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CURRENT OPINION

Reversal Strategies for NOACs: State of Development, Possible Clinical Applications and Future Perspectives

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Abstract The non-vitamin K antagonist oral anticoagulants (NOACs) are used for thromboembolic prophylaxis of patients with atrial fibrillation and in the treatment as well as secondary prophylaxis of patients with venous thromboembolism. Even though NOACs have a better safety profile than vitamin K antagonists (VKAs), there will still be bleeding complications on NOAC treatment. In some cases, stopping the NOAC and non-drug-related management such as manual compression and interventional endoscopy will be sufficient to stop the bleeding. In more serious bleeding events and before acute surgery, coagulation factor concentrates or NOAC-specific antidotes could be used. Coagulation factor concentrates can be used in patients with haemophilia and to reverse the effect of VKAs but, in NOAC-treated patients, results are inconsistent and these agents could potentially have pro-thrombotic effects. Specific antidotes for NOACs are expected to be on the market soon. Phase III clinical trials with a humanized antibody fragment directed against dabigatran (idarucizumab) and recombinant, modified factor Xa (andexanet alfa) are ongoing. A molecule (aripazine) with broad activity against various anticoagulants including NOACs is currently undergoing phase II trials. For use of these specific antidotes, it is desirable that measurements for coagulation activity with a short response delay are widely available for the different NOACs and further research in this field is needed. Furthermore, guidelines for antidote use, including general measures for the treatment of NOAC-related bleeding, should be available.

Key Points

Specific antidotes for non-vitamin K antagonist oral anticoagulants (NOACs) are expected to be available on the market soon.

Clinical trials are ongoing with idarucizumab (against dabigatran), andexanet alfa (against all factor Xa inhibitors) and aripazine (against all NOACs, fondaparinux and heparin).

Indications for use of NOAC antidotes are serious bleeding complications and before acute surgery in NOAC-treated patients.

1 Introduction

The non-vitamin K antagonist oral anticoagulants (NOACs) are used as alternatives to the vitamin K antagonists (VKAs) in thromboembolic prophylaxis of patients with atrial fibrillation (AF) and in the treatment as well as secondary prophylaxis of patients with venous thromboembolism (VTE) [1]. Treatment with NOACs has reached the guidelines in Europe for these indications and also for AF in the US.

On the market for AF and VTE are apixaban (Eliquis®), rivaroxaban (Xarelto®), dabigatran etexilate (Pradaxa®) and edoxaban (Lixiana®). Of importance for the use of

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NOACs are effective treatment options in case of serious bleeding complications on treatment, and to stop the treatment effect fast in case of a need for an operation or another procedure with a high risk of bleeding. At the moment, there is no registered antidote for these drugs, but several antidotes are under development. NOACs have a relatively short half-life in patients with normal kidney function, and therefore stopping therapy is part of the strategy in these critical situations.

The present review gives the status for the development of antidotes for NOACs, discusses the potential clinical applications and provides perspectives for the use of NOACs once the antidotes are available.

2 Pharmacological Properties of Non-Vitamin K Antagonist Oral Anticoagulants (NOACs)

Dabigatran etexilate is a prodrug that is rapidly converted by liver and plasma esterases to dabigatran, which is a competitive and reversible inhibitor of free and clot-bound thrombin. The half-life is 12–17 h and 80 % is excreted unchanged by the kidneys.

Rivaroxaban, apixaban and edoxaban are selective, competitive and direct inhibitors of both free factor Xa (FXa) and FXa associated with the prothrombinase complex, decreasing the activation of prothrombin to thrombin. The half-life of rivaroxaban is 7–11 h and 33 % is excreted unchanged by the kidneys. The half-life of apixaban is 12 h and 27 % of the drug is renally excreted unchanged. For edoxaban, the half-life is 9–11 h and 50 % is renally excreted unchanged.

3 Measurement of Plasma Concentrations

The golden standard for measurement of plasma concentrations of NOACs is liquid chromatography tandem mass-spectrometry (LC–MS/MS) [2]. Currently, the restricted availability of the test is one of the drawbacks, especially in acute situations [3].

Expected therapeutic and toxic plasma concentrations for each NOAC are shown in Table 1. Peak levels are measured 1–4 h after intake. Trough levels are measured 12 h after intake with twice daily dosing and 24 h after intake with once daily dosing, just before the next dose.

Increasing plasma concentrations of edoxaban and dabigatran showed increasing bleeding risk in clinical trials; however, these were limited by the fact that measurements of plasma concentration were not related to the time of the bleeding [4, 5]. There is evidence that NOAC plasma concentrations might not fully reflect bleeding risk, as major bleeding in some subgroups of patients (dabigatran: after implantation of a mechanical heart valve; edoxaban: in those with clinical risk factors for high bleeding risk such as reduced kidney function and body

Table 1 Plasma concentrations of NOACs

	Dabigatran[a]	Rivaroxaban[b]	Apixaban[c]	Edoxaban[d]
Trough (ng/mL)	110 mg BID: 65 (28–155) 150 mg BID: 91 (40–215) [5]	10 mg QD: 9 (1–38) [53] 20 mg QD: 26 (6–87) [54] 20 mg QD: 22 (4–96) [53]	2.5 mg BID: 32–97 5 mg BID: 63–103 [55, 56] 10 mg BID: 120 (41–335) [56]	30 mg QD: 25 60 mg QD: 48 [4]
Peak (ng/mL)	Time after last dose: 1–3 h 110 mg BID: 126 (52–275) 150 mg BID: 175 (74–383) [5]	Time after last dose: 2–4 h 10 mg QD: 125 (91–195) [53] 20 mg QD: 270 (189–419) [54] 20 mg QD: 222 (160–360) [53]	Time after last dose: 1–3 h 2.5 mg BID: 67–123 5 mg BID: 132–171 10 mg BID: 251 (111–572) [56]	Time after last dose: 1–2 h 30 mg QD: 85 60 mg QD: 175 [57]
Toxic	Trough 210 ng/mL (major bleeding rate doubled) [5]	No clinical data available	No clinical data available	Trough ca. 110 ng/mL (major bleeding rate doubled) [4]

BID twice daily, *NOACs* non-vitamin K antagonist oral anticoagulants, *QD* once daily

[a] Mean plasma concentrations are shown with 10th to 90th percentiles in parentheses

[b] Median predicted concentrations are shown with 5th to 95th percentiles in parentheses

[c] Ranges for the median of the different studies available are shown. For the 10 mg BID dose, the median is shown with 5th and 95th percentiles in parentheses

[d] Mean trough and median peak plasma concentrations are shown

weight under 60 kg) was not associated with increased plasma concentrations [4, 6]. A limitation that must be kept in mind is that the same plasma concentration can induce a variable degree of anticoagulation in different patients [7].

4 Coagulation Assays

Determining the anticoagulant activity of the drug using a coagulation assay is a more practical and possibly more accurate tool to assess a bleeding patient and the biological effect of the reversal agent. The ideal test is quick, cheap and widely available. The international normalized ratio (INR), prothrombin time (PT) and activated partial prothrombin time (APTT) are examples of such tests. Unfortunately, they are not suitable for quantitative evaluation of the anticoagulant activity of NOACs. Results of these assays vary according to the reagent used for each NOAC and the test is either insensitive or relatively insensitive already at plasma concentrations considered to be therapeutic [8]. The PT-derived INR was developed specifically for the monitoring of VKAs and cannot be used [9, 10].

Specific coagulation assays for the NOACs have been developed. These assays are currently not widely available but their use is becoming more prevalent. The Hemoclot® diluted Thrombin Time (dTT) has been advocated as the preferable assay for quantification of dabigatran [11]. The reagent for Hemoclot® dTT, however, requires an hour stabilization time before use according to the manufacturer's current recommendations[12], making this assay less suitable for use in acute clinical situations [13]. Other dTTs calibrated for dabigatran concentration are available, but clinical experience with these assays is currently lacking [14].

The Ecarin Clotting Time (ECT) and Ecarin Chromogenic Assay (ECA) have also shown strong correlations and a linear relationship with dabigatran plasma concentrations [15, 16]. The shorter incubation times of the reagents make them more useful for monitoring in acute situations. Limitations of ECT are its dependence on plasma fibrinogen and prothrombin levels [17]. These limitations are overcome by the ECA [18].

For FXa inhibitors, chromogenic anti-FXa assays with specific calibrators and controls are available and demonstrated strong correlations and linear relationships with plasma concentrations of the drug [7].

It has been suggested that Dilute Russell's Viper Venom Time (DRVV-T) can be used for monitoring of all NOACs [19], but this requires additional validation in clinical studies.

Because of large inter-individual variation in coagulation factor levels and therefore varying potency and activeness of clotting, global coagulation assays such as Thrombin Generation Assay (TGA) and thromboelastography (TEG®/ROTEM®) are better at taking the complexity of the coagulation cascade into account [20] and may be more sensitive for testing the reversal of NOAC effects [21, 22]. Drawbacks of TGA include the relatively long duration of the test and inadequate standardization for broad clinical use [23]. Use of thromboelastography is promising in studies with spiked plasma samples but requires further investigation in clinical studies [21].

Using the information currently available, for quantitative monitoring in acute situations we recommend ECA for dabigatran and anti-Xa assays for the FXa inhibitors. Further research is needed to determine the relation between results of coagulation assays and clinical outcomes.

A summary of the available coagulation assays for monitoring NOACs is shown in Table 2.

5 Clinical Application of NOAC Antidotes

Major bleeding rates in patients treated with NOACs in clinical trials are lower in comparison with VKAs (5.3 vs 6.2 %) and a substantial reduction in the rate of intracranial haemorrhage was observed (0.7 vs 1.5 %) [1]. Data from NOAC registries show even lower bleeding rates [24, 25]. Nevertheless, bleeding events will still be an important issue in patients treated with NOACs.

It can be expected that NOAC antidotes will be used in relation to serious bleeding complications on treatment with these anticoagulants as a supplement to more general measures [26]. In addition, the antidotes may be used in case of urgent surgery and procedures with a high risk of bleeding related to ongoing anticoagulant treatment with NOACs.

It is, however, important to note that the location and size of the organ lesion leading to a bleeding complication while on a NOAC and the general condition of the patient may be the most important factors for prognosis and should be taken care of simultaneously with antidote administration.

It is important in relation to the ongoing clinical studies with antidotes to focus on the risk of thromboembolic complications when anticoagulant therapy is rapidly interrupted with antidotes in patients with a high thrombogenicity related to the condition leading to anticoagulant therapy and the clinical status related to the serious bleeding or a need for urgent surgery.

The experience so far from the limited number of dabigatran etexilate-treated patients receiving idarucizumab is that no biochemical indications of a prothrombogenic state were observed [27], but a potential prognostic impact of the antidote in the treated cohort cannot be evaluated. The patients had a high mortality and a control group could not be included.

Table 2 Coagulation assays in patients treated with NOACs

	Dabigatran etexilate	Rivaroxaban	Apixaban	Edoxaban
INR	Unsuitable	Unsuitable	Unsuitable	Unsuitable
PT	Unsuitable	Not suitable for quantitative measurement	Not suitable for quantitative measurement	Not suitable for quantitative measurement
APTT	Not suitable for quantitative assessment	Unsuitable	Unsuitable	Unsuitable
dTT	Linear relationship with plasma concentration. Hemoclot® has a 1 h-reagent stabilization time before use, less suitable in acute situations	Unsuitable	Unsuitable	Unsuitable
ECT	Linear relationship with plasma concentration	Unsuitable	Unsuitable	Unsuitable
	Can be used			
ECA	Linear relationship with plasma concentration	Unsuitable	Unsuitable	Unsuitable
	Recommended			
Anti-Xa chromogenic assay	Unsuitable	Linear relationship with plasma concentration	Linear relationship with plasma concentration	Linear relationship with plasma concentration
		Recommended	Recommended	Recommended
DRVV-T	Further studies required	Further studies required	Further studies required	Further studies required
TGA	Further studies required	Further studies required	Further studies required	Further studies required
	Long test time, not suitable in acute situations	Long test time, not suitable in acute situations	Long test time, not suitable in acute situations	Long test time, not suitable in acute situations
ROTEM®/TEG®	Further studies required	Further studies required	Further studies required	Further studies required

APTT activated partial prothrombin time, *DRVV-T* Dilute Russell's Viper Venom Time, *dTT* diluted Thrombin Time, *ECA* Ecarin Chromogenic Assay, *ECT* Ecarin Clotting Time, *INR* international normalized ratio, *NOACs* non-vitamin K antagonist oral anticoagulants, *PT* prothrombin time, TGA Thrombin Generation Assay

Issues of concern regarding antidotes for NOACs might be that availability of these agents could result in a more careless attitude to timing of invasive procedures and that the agents may be subject to overuse. Patients with a prescription for a NOAC may not have circulating anticoagulant in the blood [27] in relation to serious bleeding or the need for urgent surgery and, to avoid overutilization of antidotes and a delay in the use of other more relevant interventions [26], measurement of NOAC exposure in the specific situation must be available. It is desirable that measurement of coagulation activity using different methods dependent on the type of NOAC treatment and with a short response delay is available and can be established in hospitals responsible for handling bleeding and urgent surgery of patients on treatment with oral anticoagulants including NOACs [8, 28].

For patients with normal kidney function, accumulation of NOAC in the body is not expected to occur and a low plasma concentration can be expected 12 h after the last dose. It is very important to have exact information on type of NOAC prescribed to the patient, responsible physician, indication for anticoagulant therapy and other medications with an effect on haemostasis. All patients treated with a NOAC should carry a card with this information [26].

There is no data to date evaluating a possible formation of antibodies to the antidotes leading to serious allergic reactions and/or neutralization of treatment effect on repeated antidote administration.

6 Coagulation Factor Concentrates

Prothrombin complex concentrate (PCC) can be used to reverse the effect of VKAs. In this situation, the coagulation factors in PCC substitute for the reduced concentration of coagulation factors during therapy with a VKA. It has been suggested that PCC can also be used to reverse the effect of rivaroxaban, as the administration of PCC 50 IU/

kg to healthy volunteers treated with a supratherapeutic dose of rivaroxaban (20 mg twice daily) rapidly normalized the prolonged PT [29]. In a similar Dutch study, the same effect was found on the PT, but a prolongation of the APTT was seen [30]. PCC contains a small amount of heparin to avoid coagulation factor activation during preparation and storage. The heparin content, especially when PCC is given at higher doses, could cause this APTT prolongation. Considering the prolongation of the APTT, it is questionable whether serious rivaroxaban-induced bleeding can be stopped using PCC.

The fact that PCC augments thrombin generation, however, suggests that it may have an effect [30].

When PCC is used in experiments to reverse the effect of NOACs, it is not a substitution therapy, and may also potentially lead to a prothrombotic state, as it induces a supraphysiological rise in the plasma concentration of coagulation factors that are not inhibited by the NOAC in use.

This situation is also different from the PCC treatment of patients with haemophilia that have developed an inhibitor to factor VIII or those missing another coagulation factor. For serious bleeding in these patients, PCC can be used to augment coagulation activity. For VKA-treated patients, a more potent effect can be obtained by activated PCC (Feiba®) or recombinant factor VIIa (FVIIa; NovoSeven®). For NOACs, consistent clinical data is lacking. Animal studies show conflicting results and in several studies with NOAC-induced bleeding, FVIIa and PCC improved results of coagulation assays but failed to improve haemostasis [31–33].

PCC, activated PCC and FVIIa should only be used in serious and potentially life-threatening bleeding complications during treatment with NOACs, as these patients, in contrast to patients with haemophilia, have a high risk of thromboembolic complications. Surgical and local-acting treatments should be considered and used if relevant and possible before the use of these products.

6.1 NOAC-Specific Antidotes

6.1.1 Idarucizumab

Idarucizumab is a monoclonal antibody fragment, which binds specifically to dabigatran with an affinity that is 350 times higher than is seen for thrombin [34]. Therefore, idarucizumab binds both free and thrombin-bound dabigatran and neutralizes its anti-thrombin activity [34]. The antidote has been tested in healthy young volunteers, in volunteers aged 65–80 years and in volunteers aged 45–80 years with mild or moderate renal impairment [35–37]. Idarucizumab reversed the anticoagulant effect of dabigatran immediately and completely with normalization of the Hemoclot® dTT without a procoagulant effect.

In a study including 30 pigs treated with dabigatran etexilate, idarucizumab significantly decreased total blood loss after blunt liver trauma and reduced mortality in comparison with a control group not treated with the antidote [38]. Whether these results are also applicable to humans has yet to be confirmed.

RE-VERSE AD (Reversal Effects of Idarucizumab on Active Dabigatran) is an ongoing, open, prospective cohort study in 300 patients, testing the efficacy and safety of idarucizumab 5 g intravenously for reversal of the anticoagulant effect of dabigatran in patients with severe bleeding and in patients requiring urgent surgery or intervention. An interim analysis including 90 patients has been published [27]. The primary endpoint of reversal of anticoagulation after 4 h could not be determined in 22 % of patients with severe bleeding and in 28 % of patients undergoing acute surgery, as coagulation assays (Hemoclot® dTT alone or in combination with ECT) were normal at baseline. In patients with dTT above the upper limit of normal, reversal of dTT was shown in 88–98 % of the patients within minutes. In 79 % of these patients, concentrations of unbound dabigatran remained at a level near the lower limit of quantification (20 ng/mL) at 24 h. Increases in dabigatran blood levels at 12 h were seen in 15 % (bleeding) and 21 % (surgery) of patients, respectively, probably due to redistribution of extravascular dabigatran into the intravascular compartment. In patients with severe bleeding, the median time to cessation of bleeding was 11.4 h, but this was difficult to assess in a large proportion of the patients, such as those with intracranial or retroperitoneal bleeding. Overall, there were 18 deaths (20 % of patients), ten of which were due to vascular causes (five due to cardiac arrest/circulatory collapse/ischaemic stroke and five due to fatal bleeding events). There was one thrombotic event within 72 h after idarucizumab administration in a patient in whom anticoagulants had not been reinitiated [27]. A controlled study design could have given a clearer view on clinical outcome, but seems unethical.

6.1.2 Andexanet Alfa

Andexanet alfa (PRT 064445) is a truncated human recombinant FXa, is catalytically inactive and lacks the membrane-binding domain of native FXa, but has the ability, like native FXa, to bind direct FXa inhibitors as well as low molecular weight heparin- and fondaparinux-activated antithrombin III with high affinity ([39]; Fig. 1).

Andexanet alfa acts as a decoy receptor and reverses the anticoagulant effects of all FXa inhibitors by competing with native FXa with a dose-dependent reversal of the anticoagulant effects and correction of clot formation ex vivo [39]. Reduced blood loss and restored haemostasis

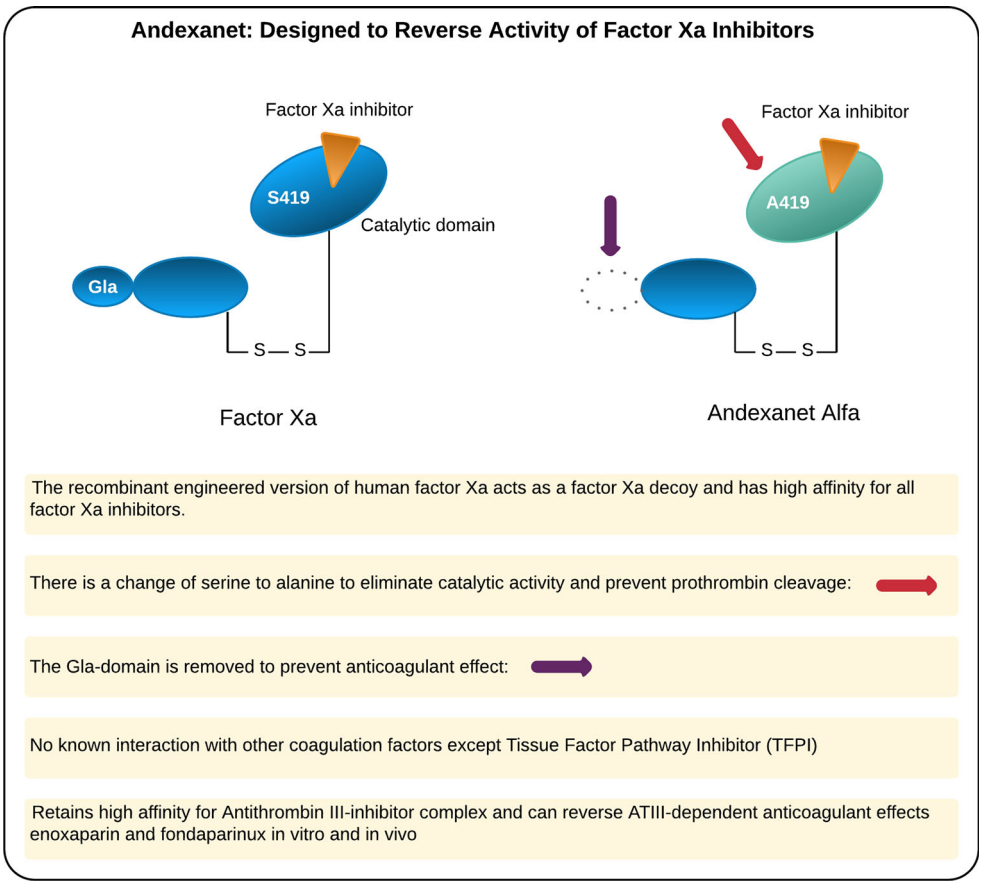

Fig. 1 Working mechanism of andexanet alfa, a reversal agent for factor Xa-inhibitors [39]

was demonstrated in animal bleeding models after rivaroxaban, fondaparinux and enoxaparin pretreatment [40–42].

No effect on normal FXa function in haemostasis and no anticoagulant activity were seen. In placebo-controlled trials, the reversal effect of andexanet alfa was tested in healthy volunteers receiving direct (apixaban, rivaroxaban and edoxaban) or indirect (low molecular weight heparin and fondaparinux) acting FXa inhibitors [43–45]. When an intravenous bolus of 240 mg with subsequent infusion of 4–8 mg/min was used, a dose-dependent reversal of the anticoagulant effect of apixaban was observed with a >90 % decrease in anti-FXa activity within 2 min [44]. The anti-FXa activity returned to placebo level 2 h after stopping infusion. Thrombin generation and clotting time were also reversed dose-dependently by andexanet alfa. No thrombotic events or adverse effects were registered.

In the ANNEXA (Andexanet Alfa a Novel Antidote to the Anticoagulant Effects of FXa Inhibitors) trials (NCT02207725 and NCT02220725), older healthy volunteers pretreated with apixaban [46] or rivaroxaban (ongoing; [47]), respectively, received andexanet alfa bolus 400 mg intravenously followed by an infusion of 4 mg/minute for 120 min [47]. A 94 % rapid reversal of the anticoagulant effect of apixaban was seen without serious adverse events or thrombotic complications in 33 volunteers aged 50–70 years [46].

A phase III, open label and uncontrolled study (NCT02329327) with andexanet alfa treatment is ongoing in patients with major bleeding episodes on treatment with an FXa inhibitor.

6.2 Synthetic Antidote

6.2.1 Aripazine

Aripazine (ciraparantag; PER977) is a small synthetic, peptide-like molecule that is designed with a distance between the positive guanidyl groups in the end of aripazine that matches the distance between the negative regions in heparin ([48]; Fig. 2). Aripazine not only binds to and reverses the anticoagulant effect of low molecular weight and unfractionated heparins, but also fondaparinux and NOACs. Aripazine does not bind coagulation factors and has no anti- or procoagulant effect.

In an animal bleeding model (rat tail transection), aripazine reduced blood loss on treatment with high doses of NOACs [48–50] and global coagulation tests were restored

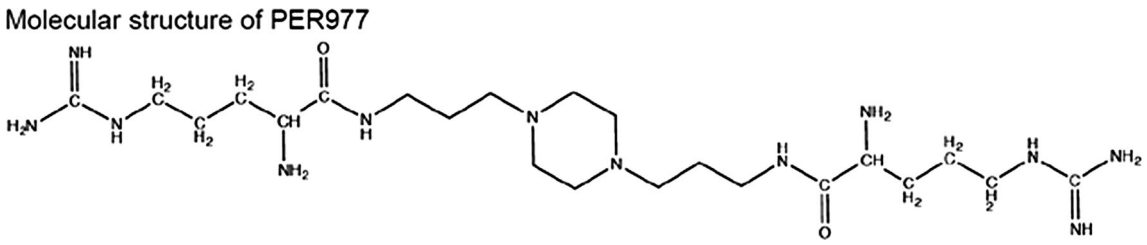

Fig. 2 Molecular structure of aripazine, a potential reversal agent for all NOACs (non-vitamin K antagonist oral anticoagulants)

to baseline levels in 20 min [48]. Aripazine safety, tolerability and pharmacokinetics were evaluated in healthy human volunteers in a phase I trial (NCT01826266) with single intravenous doses (5–300 mg) before and after a single 60 mg dose of edoxaban [51]. The anticoagulant activity of edoxaban was reversed by >100 mg of aripazine within 10 min and clot formation was restored without rebound signals for 24 h [52]. No procoagulant effect was observed and aripazine was well tolerated and safe.

With a similar design in normal human volunteers, re-anticoagulation with edoxaban and a second reversal with aripazine are currently being studied (NCT02207257).

Phase III clinical trials are needed to assess the clinical potential of this antidote in case of severe bleeding complications on treatment with NOACs.

7 Conclusion

Several types of NOAC antidotes are undergoing clinical evaluation and may, within a relatively short time period, be available for use in NOAC-treated patients with serious bleeding complications or who need urgent surgery. Availability of antidotes may lead to more frequent prescription of a NOAC in patients with an indication for anticoagulant therapy, but at the same time this will also lead to more hospital admissions with NOAC-related bleeding complications. At hospitals handling these NOAC-related bleeding complications and urgent surgery in anticoagulated patients, not only must antidotes be available, but also established coagulation assays with a rapid response rate should be on hand to test NOAC exposure, as well as guidelines for antidote use and for general measures for optimal treatment of these high-risk patients.

Ongoing studies will also show if a sudden interruption of anticoagulant therapy with antidotes increases the risk of thromboembolic complications and will demonstrate whether repeated antidote administration is safe.

Compliance with Ethical Standards

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Conflicts of interest Steen Husted has participated on the advisory board for Bayer, Bristol-Myers Squibb, Pfizer and Boehringer Ingelheim and has received speaker fees from Bayer, Bristol-Myers Squibb and Boehringer-Ingelheim. Freek Verheugt has received consulting fees from Bayer, Bristol-Myers Squibb/Pfizer, Boehringer Ingelheim and Daiichi-Sankyo. Willemijn Comuth has received the Young Thrombosis Researchers Group laboratory exchange grant from the European Society of Cardiology Working Group on Thrombosis and a hospital donation from Boehringer Ingelheim. She has received speaker fees from Bayer and Boehringer Ingelheim, and non-financial support from Bayer, Bristol-Myers Squibb/Pfizer, Boehringer Ingelheim, Stago, Siemens and ANIARA.
Steen Husted, Freek Verheugt and Willemijn Comuth have not been involved in any activities related to NOAC reversal agents, which are the topic of this article.

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