plus infusion. Blood loss after 5 h with andexanet bolus only was 1264 mL and 1202 mL with bolus plus infusion, with 100% survival. Apixaban plus vehicle animals had blood loss of 3903 mL with 100% mortality. Elevations in D-dimer levels were identified in andexanet animals, but no thrombotic events were noted on clinical or macroscopic examination.

To test for immunogenicity, various doses of andexanet were given to cynomolgus monkeys twice a day, every 3 days, for a total of 13 days, along with rivaroxaban 20 mg daily for 13 days (Lu, Hollenbach et al., 2017). Of the 40 monkeys tested, one developed an anaphylactic reaction in the highest dose group on day 13, which resolved with diphenhydramine and epinephrine. Also, no neutralizing antibodies were detected, which is encouraging with the repeated doses of a human protein to these monkeys. As seen in the porcine model, there were transient increases in D-dimer and thrombin-antithrombin complexes. These elevations were not associated with any evidence of fibrin deposition of clot formation on histopathological examination of the monkeys at necropsy. Interestingly, these elevations were attenuated when andexanet was administered with rivaroxaban. As previously discussed, this reduction in the interaction is likely due to the competition of the anticoagulant to bind to andexanet compared to TFPI, thereby limiting the andexanet-TFPI interaction.

3.3.2. Indirect Xa inhibitors

In a rat tail transection model study, andexanet 4 mg bolus plus 4 mg/h infusion completely corrected the increased blood loss with enoxaparin 8 mg/kg IV, where as a lower dose of 2 mg bolus plus 2 mg/h infusion only reduced blood loss by 42% (Lu et al., 2013). In a rabbit liver laceration blood loss model, enoxaparin-induced (8 mg/kg) blood loss was significantly reduced by 2–3 fold with andexanet 35 mg or 75 mg compared to vehicle (Pine et al., 2016). Andexanet also produced significant reductions in the elevated anti-Xa activity.

Enoxaparin given at a dose of 4.5 mg/kg IV produced a roughly 11-fold increase in blood loss in a rat tail transection model compared to placebo (Hollenbach, Lu, DeGuzman et al., 2012). Andexanet bolus doses of 1, 2, or 4 mg IV demonstrated a significant reduction in cumulative blood loss (56–62%) compared to vehicle, with complete cessation of bleeding in 70–85% of rats. Andexanet also decreased enoxaparin-induced anti-Xa activity (35–81%) and increased thrombin generation. The reduction in blood loss with andexanet in this model correlated with andexanet plasma concentrations ($r^2 = 0.80$) and reduction of anti-Xa units ($r^2 = 0.89$). While these correlations are good, they are not as strong as these seen with the direct Xa inhibitor. This is likely due to the anti-IIa activity of enoxaparin that is not significantly impacted by andexanet. In this same model, andexanet provided complete cessation of bleeding and elevated anti-Xa activity produced by fondaparinux (25 mg/kg IV). The more complete effect of andexanet on fondaparinux-induced coagulation is due to the specific anti-Xa activity of fondaparinux.

3.4. Phase 1 and 2 studies

Phase 1 data for andexanet involved subjects ($n = 32$) randomized in a 6:2 ratio to single IV bolus doses of placebo or andexanet 30, 90, 300, or 600 mg, with patients followed for 28 days (Crowther, Kitt et al., 2013). Both the C_{max} and the AUC of andexanet increased relatively proportionally with increasing doses above 30 mg. Volume of distribution was similar to that of the central blood compartment. The terminal half-life was about 6 h, which remained constant similar to clearance, with increasing dosing. There was one moderately serious adverse event, development of a bilateral pneumonia, that was not thought to be related to andexanet. There were 3 infusion reactions, none considered serious. As seen in animal models, prothrombin fragment 1 + 2, thrombin-AT complex, and D-dimer levels transiently increased with andexanet dose, with no thrombotic events reported.

The Phase 2 studies of andexanet randomized subjects in a 6:3 ratio to one of six doses of andexanet bolus only or bolus and infusion or placebo for apixaban, rivaroxaban, edoxaban, and enoxaparin (Table 4) (Crowther, Lu et al., 2014). Andexanet was administered 3 h after the last dose of the factor Xa inhibitor. Unfortunately, results of these data are divided between multiple abstracts and manuscripts and is not completely available.

Table 4
Phase 2 study of andexanet dosing for anticoagulant reversal (Crowther, Lu et al., 2014).

Factor Xa inhibitor evaluated	Andexanet dosing cohorts
Apixaban 5 mg twice daily for 5.5 days (11 doses) Rivaroxaban 20 mg daily for 6 days (6 doses) Edoxaban 60 mg daily for 6 days (6 doses) Betrixaban 80 mg daily for 6 days (6 doses) Enoxaparin 40 mg daily for 6 days (6 doses)	1. IV bolus of 90 mg over 3 min 2. IV bolus of 210 mg over 7 min 3. IV bolus of 420 mg over 15 min 4. IV bolus of 420 mg over 14 min, followed by 180 mg (4 mg/min) over 45 min 5. IV bolus of 420 mg over 14 min, followed by second bolus in 45 min over 6 min 6. IV bolus of 420 mg over 14 min, followed by 480 mg (4 mg/min) over 120 min

IV = intravenous.

Data on all 54 patients from the study with apixaban are available (Siegal et al., 2017). Within 2 min of the andexanet bolus dose total apixaban plasma concentrations increased approximately 3-fold. This coincided with reductions in unbound apixaban plasma concentrations, which were greatest with the 420 mg bolus dose (cohorts 3–6). As would be expected, subjects receiving placebo did not demonstrate any change in total or unbound apixaban concentrations. Subjects receiving the 420 mg bolus dose with the 2 h infusion (cohort 6) demonstrated a sustained reduction in unbound apixaban plasma concentrations for 3 h ($p < 0.05$ vs. pooled cohorts), which returned to placebo levels in 0.17 to 3.5 h. Reversal of anti-Xa activity followed similar results, with the greatest reduction being demonstrated with the 420 mg bolus dose (93% to 95%; $p < 0.05$ vs. baseline). Anti-Xa activity returned to placebo levels in 1 to 2.5 h in the bolus only cohorts (1–3) and in 3.3 to 4.3 h in the infusion cohorts (4–6). Restoration of thrombin generation followed these findings. As in the animal models and the Phase 1 study, no thrombotic events occurred despite the transient dose-dependent elevations of prothrombin fragment 1 + 2, thrombin-AT complex, and D-dimer levels with andexanet.

Phase 2 data evaluating andexanet reversal of rivaroxaban and enoxaparin is only available for the 210 and 420 mg bolus dose (cohorts 2 and 3) (Crowther, Mathur et al., 2013; Crowther, Levy, Lu, Conley et al., 2014). These bolus doses reduced unbound rivaroxaban plasma concentrations by 32% and 51%, respectively, as well as producing a 20% and 53% reduction in anti-Xa activity, respectively (Crowther, Mathur et al., 2013). Similarly, anti-Xa activity from enoxaparin went from 0.36 ± 0.08 to 0.12 ± 0.13 IU/mL, and inhibition of thrombin generation was reversed to baseline levels after the andexanet bolus dose (Crowther, Levy, Lu, Conley et al., 2014). These reductions were maintained for approximately 2 to 3 h after the bolus dose.

Reversal of edoxaban with an andexanet bolus of 600 mg and with an 800 mg bolus followed by 8 mg/min for 2 h has been reported (Crowther, Levy, Lu, Leeds et al., 2014). The anti-Xa activity of edoxaban was reduced by 52% and 73%, respectively, compared to baseline and returned to placebo levels approximately 2 h after completion of the andexanet regimen. Andexanet reversed edoxaban-induced inhibition of thrombin generation. With the same study design, the reversal of betrixaban with an 800 mg bolus dose alone or 800 mg bolus dose with 8 mg/min infusion for 2 h of andexanet has been reported (Crowther et al., 2016). This bolus dose of andexanet produced a 78% reduction in the anti-Xa activity and lowered unbound betrixaban plasma concentrations from 12.3 ± 5.6 to 3.6 ± 2.7 ng/mL. The use of the 2 h infusion maintained these reductions. Thrombin generation was also restored in most subjects (11 of 12) following andexanet.

3.5. Phase 3 and 4 studies

Once dosing from phase 2 trials had been determined, these doses were evaluated in the Andexanet Alfa, a Novel Antidote to the Anticoagulation Effects of FXA Inhibitors Apixaban (ANNEXA-A) and Rivaroxaban (ANNEXA-R) trials (Siegal et al., 2015). The ANNEXA –A and R trials were randomized, double-blind, placebo controlled studies in which healthy elderly subjects (50–75 years old) were enrolled, since this age group represents patients most likely to receive anticoagulant therapy. Subjects were randomized in a 3:1 ratio (ANNEXA-A) or 2:1 ratio (ANNEXA-R) to receive andexanet or placebo. Each study was conducted in two consecutive parts. Part 1 evaluated the effect of an andexanet bolus only, and part 2 evaluated the effect of an andexanet bolus plus 2 h infusion. Subjects in ANNEXA-A received apixaban 5 mg twice daily for 3.5 days (7 doses) to achieve steady state anticoagulation. At 3 h after the last dose of apixaban (peak concentration), andexanet was given as an IV bolus of 400 mg over about 15 min, followed by an infusion of 4 mg/min for 2 h (480 mg total). In ANNEXA-R subjects received rivaroxaban 20 mg daily for 4 days (4 doses) for steady state anticoagulation. At 4 h after the last dose (peak concentration), andexanet was given as an IV bolus of 800 mg over about 30 min, followed by an infusion of 8 mg/min for 2 h (960 mg total). The higher dose of andexanet with rivaroxaban is necessary due to the higher peak plasma concentrations, and the approximate 2-fold higher volume of distribution compared to apixaban.

The primary outcome of the trial was the percent change in anti-Xa activity from baseline (before andexanet or placebo) to 2 to 5 min after the andexanet bolus (part 1) or at 5 to 10 min before the end of the infusion (part 2) (Siegal et al., 2015). In patients receiving apixaban the anti-Xa activity was reduced by 94% after the bolus of andexanet compared to 21% in those receiving placebo ($p < .001$). Similar results were seen in patients receiving rivaroxaban, with a reduction in anti-Xa activity of 92% with the andexanet bolus compared to 18% with placebo (<0.001). These reductions were maintained throughout the 2 h infusion and returned to placebo level in 1 to 2 h after discontinuation of andexanet. Andexanet also produced significant reductions in unbound apixaban and rivaroxaban plasma levels, increased thrombin generation, and had significantly more patients achieve an 80% reduction in anti-Xa activity compared to placebo (Table 5).

No severe adverse events or thrombotic events were reported (Siegal et al., 2015). One patient developed hives during the andexanet infusion which lead to the discontinuation of the infusion at 35 min. The hives resolved after a single dose of diphenhydramine. No neutralizing antibodies to factor X, factor Xa, or andexanet were identified. Nonneutralizing antibodies against andexanet developed in 2% of placebo patient and 17% of andexanet patients. Antibodies typically appeared within 15 to 30 days post exposure with relatively low titer (1:640). Prothrombin fragments 1 + 2 and D-dimer levels were elevated in certain patients and these elevations returned to baseline levels in 24 to 72 h with no thrombotic events reported.

The next step in the development of andexanet was the Phase 3b/4 ANNEXA-4 trial. This trial was designed to evaluate the efficacy and safety of andexanet in patients with acute major bleeding who had received a factor Xa inhibitor within the previous 18 (Connolly et al., 2019). An andexanet bolus dose of 400 mg followed by infusion of 4 mg/min for 2 h (480 mg) was given to patients who had received apixaban at any time or rivaroxaban at least 8 h previously hours. An andexanet bolus dose of 800 mg followed by an infusion of 8 mg/min (960 mg) was given to patients receiving rivaroxaban within the last 8 h, enoxaparin, or edoxaban. These doses were developed from the Phase 2 trials, as a pharmacokinetic and pharmacodynamic model

Table 5
ANNEXA-A and ANNEXA-R Trial results (Siegal et al., 2015).

	ANNEXA – A				ANNEXA - R			
	Part 1 – Bolus only		Part 2 – Bolus + infusion		Part 1 – Bolus only		Part 2 – Bolus + infusion	
Outcome	Andexanet (n = 24)	Placebo (n = 9)	Andexanet (n = 23)	Placebo (n = 8)	Andexanet (n = 27)	Placebo (n = 14)	Andexanet (n = 26)	Placebo (n = 13)
% change in anti-Xa activity	−93.9 ± 1.7	−20.7 ± 8.6	−92.3 ± 2.8	−32.7 ± 5.6	−92.2 ± 10.7	−18.4 ± 14.7	−96.7 ± 1.8	−44.8 ± 11.7
Percent of patients with ≥80% reduction in anti-Xa activity	100	0	100	0	96.3	0	100	0
Mean change in factor Xa inhibitor free fraction to nadir (ng/mL)	−9.3 ± 3.2	−1.9 ± 1.6	−6.5 ± 2.8	−3.0 ± 1.2	−23.4 ± 6.2	−4.2 ± 2.9	−30.3 ± 8.1	−12.1 ± 5.3
Mean change in thrombin generation to peak (nM. min)	1323 ± 335.4	88.2 ± 125.8	1193.1 ± 263.3	189.4 ± 184.8	1314.2 ± 331.2	173.9 ± 104.2	1510.4 ± 344.8	264.4 ± 140.7
Percent of patients with ETP above lower limit of derived normal range	100	11.1	100	25	96.3	7.1	100	0

considering the factor Xa inhibitor peak concentrations, extravascular distribution, and factor Xa inhibitor clearance. The accuracy of the dosing model was evaluated during an interim analysis of the first 73 patients in the ANNEX-4 trial (39 apixaban and 34 rivaroxaban) (Leed et al., 2018). At this analysis, the mean observed reduction in apixaban-induced anti-Xa activity was 74.4% and the model predicted a reduction of 76.3%. Similar concordance was seen with patients receiving rivaroxaban, with a reduction in the mean observed anti-Xa activity of 83.9% and a predicted reduction of 84.1% from the model.

There were a co-primary outcomes of the trial. One was the change in anti-Xa activity, the other being clinical hemostatic efficacy through 12 h. Patients with low levels of anti-Xa activity at baseline (< 75 ng/mL for direct Xa inhibitors and < 0.25 IU/mL for enoxaparin) were included in the safety analysis, but not were not included in the efficacy analysis since they already had decreased anti-Xa levels. At the end of the trial 254 patients were enrolled in the efficacy analysis and 352 patients in the safety analysis.

Patients had a mean age of 77 ± 11 years and were mainly receiving anticoagulation for atrial fibrillation (79%) followed by venous thromboembolic disease (18%). Intracranial bleeding was most common (67%) followed by gastrointestinal bleeding (24%), and other bleeding sites (8%). The mean time from presentation to the administration of andexanet was about 5.0 h with 84% receiving the lower dose andexanet regimen.

In patients receiving rivaroxaban (n = 100), the anti-Xa activity was reduced by 92% at the end of the andexanet bolus, which was sustained to the end of the infusion. Two hours after the infusion was completed, anti-Xa activity increased, but was still 42% lower than baseline. In patients receiving apixaban (n = 134), the anti-Xa activity was reduced by 92% at the end of the bolus, which was also sustained to the end of the infusion. Two hours after the infusion was completed, the anti-Xa activity increased, but was still 32% lower than baseline. In patients receiving enoxaparin (n = 16), the anti-Xa activity was reduced by 75% at the end of the andexanet bolus and sustained during the infusion. Two hours after completing the infusion the anti-Xa level increased, but were still 46% lower than baseline. Hemostatic efficacy, as defined by the detailed criteria of the trial, was determined to be excellent or good in 82% of patients, and this finding was consistent regardless of the factor Xa inhibitor, gender, site of bleeding, age of the patient, or dose of andexanet given.

In the initial safety analysis of 67 patients, 18% of patients had a thrombotic event at 30 days with only 27% of the 67 patients having restarted anticoagulation (Connolly, Milling et al., 2016). In a follow up analysis of 104 patients, the rate of thrombotic events decreased to 12% when 40% of patients were restarted on anticoagulation (Connolly, Gibson, & Crowther, 2016). In the final analysis the rate of thrombosis dropped to 10%, when 62% restarted anticoagulation (Connolly et al., 2019). The thrombosis rate in the 62% of patients restarting any anticoagulation was only 2%.There were zero events in patients restarted on oral anticoagulation. Since these patients all have an indication for anticoagulant therapy, they are at risk of thrombotic events. Therefore, reversal of the anticoagulant activity exposes this underlying pathophysiology. While it is difficult to determine if andexanet is producing a prothrombotic state, or if it is the underlying pathophysiology, the decreasing rate of thrombotic events with increasing reinstitution of anticoagulation points to the latter. The 30 day mortality rate in the study is 15%.

3.6. Andexanet summary

Andexanet has demonstrated the ability to bind to factor Xa inhibitors and reverse their anticoagulant effect (mainly measured by anti-Xa activity) in a number of animal models as well as healthy human subjects. These results have been demonstrated in both direct and indirect inhibitors of the factor Xa molecule. In patients with acute major bleeding, the reversal of anticoagulant activity is consistent with prior trials, as well as >80% of patients achieving excellent or good hemostatic efficacy within 12 hours, which would be the goal of an anticoagulant antidote. The bolus provides this effect almost immediately, and the 2 h infusion provides time for a hemostatic plug to form at site of bleeding. Currently, the dosing regimens used in the ANNEXA-4 trial have been approved by the FDA for reversal of apixaban and rivaroxaban. Approval for reversal of other factor Xa inhibitors will require additional data from Phase 3 and 4 trials. Unlike idarucizumab, andexanet has not been evaluated for reversal of factor Xa inhibitors in patients requiring urgent major surgery. A future trial of andexanet is being planned for use prior to the need for urgent surgery. h

4. Ciraparantag

4.1. Pharmacology

The investigational drug ciraparantag has also been referred to as PER977 and aripazine throughout its development. It is a di-arginine piperazine with a chemical formula of $C_{22}H_{48}N_{12}O_2$ (Sullivan, Gad, Laulicht, Bakhru, & Steiner, 2015) (Fig. 5). Ciraparantag is a water-soluble, cationic molecule, weighing 512 Da (Ansell, Laulicht et al., 2014; Fredenburgh & Weitz, 2016). In silico modeling was used to develop the potential binding sites to target anticoagulants (Laulicht, Bakhru, Jiang, et al., 2013). The molecular structure is designed to form strong, non-covalent hydrogen bonds, as well as charge-charge interactions with its anticoagulant targets (Fredenburgh & Weitz, 2016).

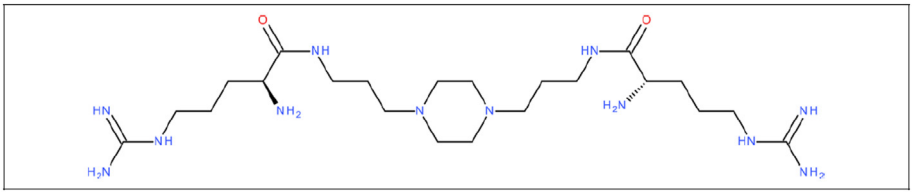

Fig. 5. Molecular structure of Ciraparantag. Chemical formula: N1, N1-[piperazine-1,4-diylbix(propane-1,3-diyl]-bis-L-argininamide (Sullivan et al., 2015).

Eight hydrogen-bonding sites were predicted using computer-aided energy minimization, focusing on four essential functional groups and their properties. Guanidine residues were predicted to interact with rivaroxaban, edoxaban, dabigatran, UFH, LMWH, and fondaparinux. The amide oxygen was predicted to interact with apixaban and edoxaban, whereas the amide nitrogen with rivaroxaban, dabigatran, UFH, LMWH, and fondaparinux. The alpha-amino group of the amide residues was predicted to interact with rivaroxaban, apixaban, dabigatran, UFH, LMWH, fondaparinux, and argatroban (Ruff, Giugliano, & Antman, 2016). Ciraparantag's mechanism is purportedly mediated through direct binding of FXa inhibitors (apixaban, rivaroxaban, and edoxaban), the thrombin inhibitor (dabigatran), and heparinoids (UFH, LMWH, and fondaparinux) (Costin, Ansell, Laulicht, Bakhru, & Steiner, 2014). Hydrogen bonding and charge-charge interactions inhibit the ability of ciraparantag's targets to interact with their respective endogenous targets.

In vitro studies using dynamic light scattering confirmed the binding specificity to enoxaparin through non-covalent hydrogen-bonds. The binding specificity for enoxaparin ranges from a ratio of 1:1 (ciraparantag to enoxaparin) up to a ratio of 10:1 (Laulicht et al., 2013). In one study, isothermal titration calorimetry assessed the affinity of ciraparantag to DOACs and heparins. Isothermal titration calorimetry did not show binding of ciraparantag to edoxaban or rivaroxaban (in a phosphate-buffered solution in the absence of citrate or other chelators) however, it did show a near-micromolar affinity with UFH and enoxaparin. The Kd for ciraparantag was similar to andexanet for UFH and enoxaparin at $2.8 \pm 0.3 \times 10^{-5}$ M and $1.7 \pm 0.3 \times 10^{-5}$ M, respectively. Interestingly, the interaction with enoxaparin was weaker when in complex with antithrombin (Kd $= 6.9 \pm 0.3 \times 10^{-5}$ M). Ciraparantag's affinity for fondaparinux was very weak with a millimolar Kd (Kalathottukaren, Creagh, Abbina, et al., 2018).

The ability of ciraparantag to directly bind DOACs has been called into question as isothermal titration calorimetry did not show a significant interaction between the molecules. Another mechanism of ciraparantag has been proposed through an interaction with factor IXa (Kd $= 3.5$ (± 0.4) x10^{-5} M). Factor IXa is responsible for the conversion of factor X to its active form in the tenase complex. The observation of ciraparantag's ability to bind factor IXa hints at the possibility that altering activity of factor IXa modulates its ability to activate factor X, similar to polylysine (Kalathottukaren et al., 2018). Ciraparantag does not reverse warfarin. It has the ability to bind argatroban but does not prevent it from interacting with thrombin and thus does not inhibit bleeding (Smythe et al., 2016).

Ciraparantag has not been shown to bind blood coagulation factors, other than factor IXa, thus is not expected to inhibit coagulation. (Sullivan et al., 2015). In later clinical trials, ciraparantag was shown to have no drug-drug binding when tested with common cardiovascular, antiepileptic and anesthetic drugs (Ansell, Bakhru, et al., 2014). The di-arginyl structure of the molecule is key in the metabolism. The primary metabolite, 1,4-bis(3-aminopropy)piperazine or BAP, is formed from a double peptide cleavage with a monoarginine piperazine intermediate. Ciraparantag undergoes rapid renal elimination with no known accumulation after 14-repeat dose studies (Sullivan et al., 2015). In clinical trials, ciraparantag was found to have no effect on CYP metabolism (Ansell, Laulicht et al., 2014). Optimal storage of this molecule will be at room temperature (Sullivan et al., 2015).

4.2. Animal studies

In an ex vivo study of human plasma spiked with rivaroxaban or apixaban at 1 or 2 times the therapeutic C_{max}, ciraparantag completely reversed the anti-Xa activity of both anticoagulants in a dose-dependent manner (Laulicht, Bakhru, & Lee, 2012). In a rat tail transection model, ciraparantag was administered IV after rats were overdosed with rivaroxaban, apixaban, edoxaban, or dabigatran, with evaluation of blood loss volume being quantified after 30 min. Ciraparantag decreased bleeding by >90% and reduced it to the same level as seen in nonanticoagulated rats. The PT, aPTT, and thromboelastography (TEG) were restored to baseline within 20 min (Bakhru et al., 2013).

In the rat liver laceration model ciraparantag fully reversed edoxaban within 10 min of administration (Bakhru et al., 2014). Ciraparantag has also been compared to andexanet in a rabbit liver laceration model (Hollenbach et al., 2014). The highest dose studied (30 mg/kg) produced a 76% reduction in blood loss that was identical to that seen with andexanet 75 mg. Interestingly, ciraparantag did not produce any change in anti-Xa activity, PT, or aPTT compared to the reductions demonstrated with andexanet. There was also no change in total rivaroxaban plasma concentrations with ciraparantag, compared with the 6-fold increase demonstrated with andexanet. The molar ratio necessary to decrease blood loss in this study with andexanet was about 1:1 and was 30:1 with ciraparantag. Overall, ciraparantag has been shown in preclinical trials to reduce bleeding from DOACs in internal (liver laceration models) and external (tail transection, human whole blood) bleeding. In the nonclinical safety assessment with rats and dogs, ciraparantag was assessed for its pharmacokinetic, pharmacodynamic, and safety properties. The maximum total daily doses of ciraparantag were 40 and 35 mg/kg in rats and dogs, respectively. In rats, nasal swelling or discharge occurred with every dose, whereas apathy and decreases in locomotive activity was seen at higher doses starting at 40 mg/kg. Death resulted in 1 out of 3 rats at approximately 3 min post administration of 60 mg/kg doses. Immediately following administration of this dose; prostration, labored breathing, and intermittent flailing was observed; surviving rats' were normal after 24 h post dose. In dogs, a dose of 47 mg/kg results in neurologic and vascular reactions. Hypersalivation and/or loss of balance was observed at 35 mg/kg. All symptoms resolved within 30 min post-dose in either species. No gross organ changes, histological changes, genotoxicity, or organ system changes (neurologic, respiratory, cardiovascular) were seen in either species after the 14-day repeat dosing of IV ciraparantag. All symptoms were very similar in the 14-day repeat dose study as the maximum tolerated dose study.

4.3. Phase I and 2 studies

Whole blood clotting time (WBCT), PT, and thromboelastography reaction time (TEG-R) are common endpoints used in the evaluation of ciraparantag's pharmacodynamic properties. The PT and TEG-R are not representative of physiologic conditions due the blood's interaction with citrate. A molar excess of anions, such as sodium citrate, oxalate, EDTA or heparin, will overwhelm the ciraparantag-anticoagulant complex. The anionic molecules will preferentially form a complex with the cationic ciraparantag molecule resulting in the release of the target

anticoagulant from this complex. Disruption of the ciraparantag-anticoagulant complex makes plasma-based assays unreliable. In addition, ciraparantag is adsorbed to activators, such as kaolin and celite, which significantly lower the concentration of active ciraparantag in a blood sample. Ciraparantag should not be evaluated in blood collected or tested with any of these anionic or chelating molecules otherwise the assay may not be representative of ciraprantag's affects (Ansell et al., 2017).

The first phase I clinical trial evaluated the ability of single-dose ciraparantag to reverse 60 mg of edoxaban (Ansell, Bakhru, et al., 2014). This study was a randomized, double-blind, placebo-controlled trial of 80 healthy subjects divided into 8 cohorts. Each cohort received an escalating dose of study drug from 5 mg up to 300 mg administered IV. Pharmacodynamic assessment of ciraparantag was evaluated using PT, TEG-R, and WBCT. As previously discussed, PT and TEG-R are poor biomarkers as they contain activators. Both biomarkers demonstrated high variability between subjects and insensitivity across time points. PT and TEG-R values in both the ciraparantag group and the placebo group showed minor variation across the following time points: pre-ciraparantag administration, 10 min post-administration, and 3.5 h post-administration. The PT value for the ciraparantag group for the above time points was 39.6 s, 39.1 s and 38.9 s, respectively. The PT value for the placebo groups was 46.5 s, 44.8 s, and 43.9 s, respectively. In the absence of an activator, WBCT is likely the most sensitive biomarker for ciraparantag. WBCT yielded reproducible values which correlated well with edoxaban pharmacokinetics. WBCT demonstrated a statistically significant reversal at 10 min post-administration of ciraparantag starting with the 50 mg dose. Clot fibrin integrity was measured using scanning electron micrographs to validate the findings of WBCT.

No procoagulant signals were significant throughout this study from evaluation of the D-dimer, TFPI, and prothrombin fragments 1.2. Visual signs of clot formations and fibrin strand diameter returned to baseline starting at the 100 mg dose. Ciraparantag was safe and well tolerated and all adverse events were considered to be mild in nature. The most common events included periorbital and facial warmth, flushing, and dysgeusia (Ansell et al., 2017).

In a similar study, ciraparantag reversal 4 h after an enoxaparin 1.5 mg/kg dose was evaluated in a single-blind, placebo controlled, ascending, repeat dose study. Forty healthy volunteers were given IV doses ranging from 100 mg to 300 mg. Enoxaparin increased the WBCT by an average of 30% above baseline. Reversal was seen with single doses of all cohorts receiving 100 mg, 200 mg and 300 mg, but not with 25 mg. Clot fibrin formation was restored to baseline within 30 min in a dose-dependent manner. Adverse events were very similar as compared to the edoxaban clinical trial, with no deaths or serious adverse events occurred (Ansell et al., 2016).

Interim data has been released for the most recent completed single-blind, placebo-controlled phase II clinical trial evaluating the effect of re-anticoagulation after ciraparantag administration. Healthy volunteers were given edoxaban 60 mg daily until steady state was achieved, followed by escalating single-doses of IV ciraparantag (25, 50, 100, 200, or 300 mg). Edoxaban continued to be given at the next scheduled dose and a subsequent reversal was performed with the same ciraparantag dose used previously. Interim data showed WBCT, measured 3 h post-administration, is reversed to pre-edoxaban levels starting with the 100 mg dose. The time to reach reversal of WBCT for the 100 mg and 300 mg doses are 60 min and 30 min, respectively. Mean WBCT was similar in re-anticoagulation as it was previous on day 3. Once again, clot fibrin structure supported the findings of decreased WBCT. The side effect profile of ciraparantag was similar across all clinical trials and mild in nature. (Laulicht, Bakhru, Steiner, et al., 2015). Ciraparantag has shown sustained effects for a duration of 24 h after administration in all clinical trials eliminating the need for infusion dosing. Ciraparantag is currently undergoing two phase 2 trials. The first is a randomized, single-blind, placebo-controlled study to assess the efficacy and safety of ciraparantag administration after rivaroxaban using WBCT [NCT03172910]. The second ongoing trial is a randomized, single-blind, placebo-controlled study to assess the efficacy and safety of ciraparantag administered after apixaban using WBCT [NCT03288454]. Currently, there are no phase III clinical trial study designs published in available literature.

4.4. Ciraparantag summary

Ciraparantag has many potential advantages as the next universal reversing agent. It is unlikely to induce immunogenic responses, can be given as a single bolus injection, and has a quick onset with prolonged effects. The less complex development of ciraparantag compared to andexanet will likely make it less costly. A major limitation includes the adaptation of WBCT testing in clinical practice and its validation in the use of other DOACs and heparins.

5. Conclusion

While the DOACs provide advantages over VKAs in terms of major bleeding, especially intracranial hemorrhage, their increased use in clinical practice forecasts that clinicians will see these bleeding episodes in more and more patients. Although simple replacement of clotting factors has been used, this approach lacks a pharmacological basis as well as any meaningful study. The specific antidotes of idarucizumab for dabigatran and andexanet for direct Xa inhibitors are pharmacologically specific agents with extensive laboratory, animal, and now human data to support their safety and efficacy. It will also be interesting to witness how ciraparantag continues it its development. These agents now provide the safety shield highly desired since the introduction of the DOACs several year ago.

Declaration of Competing Interest

Dr. Dobesh serves as a consultant for Boehringer Ingelheim, Janssen Pharmaceuticals, the Pfizer/BMS Alliance, and Portola Pharmaceuticals.

Dr. Bhatt serves as a speaker for Janssen Pharmaceuticals and Portola Pharmaceuticals.

Dr. Trujillo serves as a consultant for Portola Pharmaceuticals.

Dr. Glaubius has no potential conflicts to declare.

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