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RESEARCH ARTICLE

Reversal of Direct Oral Anticoagulants: Meeting a Need & Filling a Gap

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ABSTRACT

Presently, three reversal strategies are available to reverse the anticoagulant activity of the direct oral anticoagulants, each with various limitations or drawbacks. A new reversal agent that addresses some of these limitations or gaps in therapy is in advanced clinical trials. Ciraparantag is a small molecule specifically designed to bind non-covalently by charge-charge interaction to unfractionated heparin and low molecular weight heparin. It shows similar binding characteristics to the direct oral anticoagulants with no off-target binding of selected proteins or commonly used drugs demonstrated to date. Ciraparantag reaches maximum concentration within minutes following intravenous administration with a half-life of 12 – 19 minutes. It is primarily hydrolyzed by serum peptidases into two metabolites, neither of which has substantial activity. Ciraparantag and its metabolites are recovered almost entirely in the urine. In animal models of bleeding a single intravenous dose of ciraparantag given at peak concentrations of an anticoagulant significantly reduces blood loss. In Phase 1/2 clinical trials, ciraparantag restores coagulation in direct oral anticoagulant-treated healthy volunteers with in minutes with a sustained effect over 24 hours. The drug is well tolerated with minor side effects, the most common of which is a flush or sensation of warmth after administration. As a phase 3 trial is in preparation to clinically prove its effectiveness, ciraparantag appears to address many of the short-comings of current reversal strategies.

INTRODUCTION

Direct oral anticoagulants (DOACs) were developed without an antidote to reverse their anticoagulant activity when needed in patients with major bleeding or patients who require emergent surgery. The absence of a reversal agent was a factor in limiting the rapid uptake of these new anticoagulants because of concerns by both patients and physicians as to how to best manage major bleeding events. Ultimately, the manufacturers of dabigatran etexilate developed an antibody that specifically binds to, and reverses, the anticoagulant activity of dabigatran, and a reversal agent for the factor Xa inhibitors was developed shortly thereafter. Meanwhile, prothrombin complex concentrate (PCC), the mainstay for reversal of the vitamin K antagonists (VKA), has also been used to treat major bleeding episodes related to DOAC therapy[1]. A new reversal agent, ciraparantag, is currently in development to reverse DOAC activity. This report summarizes the mechanism of action of ciraparantag, its pharmacokinetic (PK) and pharmacodynamic (PD) characteristics, and its ability to reverse anticoagulant-induced coagulopathy and bleeding in pre-clinical and early phase 1/2 clinical trials.

IS ANOTHER DOAC REVERSAL AGENT NEEDED

Dabigatran etexilate, a direct factor II inhibitor, was the first DOAC approved for use in the United States in 2010[2]. It was followed shortly thereafter by four oral direct factor Xa (FXa) inhibitors (e.g., apixaban, betrixaban, edoxaban and rivaroxaban)[3,4,5,6]. Although these agents proved to be as effective, and in some cases more effective than warfarin, their principal benefit was their safety, with an approximate 50% reduction in intracranial hemorrhage, as well as their convenience compared to the VKAs[7,8]. However, the DOACs are still associated with an approximate annual three to four percent risk of major bleeding and an even higher rate of clinically relevant non-major bleeding[9]. They have also been associated with high rates of emergency room visits for adverse drug events in older adults[10]. Accordingly, it is clear that a reversal agent for the DOACs is desired, if not needed[11].

The characteristics of a desired reversal agent for the DOACs are summarized in Table 1[12]. There are currently three options for reversing the anticoagulant activity of the DOACs with each option having potential shortcomings.

Table 1. Characteristics of a desired reversal agent for the direct oral anticoagulants[12]

- **Readily available at the point of care**
- **Acts rapidly**
- **Produces sustained normalization of coagulation**
- **Has no significant side effects**
- **Does not induce a prothrombotic state**
- **Allows for re-anticoagulation after control of the acute bleeding event**
- **Does not induce tolerance and can be used repetitively, if needed, in the short or long term**
- **Does not induce an immune response**
- **Inexpensive or cost-effective**
- **Has the potential to improve patient outcome from the bleeding event**

Idarucizumab (Praxbind®, Boehringer Ingelheim, Germany) is a humanized murine monoclonal antibody Fab fragment with high specificity for dabigatran. Idarucizumab was studied In a single arm prospective trial (RE-VERSE AD trial) of patients with major bleeding due to dabigatran or patients requiring emergent surgery requiring dabigatran reversal[13]. It was considered effective in stopping major bleeding with a median time to cessation of bleeding of 2.5 hours. In the emergent surgery group, periprocedural hemostasis was assessed as normal in 93.4% of patients. At 90 days a small percentage of patients in each group experienced a thrombotic event (6.3% and 7.4% in bleeding and surgery groups respectively) which was considered not excessive in these patients with pre-existing prothrombotic conditions. Idarucizumab has limitations in that it is only effective against dabigatran which has a small share of the DOAC market. As a biologic, its manufacturing is complex resulting in a costly drug. It requires time to prepare for injection and two sequential injections are required.

Andexanet alfa (Andexxa®, Alexion AstraZeneca Rare Disease) is a synthesized recombinant DNA factor Xa[14]. Its enzymatic activity is modified to prevent its functioning in the coagulation cascade, but it is still recognized by factor Xa inhibitors. Thus, it acts as a decoy molecule that binds to the factor Xa inhibitor freeing up

endogenous Xa to participate in the coagulation cascade restoring normal coagulation. In a single arm prospective trial (ANNEXA-4 trial) of patients experiencing major DOAC-related bleeding, andexanet alfa was shown to result in excellent or good hemostasis in 82% of patients at 12 hours[15]. There was a 10% rate of thrombotic events in the 30 day follow up period. Andexanet alfa has limitations in that it is a biologic molecule requiring a complex manufacturing process, and thus, a costly drug. It requires time to prepare for infusion and when discontinued after a bolus dose followed by a continuous infusion (2 hours), following which, when discontinued, anticoagulation recurs based on the concentration of remaining factor Xa inhibitor. It also requires different doses for different DOACs and there is suggestive evidence that it has prothrombotic properties[16]. Antibodies to the protein occur, but do not seem to be significant (i.e., neutralizing).

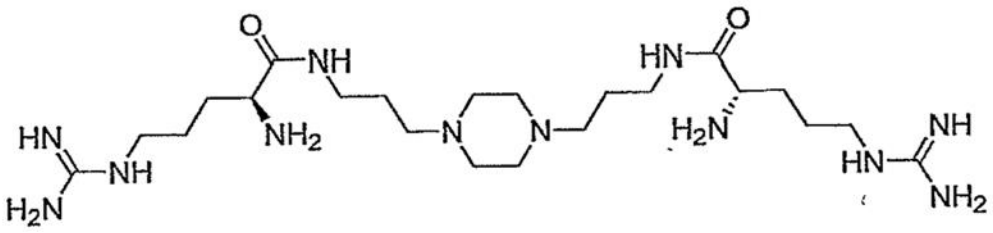
Prothrombin complex concentrates containing the four vitamin K-dependent coagulation factors (factors II, VII, IX and X) have a long history of use in reversing the activity of the vitamin K antagonists (e.g., warfarin, phenprocoumon and others). They are produced through purification of plasma from blood donations. In the absence, or unavailability of the specific reversal agents mentioned above, PCCs have been utilized to treat major DOAC-related bleedings[1,17]. It is likely that PCCs were employed because of their widespread availability, physician familiarity with them, certain limitations of the new agents, and possibly, because of their lower cost. Their effectiveness is based upon the idea that by providing excess coagulation factors, they overwhelm the specific DOAC on board and restore normal coagulation. Multiple observational and retrospective studies have evaluated the effectiveness of PCCs to reverse DOACs. In a recent systematic review[18] focusing on DOAC associated intracranial hemorrhage, outcomes with PCCs vs andexanet alfa were similar with regard to adequate hemostasis (~80%), mortality or thromboembolic events. The latter outcome is controversial with reports showing both low[17,19] and high[18,20] rates of PCC associated thromboembolism. PCCs have limitations in that they have the potential to transmit infection or incite allergic reactions, they require time to prepare, they are expensive and they are not licensed as reversal agents for DOACs.

Based on the shortcomings of available reversal agents, there is considerable interest in developing a more effective and less expensive agent.

CIRAPARANTAG

Ciraparantag, a small, synthetic, water-soluble, cationic molecule, was derived from a program of intentional molecular design searching for molecules that would bind to free unfractionated heparin through non-covalent, charge-charge interaction[21]. It is composed of naturally occurring amino acids (arginine) coupled by a piperazine ring (Figure 1).

Figure 1. Ciraparantag molecular structure ($C_{22}H_{48}N_{12}O_2$, acetate salt; M_w = 512Da) is composed of naturally occurring amino-acid derived substituents (piperazine and arginine) with short linking elements and is freely water soluble.

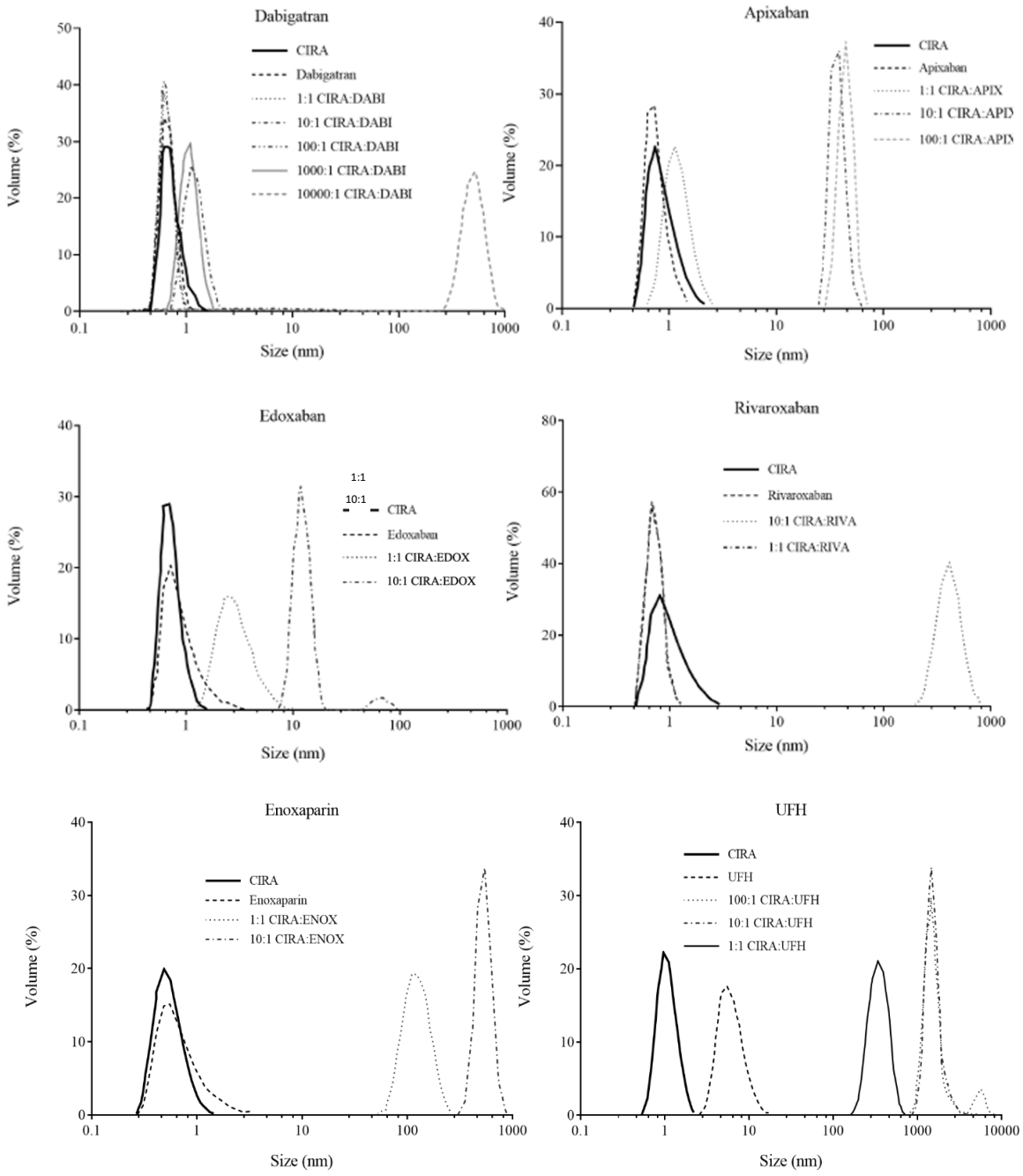


N¹,N¹'-[Piperazine-1,4-diylbis(propane-1,3-diyl)]bis-L-argininamide

Ciraparantag binding to UFH was demonstrated by strong ionic binding to a heparin affinity chromatography column. Binding to the DOACs was subsequently predicted by energy minimization modeling. Standard binding assays were not suitable for studies of ciraparantag binding to the DOACs because non-aqueous solvents disrupt physical interactions, the DOACs and ciraparantag are comparable in size and the DOACs are poorly water soluble, rendering measurements by equilibrium dialysis, mass spectrometry, and isothermal titration calorimetry unsuitable. Therefore, standard dynamic light scattering (DLS) methodology[22] was required as a

screening tool to determine the association or lack of association with the various DOACs as well as to enoxaparin and UFH (Figure 2). The data supports evidence of a physical association between ciraparantag and FXa/FIIa-inhibiting anticoagulants through ionic and/or hydrophobic, non-covalent bonding. By doing so, ciraparantag removes the DOACs from their intended target, (e.g., FXa), thereby freeing up factor Xa and allowing normal coagulation to be restored[21].

Figure 2. Dynamic light scattering (DLS) studies show the physical association of dabigatran, apixaban, edoxaban, rivaroxaban, enoxaparin, and UFH (21). Rightward migration of curves indicates a larger physical moiety representing the physical association between ciraparantag and anticoagulant. Modified from reference 21.



CIRA = ciraparantag; DABI = dabigatran; APIX = apixaban; EDOX = edoxaban; RIVA = rivaroxaban; ENOX = enoxaparin; UFH = unfractionated heparin

To assess ciraparantag's specificity, DLS protein and drug binding studies in human plasma demonstrated no binding to FIIa, FXa, and a range of commonly prescribed cardiac and non-cardiac drugs (diltiazem, digoxin, propranolol, lidocaine, metoprolol, lisinopril, propafenone, hydrochlorothiazide, triamterene and clopidogrel), antiepileptic drugs (carbamazepine, gabapentin, lamotrigine, phenytoin, and valproate), diabetic drugs (insulin, metformin), or other commonly used drugs (aspirin, atorvastatin, azithromycin, streptokinase)[21]. In addition, ciraparantag did not bind to plasma proteins in human plasma compared to compounds with known moderate to high plasma protein binding (propranolol and warfarin, respectively)

Measuring the effect of anticoagulation reversal with ciraparantag

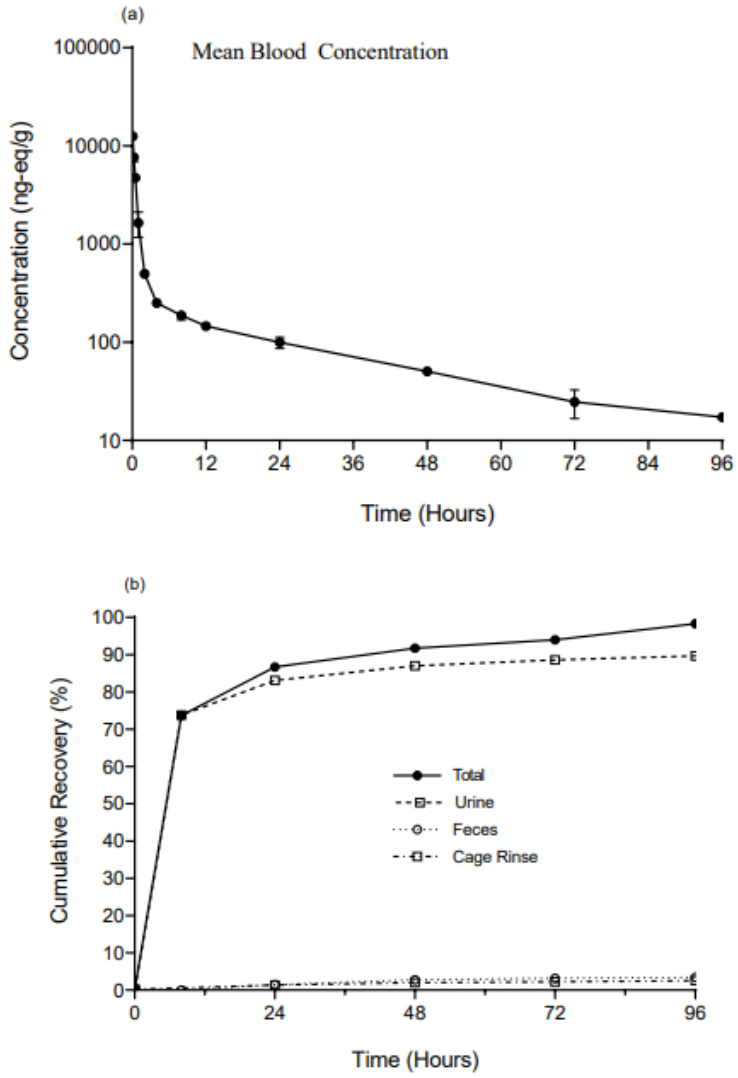
One of the challenges with ciraparantag is measuring its reversal effect with routine coagulation assays. In early animal studies, investigators noted inconsistent and widely variable results of standard coagulation assays such as the prothrombin time (PT), activated partial thromboplastin time (aPTT), and thromboelastogram (TEG) when ciraparantag was given to reverse DOAC induced-anticoagulation. This was in spite of the clear visual and quantitative reduction in blood loss ex vivo in animal models where anticoagulation was reversed with ciraparantag. The problem resided in the reagents used *in vitro* to anticoagulate whole blood in order to produce plasma to perform the assays. Ciraparantag is cationic and binds to the anionic substances in standard blood collection tubes used for coagulation testing such as sodium citrate, EDTA, oxalate and heparin. It also binds to the activators used in the traditional coagulation assays such as the aPTT (e.g., kaolin and Celite) making plasma-based assays, because of reagent interference and insensitivity, unsuitable for measurement of the ciraparantag effect. In subsequent clinical studies the manual whole blood clotting time (WBCT), which measures clotting in reagent-free collection equipment, was used as the key biomarker for ciraparantag's reversal activity. In clinical trials using the WBCT, it showed low variability (interobserver variation, 3.0%) and high reproducibility (inter-subject variation, 3.6%), and correlated well with edoxaban plasma concentrations[21].

The manual whole blood clotting time is impractical for routine clinical use. Consequently, an automated point-of-care coagulometer that replicates the WBCT has been developed that provides rapid, sensitive, accurate, and precise measures of whole blood clotting at the bedside[23,24]. At this time, this automated WBCT coagulometer is approved for clinical use in Europe[25] and poised to come before the FDA for approval in the US.

Pharmacokinetics and Metabolism of ciraparantag

The pharmacokinetics (PK), rates, and routes of excretion, with mass balance and tissue distribution of ciraparantag were determined using quantitative whole-body autoradiography (QWBA) with ^{14}C-labeled ciraparantag[21]. Following a single dose of 10 mg/kg radio-labeled ^{14}C-ciraparantag, the Tmax (peak of radioactivity) of ciraparantag in whole blood was observed at 5 minutes after the dose, the earliest time point sampled (Figure 3a). After the plasma C_{max} a steep decline through 4 hours followed (distribution phase), and a more gradual decline thereafter (elimination phase). Drug-derived radioactivity at 1 hour was widely distributed after the dose and tissue concentrations of radioactivity were highest in the kidney, urinary bladder, and urine. The main route of elimination of ^{14}C-ciraparantag was urinary excretion (Figure 3b). Approximately 82% of the total dose was recovered in the first 8 hours and most of the total dose (~93%) was recovered within the first 24 hours. Elimination via other routes was minimal.

Figure 3. (a) Elimination of [14C]-ciraparantag; The Tmax (peak of radioactivity) of ciraparantag in whole blood was observed at 5 minutes post-dose, the earliest time point sampled. The maximum plasma concentration (Cmax) was followed by a steep decline through 4 hours post-dose (distribution phase), and a more gradual decline thereafter (elimination phase). (b) Tissue recovery of [14C]-ciraparantag; Drug-derived radioactivity was widely distributed at 1 hour post dose. Tissue concentrations of radioactivity were highest in the kidney, urinary bladder, and urine. The main route of elimination of 14C-ciraparantag was urinary excretion. Approximately 82.44% of the total dose was recovered within the first 8 hours and 93% within the first 24 hours.



Pharmacokinetic data was obtained in 70 human subjects as part of the first-in-human trial of ciraparantag[21]. PK data were insufficient to estimate all PK parameters in the lowest dose cohort (5 mg). In the 15-300 mg cohorts, ciraparantag demonstrated dose proportional PKs. Ciraparantag PK parameters (Table 2) and mean serum ciraparantag concentrations vs time are provided in Figure 4a (period 1, ciraparantag alone). The Cmax for ciraparantag increased from 173 ng/mL at the 5-mg dose to 10,570 ng/mL at the 300-mg dose. Dose-proportional PK was further observed with AUC over 24 hours (AUC0-24), which increased from an average of 151 to almost 3800 ng/mL per hour with 15- and 300-mg doses, respectively. Maximum serum ciraparantag concentrations occurred within 5 to 9 minutes (Tmax), then rapidly declined, and were below the limit of quantification by 2 hours after drug administration. The $t_{1/2}$ of ciraparantag ranged from 12 to 19 minutes across cohorts. The median volume and total body CL estimates ranged from 25.4 to 34.6 L/h and 65.7 to 116.0 L/h, respectively. The PK of ciraparantag after edoxaban administration (Fig. 4b, period 2) for Tmax and other parameters (full data not shown) were similar to those of ciraparantag alone (Fig. 4a).

Table 2. Ciraparantag serum and BAP urine PK parameters from the phase 1 clinical trial, during period 1 (ciraparantag alone). From reference 21.

PK parameter, median (range)	Ciraparantag dose, mg						
	5	**15**	**25**	**50**	**100**	**200**	**300**
Ciraparantag serum							
T_{max}, h	0.08 (0.07-0.13)	0.08 (0.07-0.10)	0.10 (0.07-0.10)	0.09 (0.08-0.12)	0.15 (0.13-0.17)	0.14 (0.13-0.17)	0.15 (0.13-0.17)
C_{max}, ng/mL	173 (85-249)	634 (447-800)	861 (702-1060)	2205.0 (1680.0-2580.0)	3860 (3250-4920)	7755 (6280-10900)	10570 (9660-12200)
$t_{1/2}$, h	NA*	0.20 (0.16-0.33)	0.19 (0.16-0.29)	0.24 (0.17-0.36)	0.24 (0.20-0.31)	0.33 (0.24-0.39)	0.32 (0.24-0.42)
CL, L/h	NA	90.5 (71.6-133.7)	116.0 (67.9-126.5)	77.8 (65.3-104.3)	76.1 (58.1-92.2)	65.7 (49.4-90.4)	79.9 (63.4-100.8)
V, L	NA	25.4 (23.0-36.9)	31.0 (28.2-34.9)	26.6 (20.7-40.3)	26.1 (22.9-36.1)	30.4 (22.7-48.2)	34.6 (29.4-47.7)
BAP* urine							
A_{e0-24h}, µg	5.26 (0.00-21.59)	41.20 (0.00-77.50)	76.04 (0.00-138.60)	621.21 (249.76-1021.60)	2527.32 (130.73-27919.18)	40764.38 (4960.56-65748.24)	43654.29 (12544.68-91064.80)
F_{m0-24h}, %	0.11 (0.00-0.43)	0.27 (0.00-0.52)	0.30 (0.00-0.55)	1.24 (0.50-2.04)	2.53 (0.13-27.92)	20.38 (2.48-32.87)	14.55 (4.18-30.35)
CL, L/h	NA	NA	1.16 (0.00-2.30)	3.88 (1.16-7.26)	5.73 (0.37-48.52)	29.72 (4.55-74.70)	23.38 (8.44-48.92)

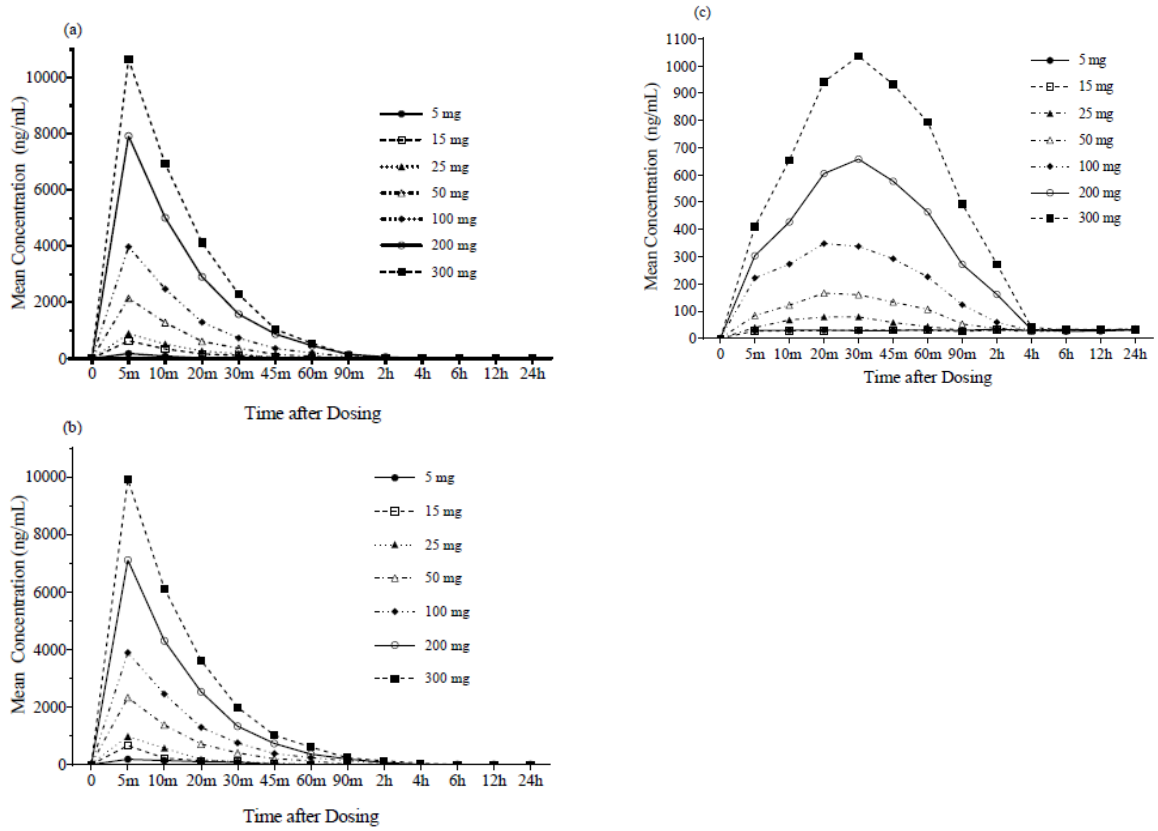
*NA = not available; *BAP is major ciraparantag metabolite (3-(4-(3amino-propul)piperazin-1-yl)propanoic acid

T_{max} = time maximum concentration; C_{max} = maximum concentration; $t_{1/2}$ = time of half-life; CL = clearance; V = vol of distribution; A_{e0-24h}, µg = cumulative amount excreted into urine from time of dosing up to 24 hr. F_{m0-24h}, % = fraction of ciraparantag dose excreted as BAP

The major metabolite of ciraparantag is 3-(4-(3amino-propul)piperazin-1-yl)propanoic acid (BAP). BAP serum exposure (AUC_{0-24} and AUC_{0-inf}) for the 25- to 300-mg dose cohorts (the 5 mg and 15 mg cohorts could not be estimated because of insufficient PK data), BAP AUC_{0-24} ranged from 29% to 52% of total ciraparantag exposure (AUC_{0-24}; without molecular weight adjustment; Figure 4c) supporting rapid hydrolytic metabolism of ciraparantag via circulating peptidase. BAP administration, at doses equivalent to large doses of ciraparantag, also reversed the anticoagulant effects of 10 mg/kg PO edoxaban. BAP (40 mg/kg IV) led to a significant reduction in blood loss that was equivalent to sham blood loss (no edoxaban). This dose of BAP is equivalent to 170 mg/kg ciraparantag on a dry powder weight basis assuming complete and immediate conversion of ciraparantag to BAP. Since the earlier study showed that ciraparantag 5 mg/kg IV fully reversed the anticoagulant effect of 10 mg/kg PO edoxaban, it is estimated that BAP has 1/34th the potency of ciraparantag on a molar basis with respect to edoxaban reversal.

The excretion of ciraparantag (Ae0-24) in urine was unchanged. Main urine BAP PK results (Table 2) showed that the median cumulative amount of BAP (Ae0-24) increased from 5.26 mg (5-mg dose) to 43,654.3 mg (300-mg dose). Up to 20% (Fm0-24) of the ciraparantag dose was recovered in urine as BAP. Median BAP CLr across the 25- to 300-mg doses, where data were available, ranged from 1.2 to 29.7 L/h.

Figure 4. Mean serum concentration versus time by dose cohort. (a) ciraparantag; (b) ciraparantag after edoxaban; (c) ciraparantag metabolite BAP. From reference 21.



Initial proof of concept and pre-clinical trials

The four DOACs plus UFH and enoxaparin were studied in animal models of bleeding induced by an anticoagulant and results support the binding of ciraparantag to DOACs and heparins (Figure 5, graphs a-h)[21]. In a rat tail transection model of bleeding induced by edoxaban (10 mg/kg, orally), ciraparantag given after edoxaban but prior to rat tail transection at doses of 1.25 and 2.5 mg/kg IV had no effect on reduction of blood loss. With higher doses (5 and 10 mg/kg), blood loss was significantly reduced and similar to sham rats that received no edoxaban (Figure 5a,b). Ciraparantag at 30 mg/kg, when given after the rat tail transection (i.e., during active bleeding), also reduced blood loss (Figure 5c).

In rats dosed with apixaban (3.125 mg/kg PO), the administration of ciraparantag (12.5 mg/kg IV before tail transection) significantly reduced blood loss compared with baseline blood loss with no ciraparantag, indicating a full reversal of apixaban activity (Figure 5e). Similarly, ciraparantag (6.25 mg/kg IV before tail transection) significantly reduced bleeding compared to a saline control in rats dosed with rivaroxaban (5 mg/kg PO). At a dose of 31.25 mg/kg, ciraparantag fully reduced blood loss to baseline levels (Figure 5f). Lastly, high dose ciraparantag (31.25 mg/kg IV before tail transection) fully reversed the anticoagulant effects of dabigatran (37.5 mg/kg PO) in the rat tail transection model similar to the levels observed in control animals (Figure 5d).

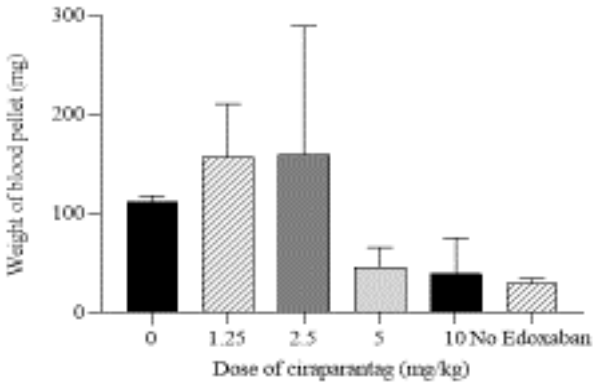
Ciraparantag's ability to reverse the anticoagulant effects of UFH was also compared to protamine sulfate, a standard reversal agent for UFH (Figure 5g). In animals given UFH (1 mg/kg IV), the administration of ciraparantag (20 mg/kg IV before tail transection) significantly reduced blood loss. However, protamine sulfate (10 mg/kg IV) did not lead to a significant change in blood loss compared to saline controls. Protamine sulfate reduced the UFH concentration to baseline (based on an anti-FXa activity assay) and significantly reduced aPTT to baseline, while ciraparantag did not significantly decrease aPTT levels.

Similarly, in studies with enoxaparin, ciraparantag (30 mg/kg IV before tail transection) fully reversed the anticoagulant activity of enoxaparin (10 mg/kg IV) as shown by blood loss

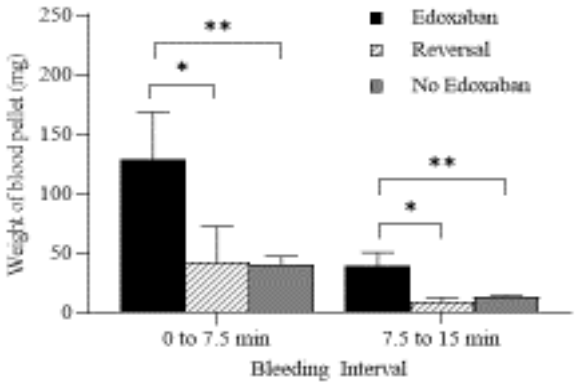
being restored to that of sham animals (Figure 5h). Protamine sulfate, at the recommended dose of 10 mg/kg IV, did not lead to a significant change in blood loss following enoxaparin when compared with saline-treated animals. The aPTT returned to control values after protamine (despite having no apparent effect on increased bleeding) while the aPTT was not altered by ciraparantag (despite reversing the increase in bleeding after enoxaparin). These results indicate that standard coagulation assays (anti-FXa activity assay and aPTT) are an ineffective measure of the ciraparantag effect and may not be appropriate in monitoring effectiveness of ciraparantag in this species with this model.

Figure 5. Effect of ciraparantag to reverse anticoagulation in a rat tail transection model: edoxaban (a, b, c), dabigatran (d), apixaban (e), rivaroxaban (f), unfractionated heparin (g), and enoxaparin (h). From reference 21.

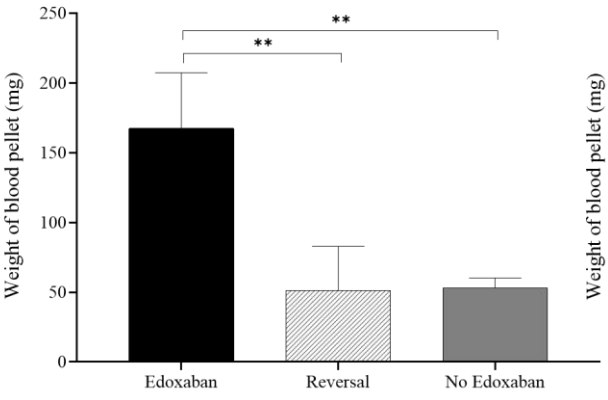
(a) Blood loss after edoxaban (10 gm/kg PO) with increasing doses of ciraparantag given before rat tail transection



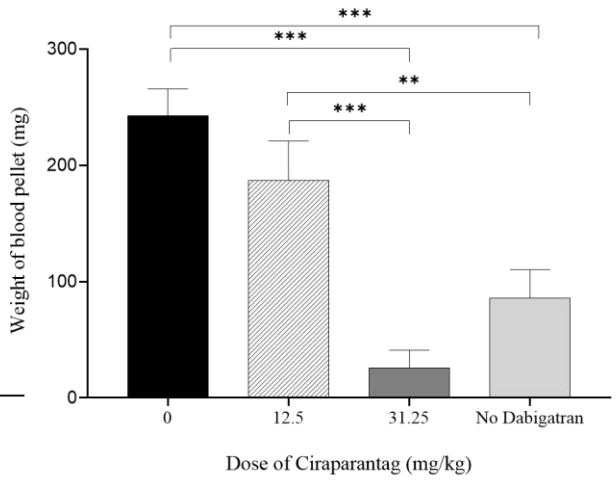
(b) Blood loss during specific collection periods *p<0.05; **p<0.01



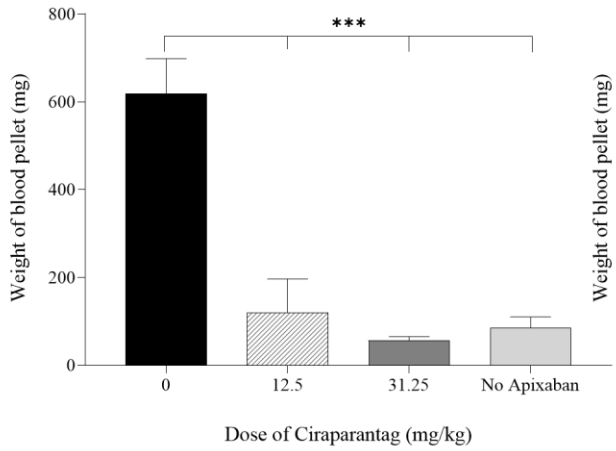
(c) Blood loss when ciraparantag (30 mg/kg) was given after rat tail transection **p<0.01



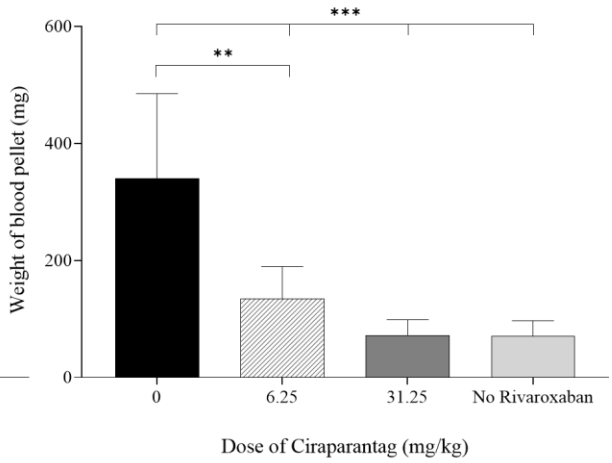
(d) Blood loss after dabigatran (37.5 mg/kg PO) ***p<0.001; **p<0.01



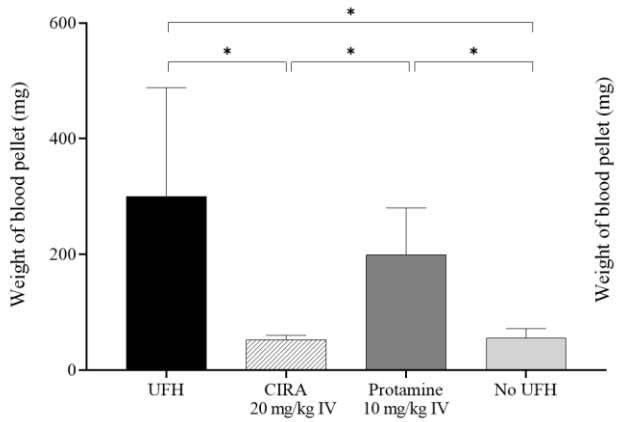
(e) Blood loss after apixaban (3.125 mg/kg PO). ***p<0.001



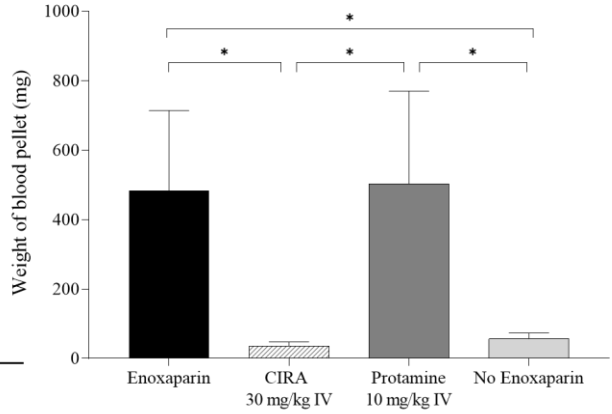
(f) Blood loss after rivaroxaban (5 mg/kg PO). **p<0.01; ***p<0.001



(g) lood loss after unfractionated heparin (UFH) dosing compared with protamine dosing; *p<0.05



(h) Blood loss after enoxaparin dosing compared with protamine dosing; *p<0.05



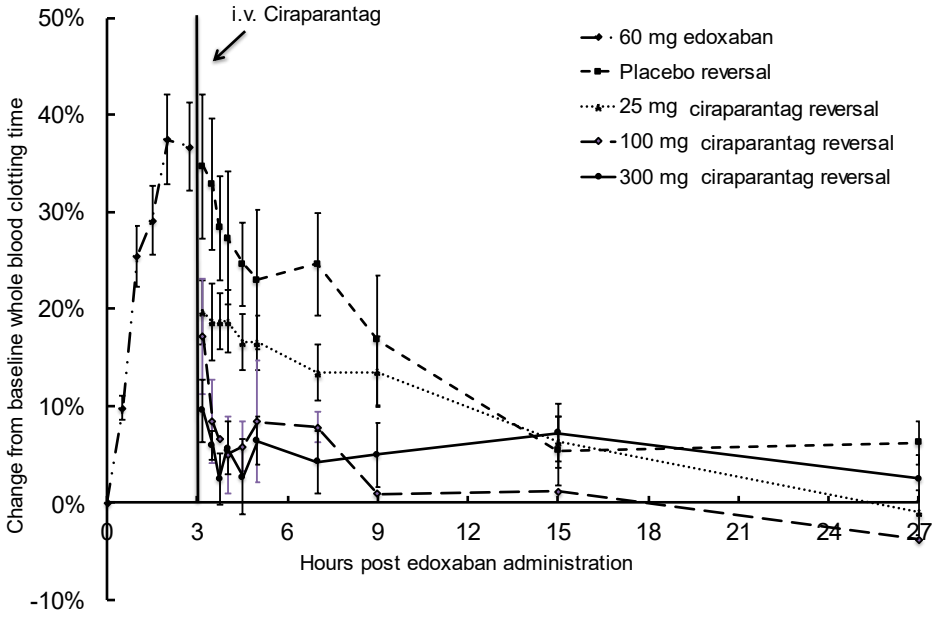
Phase 1/2 clinical trials

The pharmacodynamic effect of escalating, single intravenous doses of ciraparantag (5 to 300 mg) administered after a 60-mg oral dose of edoxaban was studied in a double-blind, placebo-controlled trial involving 80 healthy persons[26]. After individual doses of ciraparantag (100 to 300 mg) given 3 hours after the administration of edoxaban, the whole-blood clotting time decreased to within 10% above the baseline value in 10 minutes, whereas 12 – 15 hours elapsed before placebo patients returned to baseline (Figure 6a). The whole-blood clotting time remained within 10% above or below the baseline value for 24 hours after the administration of a single dose of ciraparantag.

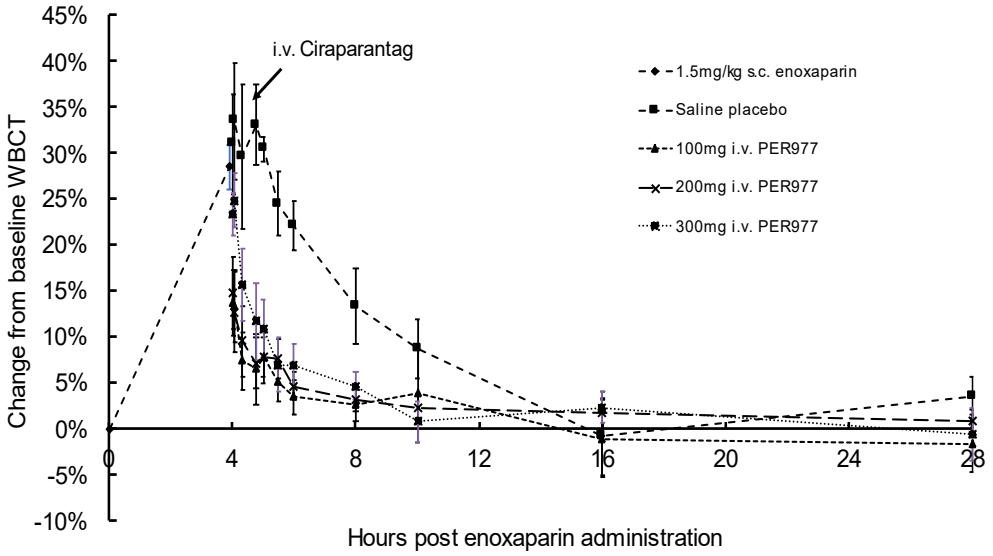
Similar results were obtained in subjects given enoxaparin[27]. Complete reversal of enoxaparin anticoagulation was achieved in all subjects who received a single ciraparantag dose ranging from 100 mg to 300 mg compared to placebo approximately 4 h after a single subcutaneous dose of enoxaparin, 1.5 mg/kg (Figure 6b). Anticoagulation reversal occurred rapidly and persisted for the duration of the study. At 12 h and 24 h, the differences in whole blood clotting time in the treated group compared to placebo were no longer significant, consistent with the decline in enoxaparin concentrations and anticoagulation effects. No procoagulant signals were detected as measured by D-dimer, F1.2, and tissue factor pathway inhibitor levels.

Figure 6. Dose response reversal of (a) edoxaban anticoagulation and (b) enoxaparin in healthy volunteers (n=80). Figures 6a and 6b from references 26 and 27 respectively.

(a)



(b)



Two phase 2 randomized, placebo-controlled, dose-ranging trials were conducted in healthy subjects aged 50–75 years to assess reversal of apixaban and rivaroxaban[28]. Apixaban was administered 10 mg orally twice daily for 3.5 days or rivaroxaban 20 mg orally once daily for 3 days. At steady-state the anticoagulated volunteers were randomized 3:1 to a single intravenous dose of ciraparantag (Study 1: 30, 60, or 120 mg; Study 2: 30, 60, 120, or 180 mg) or placebo (note: doses expressed as pure ciraparantag rather than the acetate form expressed in previous studies). Complete reversal of WBCT within 1 h post-dose and sustained through 5 h (apixaban) or 6 h (rivaroxaban) was dose related and observed with apixaban in 67%,

100%, 100%, and 17% of subjects receiving ciraparantag 30 mg, 60 mg, 120 mg, or placebo, respectively; and with rivaroxaban in 58%, 75%, 67%, 100%, and 13% of subjects receiving ciraparantag 30 mg, 60 mg, 120 mg, 180 mg, or placebo, respectively (Figure 7,8).

Figure 7. Correction of whole blood clotting time (WBCT) vs dose of ciraparantag. Proportion of subjects with complete and sustained reversal of steady-state anticoagulation induced by apixaban (Study 1) or rivaroxaban (Study 2). Complete and sustained reversal is a WBCT $\leq$ 10% above baseline within 1 hour after ciraparantag/placebo dose and sustained through at least 5 or 6 hours for apixaban or rivaroxaban, respectively. From reference 28.

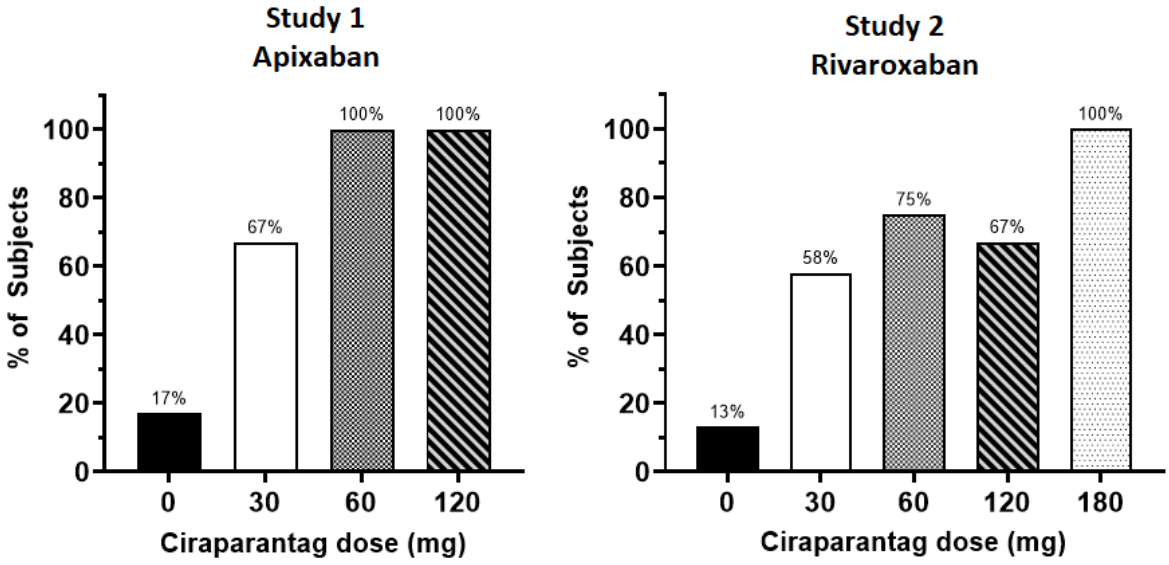
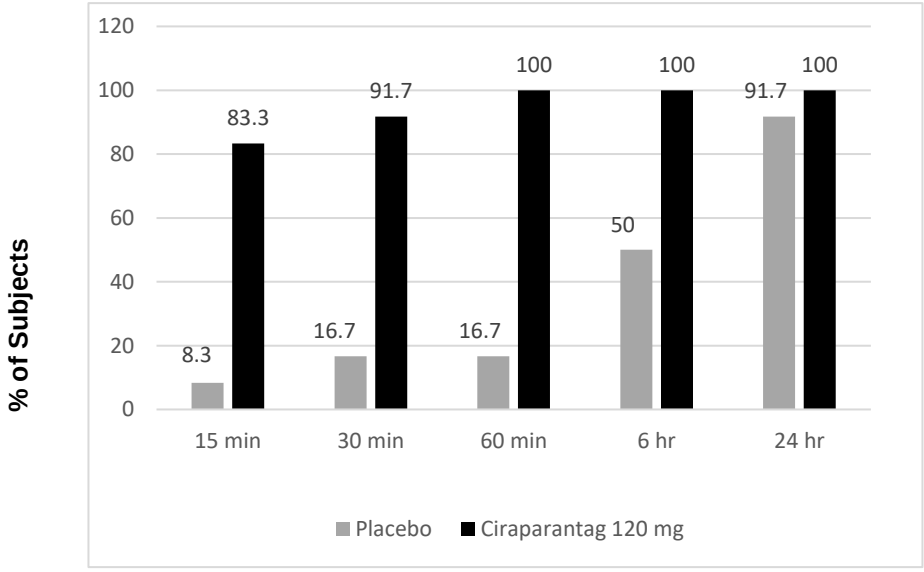
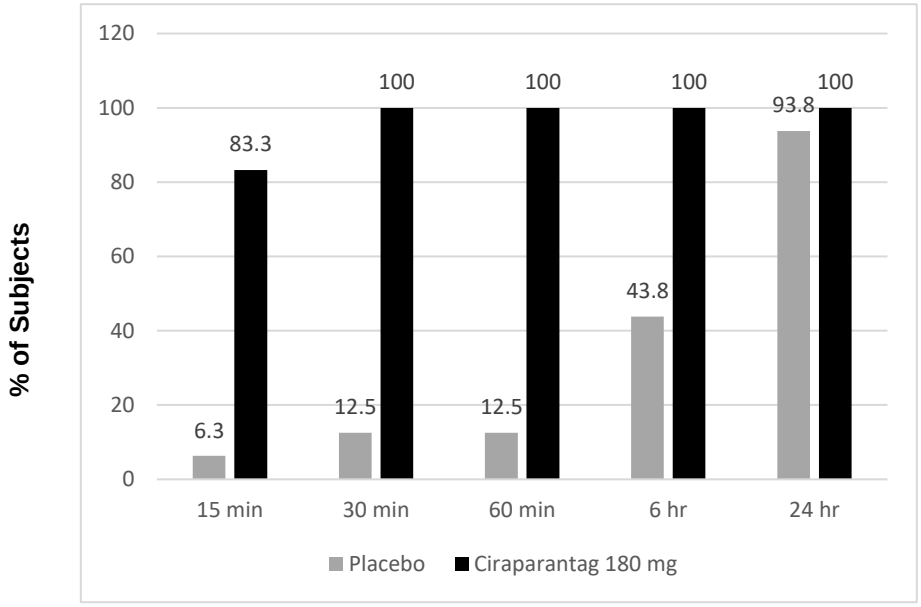


Figure 8. Correction of whole blood clotting time (WBCT) vs time after ciraparantag application Proportion of subjects with complete reversal of steady-state anticoagulation induced by apixaban (Study 1) or rivaroxaban (Study 2). Complete reversal is defined as a WBCT $\leq$ 10% above baseline at any time point within 1 hour of ciraparantag/placebo dose. The dose of ciraparantag for apixaban was 120 mg and for rivaroxaban was 180 mg. From reference 28.

Study 1 - Apixaban



Study 2 - Rivaroxaban



In the first-in-human trial noted above[26] and in the phase 2 studies of reversal of apixaban and rivaroxaban[28], adverse events related to ciraparantag were similar. Table 3 summarizes the adverse events in the phase 2 trials. In these trials, ciraparantag was well tolerated across all range of doses. Mild, transient sensations of warmth (i.e., hot flashes or flushing) were dose related and the most common complaint. These events occurred soon after drug administration and mostly resolved with less than one hour. Ciraparantag did not produce any clinically significant change in routine laboratory measurements (CBC, chemistry, liver, renal function, lipids), vital signs or ECG parameters. Importantly, in all human studies, when measured, there were no consistent changes in prothrombotic markers such as prothrombin fragment 1.2, D-dimers or tissue factor pathway inhibitor.

Table 3. Adverse events (AE) in human trials with ciraparantag from reference 28

	Study 1 Apixaban		**Study 2 Rivaroxaban**	
Subjects with:	Ciraparantag n=36 n (%)	Placebo n=13 n (%)	Ciraparantag n=48 n (%)	Placebo n=16 n (%)
Treatment-emergent AEs (TEAEs)	13 (36.1%)	0	20 (41.7%)	2 (12.5%)
TEAEs in >1 subject				
Hot flush	8 (22.2%)	0	9 (18.8%)	0
Feeling hot	3 (8.3%)	0	2 (4.2%)	0
Feeling cold	1 (2.8%)	0	4 (8.3%)	0
Paresthesia	0	0	3 (6.3%)	0
Flushing	0	0	2 (4.2%)	0
Dizziness	0	0	2 (4.2%)	0
Dysgeusia	0	0	2 (4.2%)	0

CIRAPARANTAG COMPARED TO EXISTING REVERSAL ANGENTS

Since the introduction of the DOACs there has been some controversy as to the necessity of a reversal agent given the short half-life of each of the DOACs (29,30). Unfortunately, there have been no randomized trials of the available reversal agents versus no reversal agent or standard of care. Given the short comings of the two specific available reversal agents, the PCCs, which have always been widely available, by default, may have become the standard of care although they are not licensed for use in DOAC treated patients

so far. Of note, some studies show no difference in outcomes with PCCs versus andexanet alfa (18).

Compared to the existing reversal agents in clinical use, ciraparantag has unique, favorable attributes including its broad-spectrum activity reversing oral Factor Xa inhibitors and parenteral Xa/IIa inhibitors, its ability to be rapidly and easily administered, and its long functional pharmacodynamic half-life (Table 4).

Table 4. Comparative attributes of ciraparantag with existing reversal agents.

Reversal Agents	How Given	Drawbacks	Advantages
Idarucizamab (Praxbind®) *Binds dabigatran and restores anticoagulation*	-Given as 2 sequential IV infusions	-Large monoclonal AB (biological) -Only reverses dabigatran -Requires storage and preparation -Costly	-No prothrombotic effect -One short treatment -Sustained effect over 24 hrs -No significant side effects
Andexanet alfa (Andexxa®) *Binds the Xa inhibitors and restores anticoagulation*	-Given as a bolus dose followed with continuous infusion over 2 hrs	-Large recombinant protein (biological) -Requires storage and preparation -Preparation cumbersome -Different doses per Xa inhibitor -Requires a 2 hr infusion -When stopped, anticoagulation returns based on concentration of remaining anticoagulant -Is not licensed for use in emergency surgery -Is not licensed for use in edoxaban-treated patients -Reversal effect cannot be monitored by commercially available (diluted) antiXa assays -May have a prothrombotic effect -Costly	-Reverses all of the direct factor X inhibitors and low molecular weight heparin
Prothrombin Complex Concentrates *Replaces vitamin K -dependent coagulation factors*	-Given as a brief infusion	-Purified human coagulation factors - Contains factor II, VII, IX and X -Requires storage and preparation -Risk of allergic, infectious reactions -May have a prothrombotic effect -Is not licensed for use in DOAC treated patients -Costly	-Great familiarity with product -May be as effective as andexanet alfa at less cost
Ciraparantag *Binds the Xa inhibitors and restores anticoagulation*	-Given as a brief infusion	-Reversal effect cannot be measured by standard coagulation tests but can be measured by whole blood clotting time	-Reverses all direct oral factor X inhibitors, low molecular weight heparin and unfractionated heparin -Small synthesized molecule -Sustained effect over 24 hrs -Storage and preparation simple -Long shelf-life -Fixed dose for all Xa inhibitors -Cost not yet known, but likely to be less expensive option

CONCLUSION

Animal models of bleeding and initial human studies in healthy volunteers show the ability of ciraparantag to rapidly reverse DOAC induced bleeding (oral FXa inhibitors - apixaban, edoxaban, rivaroxaban; and the parenteral IIa/Xa inhibitors, enoxaparin and UFH) or coagulation defect in a dose-dependent manner without significant safety signals.

The clinical efficacy, safety and potential advantages of ciraparantag need to be confirmed by phase 3 clinical trials in patients with major bleeding or needing urgent surgery which are currently in the planning stages.

CONFLICTS OF INTEREST

E. Lindhoff-Last has received lecture honoraria and advisory fees from Bayer AG, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, Daiichi-Sankyo, Portola, CSL Behring, Norgine Pharmaceuticals, Viatris and Aspen and institutional research support from Bayer AG, Bristol-Myers Squibb/Pfizer, Daiichi-Sankyo and CSL Behring for a different research project.

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