Reversal of anticoagulants: an overview of current developments

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Summary

Several new anticoagulants have entered the clinical arena or are under clinical development. These drugs include indirect (fondaparinux) and direct oral factor Xa inhibitors (rivaroxaban, apixaban, edoxaban, betrixaban), and the direct thrombin inhibitor dabigatran. Especially the oral direct FXa and FIIa inhibitors overcome many of the shortcomings of heparins and vitamin K antagonists (VKAs). They are administered orally at a fixed dose; regular monitoring is not necessary; interaction with other drugs or nutrition occur less than with VKAs and they are at least as effective as VKAs for most indications tested. They are associated with about 50 % less intracranial bleeding than VKAs. Nevertheless, they are still associated with bleeding complications. Bleeding can occur spontaneously or as a result of trauma or urgent surgery. In such situations rapid reversal of the anticoagulant effect is highly desirable. For unfractionated heparin protamine, and for VKAs prothrombin complex concentrates are available as specific antidotes. Under clinical development are: for the direct and indirect FXa inhibitors a modified recombinant FXa (andexanet alpha), which lacks enzymatic activity; and for dabigatran a Fab fragment of a monoclonal antibody (idarucizumab). In addition a small molecule (aripazine) has entered phase I clinical trials, which seems to inhibit nearly all anticoagulants but VKAs and argatroban. This review summarises the current options and strategies in development to antagonise anticoagulants with a focus on the status of the development of antidotes for the oral direct FXa and FIIa inhibitors.

Keywords

New anticoagulants, antidotes, apixaban, dabigatran, rivaroxaban

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Introduction

Longer-term therapeutic dose anticoagulation is indicated primarily for prevention of stroke in patients with atrial fibrillation, for deep-vein thrombosis and pulmonary embolism, and in patients with heart valve disease. The risk for these prothrombotic disorders increases with age. Due to the demographic change in the Western World with an ageing population the number of individuals with an indication for therapeutic dose anticoagulation constantly increases. However, with the therapeutic benefit, treatment with anticoagulants also bears risks. For example, after anti-diabetic drugs, warfarin and antiplatelet agents accounted for most emergency hospitalisations in the elderly in the US between 2007 and 2009 (1). Treatment with anticoagulants and/or with antiplatelet drugs increases the risk for major bleeding (►Table 1A und ►Table 1B), especially when anticoagulants and antiplatelet drugs are combined (2). Bleeding can occur spontaneously or as a result of trauma or urgent surgery.

During the last decade, several new anticoagulants have entered the clinical arena or are under clinical development. These drugs include the synthetically produced, antithrombin catalysing pentasaccharide fondaparinux (3), the oral, direct factor Xa inhibitors rivaroxaban, apixaban, edoxaban, betrixaban, the direct thrombin inhibitor dabigatran, which is given as the oral agent dabigatran

etexilate. Especially the oral direct FXa and FIIa inhibitors overcome many of the shortcomings of heparins and vitamin K antagonists (VKAs). They are administered orally at a fixed dose; regular monitoring is not necessary; interaction with other drugs or nutrition is much less an issue than with VKAs and they are at least as effective as VKAs for most indications tested, except for mechanical heart valves (4). Most prominently, the direct oral FXa and FIIa inhibitors have a risk for intracranial haemorrhage that is ~50 % lower than for VKAs (5, 6) (►Table 1A). This important advantage is a robust and constant finding through all studies irrespective of the indication or the anticoagulant used, and the increased risk for intracranial haemorrhage seems to be a class effect of VKAs.

Regardless which anticoagulant is involved, management or prevention of bleeding can be a major issue for the individual patient and for the treating physician. Especially, if an acute trauma occurs or if emergency interventions are necessary, rapid reversal of the anticoagulant effect is highly desirable.

Based on the different modes of action of these different anticoagulants, it is difficult to foresee an universal "one for all" antidote for all anticoagulants. This will add a new layer of complexity to the management of patients requiring therapeutic dose anticoagulation. In the following we will review the current options and strategies in development to antagonise anticoagulants with a

Table 1A: Risk of major bleeding for long-term anticoagulant treatment. Bleeding rates between studies are not directly comparable because of the variable study designs and patient populations.

	Agent	Comparator(s)	Indication	Dose	Treatment duration	Patients (n)	Incidence rate, % of patients or otherwise indicated (intracranial bleeding)	Hazard ratio (95 % CI; p-value) unless otherwise indicated (intracranial bleeding)	References
Major bleeding	Vitamin K antagonists	Dabigatran Rivaroxaban Apixaban Edoxaban	DVT treatment (combined analysis from RE-COVER, RE-COVER II, EINSTEIN-DVT, EINSTEIN-PE, AMPLIFY, Hokusai-VTE)	INR 2–3	≥3 months, (more than 50 % of patients 6 months)	13,481	**1.8 (0.3)**	**	(6)
		Dabigatran Rivaroxaban Apixaban Edoxaban	Atrial fibrillation (Metaanalysis RE-LY, ROCKET AF, ARISTOTLE, and ENGAGE AF–TIMI 48)	INR 2–3	≥12 months	29,211	**6.17 (1.45)**	***	(5)
	LMWH	Vitamin K antagonist++	DVT/ tumour	Tinzaparin 175 IU/kg	3 months	100	**7.0**	NS	LITE (53)
		Vitamin K antagonist	DVT/ tumour	Dalteparin 150 IU/kg	6 months	336	**6.0**	NS	CLOT (54)
		NS	DVT enoxaparin dalteparin tinzaparin				**2.1 %/ year 6 %/ year 2.0 %/ year**	NS	(55)
	Fondaparinux		No long-term treatment studies						
	Danaparoid		No long-term treatment studies						
	Apixaban	Enoxaparin (initial), warfarin	DVT, PE	10 mg bid for 7 days, 5 mg bid	6 months	2,691	**0.6 (0.1)**	RR 0.31 (0.17–0.55; p<0.001)	AMPLIFY (56)
		Placebo	DVT long-term prophylaxis #	2.5 mg bid	12 months	840	**0.2**	RR 0.49 (0.09–2.64)	AMPLIFY-EXT (57)
		Warfarin	Atrial fibrillation	5 mg bid	≥12 months	9,088	**2.13 %/ year (0.33)**	0.69 (0.60–0.80; p<0.001) 0.42 (0.30-0.58; p<0.001)	ARISTOTLE (58)
		Aspirin	Atrial fibrillation	5 mg bid	≥12 months	2,808	**1.4 %/ year (0.4)**	1.13 (0.74–1.75; p=0.57) 0.85 (0.38-1.90; p=0.69)	AVERROES (59)
	Rivaroxaban	Enoxaparin (initial), warfarin	DVT	15 mg bid 3 weeks, 20 mg once daily	≥3 months	1,731	**0.8**	0.65 (0.33–1.30; p=0.21)	Einstein-DVT (60)
		Enoxaparin (initial), warfarin	PE	15 mg bid 3 weeks, 20 mg once daily	≥3 months	2,412	**1.1**	0.49 (0.31–0.79; p=0.003)	Einstein-PE (61)

Table 1A: Continued

	Agent	Comparator(s)	Indication	Dose	Treatment duration	Patients (n)	Incidence rate, % of patients or otherwise indicated (intracranial bleeding)	Hazard ratio (95 % CI; p-value) unless otherwise indicated (intracranial bleeding)	References
Major bleeding		Placebo	DVT long-term prophylaxis	20 mg once daily	≥6 months	598	**0.7**	NS	Einstein-Extension (60)
		Warfarin	Atrial fibrillation	20 mg once daily	12 months	7,111	**3.6 %/ year (0.5)**	1.04 (0.90–1.20; p=0.58) 0.67 (0.47-0.93; p=0.02)	ROCKET AF (62)
	Edoxaban	Warfarin	DVT*	60 mg once daily	≥3 months	4,118	**1.4**	0.84 (0.59–1.21; p=0.35)	Hokusai-VTE (63)
		Warfarin	Atrial fibrillation	30 mg once daily	24 months	7,002	**1.61 %/ year (0.26)**	0.47 (0.41–0.55; p<0.001) 0.30 (0.21-0.43; p<0.001)	ENGAGE AF-TIMI 48 (64)
				60 mg once daily		7,012	**2.75 %/ year (0.39**	0.80 (0.71–0.91; p<0.001) 0.47 (0.34-0.63; p<0.001	
	Betrixaban	Warfarin	Atrial fibrillation	40 mg once daily 60 mg once daily 80 mg once daily	≥3 months	127 127 127	**0** **0** **2.4**	N/A N/A HR 0.609 (0.145–2.557)	EXPLORE-Xa (65)
	Dabigatran	Warfarin	DVT*	150 mg bid	6 months	1,274	**1.6**	0.82 (0.45–1.48)	RE-Cover I (66)
		Warfarin	DVT*	150 mg bid	6 months	1,279	**1.2 (0.15)**	0.69 (0.36–1.32)	RE-Cover II (67)
		Warfarin Placebo	DVT long-term prophylaxis#	150 mg bid	≥6 months	1,430 681	**0.9** **0.3**	0.52 (0.27 to 1.02; p=0.06)	RE-MEDY/ RE-SONATE (68)
		Warfarin	Atrial fibrillation	110 mg bid	2 years	6,015	**2.7 %/ year (0.23)**	RR 0.80 (0.70 to 0.93; p=0.003) 0.31 (0.20-0.47; p<0.001)	RE-LY (69) RE-LY
				150 mg bid	2 years	6,076	**3.1 %/ year (0.3)**	RR 0.94 (0.82 to 1.08; p=0.41) 0.40 (0.27-0.60; p<0.001)	
	Argatroban		No long-term treatment studies						

LMWH: low-molecular-weight heparin; NS: Not stated; RR: relative risk; # after 3–6 months treatment phase; * after initial treatment with heparin/warfarin for 5–10 days; ++after initial treatment with unfractionated heparin; **RR (95% CI) for NOAC vs VKA: 0.61 (0.45-0.83; p=0.002) and 0.37 (0.21-0.68; p=0.001) for major bleeding and intracranial bleeding; ***RR (95% CI) for NOAC vs VKA: 0.86 (0.73-1.00; p=0.006) and 0.48 (0.39-0.59; p<0.0001) for major bleeding and intracranial bleeding.

Table 1B: Risk of clinically relevant-non major bleeding during long-term anticoagulant treatment. Bleeding rates between studies are not comparable because of the variable study designs and patient populations.

	Agent	Comparator(s)	Indication	Dose	Treatment duration	Patients (n)	Incidence rate, % of patients or otherwise indicated	Hazard ratio (95 % CI; p-value) unless otherwise indicated†	References
Clinically relevant– non major bleeding	Vitamin K antagonists	Dabigatran Rivaroxaban Apixaban Edoxaban	Combined analysis from phase 3 trials for DVT treatment (RE-COVER, RE-COVER II, EINSTEIN-DVT, EINSTEIN-PE, AMPLIFY, Hokusai-VTE)	INR 2–3	≥3 months, (more than 50 % of patients 6 months)	13,481 10,287	8.0	**	(6)
		Dabigatran AZD0837 Ximelagatran	Atrial fibrillation	INR 2–3	≥3 months		6.25 (fatal and non-fatal bleeding)	***	(70)
	LMWH						No data		
	Fondaparinux		No long-term treatment study				No data		
	Danaparoid		No long-term treatment study				No data		
	Apixaban	Enoxaparin (initial), warfarin	DVT, PE	10 mg bid for 7 days, 5 mg bid	6 months	2,691	3.8	RR 0.48 (0.38–0.60)	AMPLIFY (56)
		Placebo	DVT long-term prophylaxis	2.5 mg bid	12 months	840	3.0	RR 1.29 (0.72–2.33)	AMPLIFY-EXT (57)
				5 mg bid		813	4.2	RR 1.82 (1.05–3.18)	AMPLIFY-EXT (57)
		Warfarin	Atrial fibrillation	5 mg bid	≥12 months	9,088	4.07 %/year (incl. major bleeding)	0.68 (0.61–0.75; p<0.001)	ARISTOTLE (58)
		Aspirin	Atrial fibrillation	5 mg bid	≥12 months	2,808	3.1 %/ year	1.15 (0.86–1.54; p=0.35)	AVERROES (59)
	Rivaroxaban	Enoxaparin (initial), vitamin K antagonist	DVT	15 mg bid 3 weeks, 20 mg	≥3 months	1,731	7.3	NS	Einstein-DVT (60)
		Enoxaparin (initial), vitamin K antagonist	PE	15 mg bid 3 weeks, 20 mg	≥3 months	2,412	9.5	NS	Einstein-PE (61)
		Placebo	DVT long-term prophylaxis	20 mg once daily	≥6 months	598	5.4	NS	Einstein-Extension (60)
		Warfarin	Atrial fibrillation	20 mg once daily	12 months	7,111	11.8 %/ year	1.04 (0.96–1.13; p=0.35)	ROCKET-AF (62)
	Edoxaban	Warfarin	DVT	60 mg once daily	≥3 months	4,118	7.2	0.80 (0.68–0.93; p=0.004)	Hokusai-VTE (63)

Table 1B: Continued

	Agent	Comparator(s)	Indication	Dose	Treatment duration	Patients (n)	Incidence rate, % of patients or otherwise indicated	Hazard ratio (95 % CI; p-value) unless otherwise indicated†	References
Clinically relevant– non major bleeding		Warfarin	Atrial fibrillation	30 mg once daily	24 months	7,002	6.60 %/ year	0.66 (0.6–0.71; p<0.001)	ENGAGE-AF-TIMI 48 (64)
				60 mg once daily		7,012	8.67 %/ year	0.86 (0.79–0.93; p<0.001)	
	Betrixaban	Warfarin	Atrial fibrillation	40 mg once daily	≥3 months	127	0.8	0.264 (0.030–2.364)	EXPLORE-Xa (65)
				60 mg once daily		127	3.9	1.257 (0.337–4.684)	
				80 mg once daily		127	1.6	0.538 (0.098–2.937)	
	Dabigatran	Warfarin	DVT	150 mg bid	6 months	1,274	5.6 (including major bleeding)	0.63 (0.47–0.84; p=0.002)	RE-COVER I (66)
		Warfarin	DVT long-term prophylaxis	150 mg bid	≥6 months	1,430	5.6	0.54 (0.41 to 0.71; p<0.001)	RE-MEDY RE-SONATE (68)
		Placebo				681	5.3 (including major bleeding)	2.92 (1.52–5.60; p=0.001)	
		Warfarin	Atrial fibrillation	150 mg bid 110 mg bid	24 months 24 months	6,076 6,015	Not assessed Not assessed	N/A N/A	RE-LY (69) RE-LY
	Argatroban		No long term treatment study				No data	N/A	

† NS: Not stated; RR. Relative Risk. **RR (95% CI) for NOAC vs VKA: 0.73 (0.58-0.93, p=0.01) for clinically relevant non-major bleeding. ***Odds Ratio (95% CI) for NOAC vs VKA: 0.87 (0.78-0.97, p=0.05) for fatal and non-fatal haemorrhage.

focus on the development of antidotes for the oral direct FXa and FIIa inhibitors. We searched NCBI PubMed and the special abstract issues of the international congresses ASH, ISTH, AHA, ESC between 2011–2014 for the items antidote, reversal, idarucizumab, PER977, aripazine, PRT064445, andexanet alpha, aDabi-Fab, yT-S195-antithrombin. In addition, the www.clinicaltrials.gov website was screened for the terms andexanet alpha, idarucizumab, and PER977. Of note, many references of preclinical as well as clinical studies of the antidotes summarised here are abstracts and not peer reviewed publications.

Parenteral anticoagulants

The anticoagulant that can be rapidly reversed is unfractionated heparin. Intravenously infused protamine binds heparin and hereby neutralises its anticoagulant effects. This makes unfractionated heparin still the anticoagulant of choice in situations requiring rapid reversal of the drug, e. g. anticoagulation during cardiac surgery or therapeutic dose anticoagulation in critically ill patients. However, unfractionated heparin does not play a substantial role in long term anticoagulation, where low-molecular-weight heparins (LMWH) or fondaparinux are preferentially used. LMWH can partly be neutralised by protamine (30–40 %) which binds to the fraction of longer heparin molecules in LMWHs and neutralises their anti-IIa activity (7). For the residual smaller molecules in LMWH with anti-FXa activity and for the very small molecule fondaparinux currently no antidote is available. However, a potential antidote is under clinical development (►Table 2). The same is true for danaparoid, a mixture of heparan sulfate, chondroitin sulfate, and dermatan sulfate. Especially for fondaparinux and danaparoid the lack of an antidote can be an issue due to their long half-lives (►Table 2), which is further aggravated by their potential accumulation in patients with renal insufficiency.

For the two parenteral direct thrombin inhibitors argatroban and bivalirudin there is also no specific antidote available, although this may be less needed as their half-lives are relatively short (▶ Table 2).

Oral anticoagulants

For VKAs, which inhibit vitamin K-dependent gamma-carboxylation of the clotting factors II, VII, IX, and X, enhancement of carboxylation by vitamin K (10–20 mg orally or i. v.) is an option to increase the availability of active clotting factors. However, it requires 12–24 hours (h) after vitamin K is given (8), until a sufficient amount of these clotting factors is carboxylated to reconstitute sufficient haemostasis. Immediate reconstitution of the clotting system can be achieved by infusion of the carboxylated clotting factors as prothrombin complex concentrates (PCC), while transfusion of plasma is less efficient (9). Indeed, replacement of carboxylated clotting factors is the only option to rapidly reverse VKAs. Transfusion of PCCs should be combined with vitamin K application because of the shorter half-life of these clotting factors compared to the half-life of VKAs (especially factor VII -$T_{1/2}$ ~7h). When vitamin K is given together with PCCs it allows carboxylation of endogenous clotting factors, especially FVII, maintaining sufficient haemostasis when the transfused FVII is consumed or cleared. PCCs can be prothrombotic and no more than 40–50 U/kg body weight should be given as initial treatment (10, 11).

For the other oral anticoagulants with direct anti-clotting factor activity, no specific antidotes are currently available. Prothrombin complex concentrates (12) and activated prothrombin complex concentrates (FEIBA) have been proposed in case of severe bleeding to overcome the anti-FXa or anti-FIIa activities by increasing the plasma concentration of these clotting factors. In addition recombinant FVIIa (NovoSeven, Novo Nordisk, Copenhagen, Denmark) has been considered to improve haemostasis by increasing thrombin generation (13). There are no clinical studies

Table 2: Anticoagulants and their half lifes and potential antagonists (71–76). *approved and available; # volunteer studies available; + patient studies running.

	Drug	mode of action	Elimination half-life	Clearance	Specific direct antagonists
Oral anticoagulants	phenprocoumon	inhibition of vitamin K-–dependent γ-carboxylation of clotting factors	100–200 h (~7 days until INR-normalisation)	hepatic	vitamin K*, PCC*
	warfarin	inhibition of vitamin K-–dependent γ-carboxylation of clotting factors	36–42 h	hepatic and renal	vitamin K*, PCC*
	dabigatran	direct thrombin inhibition	12–14 h	80 % renal	idarucizumab#+, modified thrombin§, aripazine (PER977)
	rivaroxaban	direct factor Xa inhibition	9–13 h	66 % renal	andexanet alpha (PRT064445)#,+ aripazine (PER977)#
	apixaban	direct factor Xa inhibition	8–15 h	25 % renal	andexanet alpha (PRT064445)#,+ aripazine (PER977)#
	edoxaban	direct factor Xa inhibition	10–14 h	35 % renal	andexanet alpha (PRT064445), aripazine (PER977)#
	betrixaban	direct factor Xa inhibition	19–27 h	85 % hepatic, <8 % renal	andexanet alpha (PRT064445), aripazine (PER977)
Parenteral anticoagulants	unfractionated heparin	antithrombin-mediated inhibition of serine proteases (predominantely factor IIa and Xa inhibition)	30–60 min	phagocytosis	protamine*, aripazine (PER977)
	low-molecular-weight heparin	antithrombin-mediated inhibition of serine proteinases (predominantely factor Xa inhibition)	3–6 h	renal	high molecular weight molecules by protamine*, low-molecular-weight molecules by andexanet alpha (PRT064445)#, aripazine (PER977)#
	fondaparinux	antithrombin-mediated factor Xa inhibition	17 h	renal	andexanet alpha (PRT064445)#, aripazine (PER977)#
	danaparoid	antithrombin-mediated factor Xa inhibition	24 h	renal	andexanet alpha (PRT064445)
	argatroban	reversible factor IIa inhibition	45 min	hepatic	modified thrombin
	bivalirudin	factor IIa inhibition	30 min	renal	none

evaluating the efficacy of rFVIIa as antidote for anticoagulants other than VKAs. Anecdotal reports, *in vitro* and *ex vivo* data, and volunteer studies (14, 15) indicate that these clotting factor concentrates may reduce the bleeding tendency (16, 17).

Dialysis (18) or haemofiltration are options to remove dabigatran, which binds moderately to plasma proteins. But these procedures take time and are difficult to perform in emergency situations, especially in acutely bleeding patients (19). Dialysis is most efficient in patients who present with both, high plasma levels of dabigatran and renal impairment. Edoxaban, which also reaches considerable free plasma levels, cannot be removed efficiently by dialysis (20).

For all strategies to antagonise the direct oral FXa or FIIa inhibitors it must be considered that these drugs do not only distribute within the intravascular but also in the extravascular compartment. From there they will redistribute, even if the drug in the intravascular compartment has been removed or antagonised. This is important for dosing and application of the antidote, which has to take the extravascular drug amount into account and needs to be considered when interpreting *in vitro* data using human plasma. *In vitro* data do not reflect redistribution of the active drug from the extravascular compartment. For the same reason, when dialysis is used to remove dabigatran, it should be further continued after low drug levels have been reached to further eliminate redistributing dabigatran (19).

Approaches of antidote development for the direct oral FXa and FIIa inhibitors

An antidote in development for the direct FXa inhibitors (rivaroxaban, apixaban, edoxaban, betrixaban) is a modified, recombinant activated FX (rFXa) (21). This recombinant protein (andexanet alpha, PRT064445, Annexa™-A, Portola Pharmaceuticals, Inc., San Francisco, CA, USA) is catalytically inactive and lacks the membrane-binding γ-carboxyglutamic acid domain (Gla domain) of native FXa. But it retains the ability to bind direct FXa inhibitors as well as antithrombin activated by LMWH or fondaparinux.

For dabigatran, which inhibits thrombin, two different approaches have been used, the one is based on a modified thrombin molecule (22); the other is based on a Fab-fragment of a humanised monoclonal antibody, which directly binds and thereby inhibits dabigatran and which is currently in phase 3 clinical development (idarucizumab; Boehringer Ingelheim, Ingelheim, Germany).

The third approach is a small molecule (aripazine; PER977; Perosphere Inc., Bedford, MA, USA) which has been reported to antagonize the effects of all anticoagulants tested except VKAs and argatroban, while at the same time no binding to other frequently used drugs should occur.

Factor Xa inhibitor antidote (andexanet alpha, PRT064445, Annexa™-A)

This modified rFXa is produced in Chinese hamster ovary cells. It has no intrinsic procoagulatory activity because the amino acid serine at position 419 is replaced by alanine. This modification still allows the molecule to bind the direct anti-FXa inhibitors and heparin-antithrombin-complexes while lacking its enzymatic activity. However, the risk of a modified, functionally inactive rFXa is that it could still compete with the active FXa molecules in an activated clotting cascade, thereby inhibiting normal coagulation. Therefore, the molecule is expressed without the Gla domain, which contains the COOH group allowing binding of FX to phospholipids. This prevents the modified rFXa from binding to the phospholipid surface of cells and thereby to form complexes with tissue factor and FVIIa. Thus it does not interfere with the activation of endogenous FX to FXa by the complex of tissue factor and FVIIa. The modified rFXa andexanet alpha also binds antithrombin complexed with LMWH or fondaparinux, thereby inhibiting inactivation of native FXa by antithrombin. For LMWHs, andexanet alpha is only a partial antidote which blocks their anti-FXa activity but not their remaining activity against FIIa. Thrombin inhibition, which is predominantly associated with longer heparin chains of LMWH, could be additionally blocked by protamine, if necessary.

Animal experiments show efficacy of andexanet alpha to inhibit the direct FXa inhibitors as well as fondaparinux, but it was somewhat less efficacious to inhibit the LMWH enoxaparin (21, 23).

In a phase 1 study (24), 32 volunteers were randomised to andexanet alpha (between 30 and 600 mg; n=24) or placebo (n=8). When their plasma was spiked *ex vivo* with rivaroxaban, the effects of rivaroxaban on thrombin generation and anti-FXa activity were reversed dose dependently.

In several phase 2 double-blind, placebo-controlled studies, volunteers received apixaban, rivaroxaban, betrixaban, and the LMWH enoxaparin for six days, followed by an intravenous bolus of andexanet alpha with four different doses (25). First results are available with a dose of 90 mg, which decreased the anti-FXa activity of apixaban within 2 minutes (min) by ~ 65 % (n=4) (25). At a dose of 420 mg andexanet alpha neutralised 91 % of the apixaban anti-FXa-effect (compared to placebo) and 53 % of the rivaroxaban anti-FXa effect, respectively (26, 27).

In a study with healthy volunteers receiving apixaban 5 mg b.i.d. for six days, a bolus of andexanet alpha (420 mg) followed by an infusion of 4 mg/min for 120 min, resulted in complete reversal of the inhibition of thrombin generation which remained within the normal range after stop of andexanet alpha (27). Edoxaban 60 mg once daily was reversed by 52 % after a bolus of 600 mg andexanet and by 73 % by a bolus of 800 mg, each followed by 8 mg/min infusion for 1 h (28). Interestingly, within 2 h after stop of andexanet alpha infusion, anti-FXa levels returned to the drug levels measured in the group receiving edoxaban and placebo.

Currently a further trial has been started testing reversal of apixaban with a dose of 400 mg andexanet alpha (NCT02207725) and

reversal of rivaroxaban with a dose of 800 mg andexanet alpha (NCT02220725) in elderly healthy volunteers. The design of this placebo controlled trial in subjects 50–75 years of age treated with apixaban has been reported as an abstract in late 2014. In the first part of this study a bolus regimen of 400 mg andexanet i.v. is tested and in the second part a bolus of 400 mg followed by a 2 h infusion (29). This trial is labelled phase 3; however, it is a volunteer study testing surrogate markers and not the effect of the antidote on bleeding. In the opinion of the authors, the trial is therefore rather a phase 2 trial. A phase 3 study to evaluate the effect of andexanet in bleeding patients receiving FXa-inhibitors just started (NCT02329327, status March 2015).

Challenges for andexanet alpha

The currently available data suggest that different doses of andexanet alpha seem to be required for the two direct FXa inhibitors apixaban and rivaroxaban, and it will be interesting to see the first results for edoxaban. For the indirect FXa inhibitors, fondaparinux and the LMWHs likely again different doses are needed (25). This may make it challenging to apply the appropriate dose under emergency conditions clinically.

In the phase 2 studies, andexanet alpha given to volunteers in the absence of an anticoagulant was temporally associated with a transient reduction of tissue factor pathway inhibitor, a slight increase of fibrinogen F1+2 fragments, and of D-dimer. No thrombotic events were observed in the healthy volunteers (data presented orally by Dr Marc Crowther at the ISTH 2013 congress Amsterdam). However, this possible prothrombotic effect might be of minor relevance in a situation where the antidote is given to reverse anticoagulants.

As with all structurally modified proteins, immunogenicity might become an issue. Although no anti-andexanet alpha antibodies have been reported yet, a modified human protein always bears the risk to induce antibodies. These antibodies are relatively unproblematic if they bind only to the modified protein, but the lessons learned from other pharmacologically produced human proteins are that an immune response raised against a modified human protein can result in antibodies which cross-react with the native endogenous protein. This has been shown previously e.g. for recombinant erythropoietin (30) and thrombopoietin (31), where the drug induced antibodies caused red cell aplasia and hypoproliferative thrombocytopenia, respectively. In a worst case scenario, such an antibody could lead to autoimmune FX deficiency.

Factor IIa inhibitor antidote

Idarucizumab

The antidote against the direct FIIa inhibitor dabigatran is derived from a monoclonal antibody which has been induced in mice using dabigatran bound to carrier proteins. The Fab sequences of the mouse antibody were then humanised and the Fab fragments are now produced recombinantly (32). The Fab fragment directly binds dabigatran with a very high affinity, which is ~350 times greater than that of thrombin. The complex of the Fab and dabigatran can no longer bind into the cleft of the active center of thrombin. Idarucizumab inhibits dabigatran very effectively within minutes *in vitro* (33) as well as *in vivo* in animals (32) and healthy volunteers. In a large animal, porcine trauma model of severe bleeding, the antidote effectively inhibited dabigatran as shown *ex vivo* by normalisation of all clotting tests applied (34) and *in vivo* by significant reduction of the blood loss (35). This pig model is rather promising as it closely reflects antagonising dabigatran in an emergency situation of trauma or major surgery, a situation which cannot be studied in human volunteers.

It was a concern that idarucizumab could also bind substrates of thrombin due to conformational similarities, which has been excluded by in vitro assays. Here the Fab-fragments neither bound to various clotting factors nor they had an effect on platelets, e.g. by binding to PAR-1, the platelet thrombin receptor (32).

The complexes of idarucizumab and dabigatran are filtered by the glomerula, where the Fab fragments (as all Fab fragments) are degraded and dabigatran is eliminated with the urine. This is the reason for the short half-life of the Fab fragments (45 min) compared to a complete IgG-antibody which has a half-life of ~21 days.

The phase 1 study with idarucizumab was performed in 110 volunteers who received increasing doses of the antidote (20 mg to 8 g). When the Fab fragments were shown to be well tolerated, 35 volunteers received 220 mg dabigatran b.i.d. for four days, followed by an infusion of 1 g, 2 g, or 4 g of idarucizumab over 5 min. Clotting tests (aPTT, PT, thrombin time, ecarin clotting time, diluted thrombin time) normalised immediately after the infusion in a dose-dependent manner with a complete and sustained effect in all subjects receiving the 4 g dose (37). In a study enrolling 46 volunteers receiving dabigatran 220 mg bid (healthy subjects) and 150 mg bid (moderately impaired renal function), idarucizumab was given at increasing doses from 1 g to 5 g 2 h after the last dabigatran intake. Sustained reversal of dabigatran was seen with the 2.5 g, the 5 g and the 2×2.5 g dosing regimens. Rechallenge of the volunteers two months later with the same dose again resulted in successful reversal of dabigatran. When dabigatran was given 24 h after the idarucizumab infusion, again therapeutic dose anticoagulation was achieved (38).

The only volunteer study testing the *in vivo* effect of idarucizumab on bleeding used a forearm incision model. There was a dose dependent return of fibrin formation with increasing doses of idarucizumab to 24%, 45%, and 63% of the baseline values with 1 g, 2 g and 4 g idarucizumab, respectively (39).

The short half-life of idarucizumab raised the concern that there might be a reappearance of anticoagulation when dabigatran is redistributed from the extravascular compartment into the intravasal space. It was therefore an interesting observation that within minutes after application of idarucizumab the intravasal concentration of total dabigatran rapidly increases (this includes protein-bound, idarucizumab-bound and any unbound dabigatran). In parallel, anticoagulant activity measured using clotting tests was normalised. This paradox can be explained by the fact that the gradient, which determines the diffusion velocity of

dabigatran from the extravasal to the intravasal compartment involves primarily free (unbound) dabigatran. As all dabigatran, which redistributes into the intravasal compartment, is immediately neutralised by idarucizumab, a high diffusion gradient is maintained until all active dabigatran is inactivated. The idarucizumab-dabigatran complexes are then cleared by the kidneys. Although in case of renal impairment the idarucizumab-dabigatran complexes are less efficiently cleared, the almost irreversible binding of dabigatran to idarucizumab (very slow off rate >260h) still prevents any relevant rebinding of dabigatran to thrombin (32).

In summer 2014 an international multicentre phase 3 study was started testing idarucizumab infusion of 5 g in patients who experience life-threatening or uncontrolled bleeding or who require emergency surgery or procedure (NCT02104947). The study targets to recruit 250–300 patients (with a pre-specified interim analysis) and has enrolled >100 patients already in March 2015.

Challenges for idarucizumab

About 15% of normal individuals have natural antibodies binding to the cleavage site of Fab fragments, thus immunogenicity of idarucizumab might become an issue. Based on the experience with other therapeutically used Fab fragments, e.g. abciximab (antibody against the fibrinogen receptor glycoprotein IIbIIIa) (40, 41), these natural anti-Fab-antibodies are usually of minor clinical relevance. In particular, they do not block the drug effect. As dabigatran does not bind to cells, complexes of dabigatran, idarucizumab and possible anti-Fab antibodies will unlikely attach to cells causing cell alterations (42).

One potential problem in patients with preexisting anti-Fab antibodies might be that the complexes of dabigatran, idarucizumab and the anti-Fab antibody will no longer be filtered by the kidney, because they are too large. This may prolong the resistance to new doses of dabigatran. However, this can be easily overcome by changing the anticoagulant, if further anticoagulation is required.

A further theoretical risk is the formation of anti-idiotype antibodies (43). Their existence has been hypothesized, e.g. for HLA-receptor-antibodies (44), for anti-alprenolol antibodies (45), for vaccines (46) and recently described in intravenous immunoglobulin G preparations against amyloid-beta antibodies in Alzheimer`s disease (47). Anti-idiotype antibodies bind to the variable region of an antibody and could thereby inactivate the dabigatran antidote, if this has to be given again. Even more problematic is that anti-idiotype antibodies can sterically mimick the original antigen of the idiotype antibody. Thus an anti-idiotype antibody against idarucizumab may mimick sterically dabigatran and could become an endogenous thrombin inhibitor. However, this risk is presumably very low, especially as the antidote is typically given only once.

Table 3: Current status of antidote development.

Antidote	Data available for	Ex vivo	Animal	Phase 1 & 2 trials*	Phase 3	References	ClinicalTrials.gov numbers
andexanet alpha, PRT064445	apixaban	+	+	+[#]	+	21,25,27,29[#]	NCT02207725 NCT02220725 NCT02329327
	betrixaban	+	+	+	+	21,25	
	rivaroxaban	+	+	+	+	21,23,24,26	
	edoxaban	n. d.	n. d.	+	+	27	
	fondaparinux	+	+	n. d.	+	21	
	enoxaparin	+	+	+	+	21,25	
idarucizumab	dabigatran	+	+	+	+	32–39	NCT02028780 NCT02104947
modified thrombin (γT -S195A-IIa)	dabigatran	+	+	n. d.	n. d.	22	
aripazine (PER977)	apixaban	+	+	n. d.	n. d.	48,49,50,51	NCT02206100 NCT02205905 NCT01826266 NCT02207257 NCT02206087
	rivaroxaban	+	+	n. d.	n. d.	23,48,50,51	
	edoxaban	+	+	+	n. d.	49,50,51,52	
	enoxaparin	+	+	n. d.	n. d.	49,50	
	dabigatran	+	+	n. d.	n. d.	48,51	
	heparin	n. d.	n. d.	planned	n. d.	n. d.	

*as these antidotes are all tested in volunteers in whom real bleeding studies are not possible, the differentiation between phase 1 and 2 studies is difficult. # this trial is named phase 3 trial but it enrolls elderly volunteers and measures surrogate markers and not the efficacy of the antidote in patients with acute bleeding or acute invasive interventions. n. d.: no data available; planned: study does not recruit patients; +: completed or ongoing studies.

Modified thrombin

In a similar approach as with modified FXa, modified thrombin molecules have been generated as potential antidotes for dabigatran (and other thrombin inhibitors) – an active site-mutated S195A thrombin (S195A-IIa) and its trypsinised derivative (γT -S195A-IIa). γT -S195A-IIa was more effective than S195A-IIa in antagonising dabigatran effects on clotting tests in human plasma *in vitro* and in a mouse model (both modified thrombins given at a dose of 6 mg/kg) (22). Finally, the modified thrombin will face similar immunogenic challenges as modified FXa (see above). To date, no clinical data have been reported for this potential antidote.

Aripazine (PER977)

Aripazine, or PER977, is a small molecule which binds non-covalently to anticoagulants, inhibiting the anticoagulant effects of LMWH, fondaparinux, the direct oral FXa inhibitors and dabigatran (48, 49). Information about this potential antidote is currently only available in abstract or correspondence format. *In vitro* studies showed no major interactions with other coagulation factors or albumin (49). In a rat model PER977 reduced bleeding by >90% when given in a situation where the rats received a 100 times overdose of dabigatran, rivaroxaban, apixaban, or edoxaban (48).

In vitro, PER977 also inhibited the effects of rivaroxaban, apixaban, and enoxaparin in human plasma (48). In a rabbit liver laceration model, rivaroxaban-induced blood loss was reduced to a similar extent when 30 mg/kg PER977 were given, as compared to a dose of 75 mg andexanet alpha/rabbit, despite PER977 having no effect on clotting assays (23). Another report challenges that PER977 binds directly to anticoagulants, but indicates that it rather acts procoagulatory similar to polycationic molecules (50). Also reversal of edoxaban by PER977 was tested in a rat liver laceration model where it reversed bleeding (51).

First human *in vivo* data with PER977 were reported in volunteers (n=80) who received an i.v. bolus of PER977 in doses ranging between 5 and 300 mg (52). The volunteers were either untreated or pretreated with 60 mg edoxaban. PER977 alone did not induce serious adverse events and no procoagulant signal (measured by D-dimer, TFPI, prothrombin fragments 1.2). A single bolus of 300 mg PER977 i. v. normalised the whole blood clotting time (no further specification) in volunteers who were pretreated with 60 mg edoxaban. This effect remained stable over 24 h. An overview of the phase 2 trials can be found under (www.clinicaltrials.gov/ct2/results?term=per977); in January 2015 no phase 3 trial had been registered yet.

Challenges for aripazine

In the opinion of the authors, currently the biggest challenge for aripazine is its unclear mode of action. It remains somewhat difficult to understand, how this compound should show specificity for a broad range of structurally very different anticoagulants, while at the same time not interfering with other biologically relevant molecules or other drugs. Furthermore, an animal study comparing PER977 with andexanet alpha indicates that the molecule may re-verse bleeding without reversing the altered clotting assays. This might become an issue for monitoring of reversal therapy.

Perspective

The new oral direct FXa and FIIa inhibitors have major advantages for longer-term therapeutic dose anticoagulation. They can increase patient safety by reducing the risk of intracranial haemorrhage by about 50% in comparison to VKAs. This is true, whether they are given for atrial fibrillation (5) or for treatment or secondary prophylaxis of deep-vein thrombosis (6). Their most important downside is the lack of specific antidotes in case of emergency situations (trauma; stroke requiring thrombolysis; urgent surgery), while VKAs can be directly antagonised with PCCs. This still makes physicians sometimes reluctant to prescribe one of the direct oral FXa inhibitors or the direct oral FIIa inhibitor. With the development of antidotes for these new drugs, and hopefully clinical availability in the near future, also the rare but often dramatic situations in which rapid reversal of the anticoagulant is required can be better managed. An overview of the developmental stage of these antidotes is given in ▶ Table 3 (as per March 2015).

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Conflicts of interest

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