

Availability, Use and Development of Animal Models for the Assessment of Drug-Drug Interactions and Safety of Direct Oral Anticoagulants

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Abstract

Direct oral anticoagulants (DOACs) are novel, direct acting drugs that are selective for either thrombin or activated factor X. Due to their obvious benefits for patients (broader therapeutic window, are not routinely monitored etc.), they are increasingly used as an alternative to vitamin K antagonists. One of the major indications for the use of DOACs is the stroke prevention in patients with atrial fibrillation (AF). Although, the DOAC use becomes extensive in the clinical area especially cardiology, many drug drug interactions occur when DOACs are used with other drugs. Also, the safety profile of DOACs is still to be investigated. Animal models can be used to investigate the drug drug interactions and safety of DOACs under standart laboratory conditions. Unfortunately, there is not sufficient data that investigates the drug drug interactions and safety of DOACs in animal models. The focus of this review will be the availability, use and development of animal models to assess drug drug interactions and safety profile of DOACs.

Keywords: DOACs, Animal models, Drug-drug interaction, Safety

Yeni Nesil Oral Antikoagulanların İlaç İlaç Etkileşimleri ve Güvenliliğinin Değerlendirilebilmesi İçin Hayvan Modellerinin Geliştirilmesi, Kullanımı ve Bulunabilirliği

Öz

Direkt oral antikoagulanlar (DOAK'lar), yeni, thrombine veya aktive faktör X'a seçtif olan direkt etkili ilaçlardır. Bu yeni oral antikoagulan ilaçların, klasik antikoagulan ilaçlara göre hastalar açısından çok sayıda avantajları vardır (izleme ihtiyacının olmaması, geniş tedavi penceresi vbg.). Yakın geçmişte, direkt oral antikoagulanlar, tromboembolizmi önleme ve tedavisinde K vitamini antagonistlerine alternatif olarak sunulmuşlardır. DOAKların ana kullanım alanı, atrial fibrilasyonlu hastalarda inmenin önlenmesidir. Ancak, öte yandan, DOAK'ların diğer ilaçlar ile kombine kullanımı sırasında ilaç-ilaç etkileşimleri oluşabilmekte ve güvenlilik profilleri henüz yeterli düzeyde bilinmemektedir. Günümüzde, DOAK'ların ilaç-ilaç etkileşimi ve güvenliliğini araştırmada kullanılan hayvan modelleri henüz yeterli değildir. Bu derlemenin amacı, DOAK'ların ilaç-ilaç etkileşimi ve güvenliliğini araştıran hayvan modellerinin daha da geliştirilmesi, kullanımı ve bulunabilirliğidir.

Anahtar sözcükler: DOAK, Hayvan modelleri, İlaç-ilaç etkileşim, Güvenlilik

INTRODUCTION

Vitamin K antagonists (VKAs) were the only available oral anticoagulants for more than 50 years. They are proven effective in preventing stroke. There are already known disadvantages of vitamin K antagonists. These disadvantages can be explained as: they have narrow therapeutic window, there is a need for regular monitoring

of the international normalized ratio (INR) and have potential to interact with various drugs and food products ^[1].

In recent years, the non-vitamin K dependent new direct oral anticoagulants (DOACs) dabigatran, rivoraxaban, apixaban and edoxaban have been introduced into the market worldwide. Due to their clear benefits for patients, they have gained popularity as alternatives to VKAs in



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various clinical areas. In major trials, DOACs have been found effective in preventing stroke. DOACs are increasingly used as an alternative to warfarin, predominantly for indications such as stroke reduction in atrial fibrillation, prevention and treatment of venous thromboembolism and pulmonary embolism [2].

DOACs have been developed in response to the limitations of VKAs. Compared to VKAs, DOACs have several advantages. They are characterized by more defined pharmacokinetic and pharmacodynamic profiles. They have more predictable half-life and elimination. In a lot of indications, DOACs are rapidly replacing vitamin K antagonists due to their ease of use for patients such as without the need for laboratory monitoring. DOACs have improved efficacy and safety ratio and non inferior bleeding risk than VKAs [1].

It is already known that DOACs have fewer drug-drug interactions compared to VKAs. In a recent review about the interactions and safety of DOACs with antiarrhythmic drugs, even though DOACs have fewer interactions with antiarrhythmic drugs than vitamin K antagonists, it has been reported that, required dose adjustments and contraindications are still important to be kept in mind [3].

Despite many favoring properties in clinical trials, the use of DOACs has been relatively modest. In terms of safety, meta analyses of randomized controlled trials suggest that DOACs are noninferior to VKAs for overall risk of bleeding complications. In case of bleeding events, there is a need for an effective anticoagulation reversal strategy by using an antidote [4].

The reversal of DOACs in animal models are already explained in the previous literature. There are suitable animal models for the reversal of DOAC effects. Interestingly, there is not sufficient data that investigates the safety and drug-drug interactions of DOACs in animal models.

This review tries to explain the questions that could be asked in this area:

1. What are the animal models available?
2. What are the perspectives and limitations of the animal models currently in use?
3. How can we investigate safety on animals?
4. Which are the most promising models currently in use? How can they be refined?
5. How could findings generated by the animal models be translated clinically?

The focus of this review will be the availability, use and development of animal models for the critical assessment of drug-drug interactions and safety of DOACs.

DIRECT ORAL ANTICOAGULANTS (DOACs)

a. Direct Thrombin Inhibitor: Dabigatran

Dabigatran etexilate is a potent and direct inhibitor (competitive) of thrombin, both free and bound to fibrin. It is a prodrug with a bioavailability of 6%. After oral administration it is rapidly converted to dabigatran by serum esterases. Dabigatran has a serum half life of 12-17 h and 80% of the drug is excreted by the kidneys [5].

The RE-LY trial showed that dabigatran at a dose of 150 mg BID was superior to warfarin in preventing stroke and systemic embolism with a similar risk of major bleedings [6].

b. Direct Factor Xa Inhibitors: Rivaroxaban, Apixaban and Edoxaban

Rivaroxaban and apixaban are the two direct inhibitors of factor Xa. These agents reversibly inhibit free and clot bound factor Xa. They prevent the conversion of prothrombin to thrombin and subsequent fibrin clot formation. They have a high bioavailability (around 50% in apixaban, 62% in edoxaban to 100% in rivaroxaban when taken with food) [7].

ANIMAL STUDIES IN DOAC RESEARCH

This part of the review will try to explain what are the animal models available and what are the perspectives and limitations of the animal models currently in use?

Research animals that are used in experimental studies are valuable tools for understanding the pathophysiology of a situation or in developing therapeutic interventions for a disease. Feasibility, similarities to human and drug safety are the basic reasons that animals are used in biomedical research. Animal models are relatively easy to manage. The dietary intake and environmental factors (temperature and lighting) can be controlled easily in standard laboratory conditions. Compared to human studies, there is relatively less environmental variation. Many animals are suitable due to their similarity in anatomical basis and physiological functions with humans. Before the compounds are used in humans, preclinical toxicity testing, pharmacodynamics and pharmacokinetics profile of drugs may be investigated on animals. Prior to testing on humans, the effectiveness of a drug as potential treatment needs to be carried out on animals [8].

Animal models can provide a means to investigate "the safety and drug-drug interactions of DOACs" by different strategies under standard laboratory conditions. As DOAC use becomes extensive in the clinical area, the concept of the safety and drug-drug interactions of DOACs becomes more important.

The concept of the safety and drug-drug interactions of DOACs in animal models becomes mandatory in defining appropriate treatment strategies. All relevant research from the pubmed were checked via using the following key search terms: *safety, drug-drug interactions, DOACs, antidotes, animal models*. In the present literature, mouse, rat, rabbit, pig and baboons are the mostly used species in anticoagulation studies.

There are advantages and disadvantages of the species used for the assessment of anticoagulation reversal: In small animal models (for example: rodents and rabbits) high numbers can be used for the selection of dose. Due to lower body weight of small animals, the drug requirements are lower. With rabbits, the sample collection and regional tissue assessment are more simple.

In the use of large animals (pigs) organ size, blood volume and hemodynamic response are comparable to humans. The sample collection is simple and polytrauma can be inflicted. There may be disadvantages of small animal use (rodent and rabbit) such as small animals have low blood volume and might be less suitable for use in trauma studies. The disadvantages of the use of large animals may be the ethical approvals that can be harder to take and standardization of the study may be more difficult^[9].

Animal studies have shown that specific antidotes are likely to be effective means of reversing the anticoagulant effects of DOACs. The most informative experiments for studying DOAC anticoagulation and its reversal are those conducted in large animals with relevant trauma and severe bleeding with hemorrhagic shock and animal species with pharmacological relevance. The animal species and the DOACs used in previous studies are summarized in *Table 1*.

In terms of the assessment of anticoagulation and its reversal, in the review paper of Honickel et al.^[9], it was considered that "bleeding volume" should be regarded as the gold standard of outcomes. The "comparisons of bleeding volume versus bleeding time" have been found as a more sensitive and reliable outcome.

There are different "animal models" that has been used in experimental studies of DOACs that can be summarized as:

1. Intracerebral hemorrhage (ICH)^[10]

2. Tail bleeding^[11-13]

3. Carotid artery occlusion^[13]

4. Standardized kidney incision^[14]

5. Blunt liver injury^[15,16]

6. Mesenteric bleeding^[17]

7. Liver laceration^[18]

Standardized animal models with standardized outcomes are essential to understand the effects of DOAC reversal research and treatments. The models already used in DOAC reversal research can be explained herein after as:

In a murine intracerebral hemorrhage model associated with rivaroxaban, the reversal of rivaroxaban was studied, and prothrombin complex concentrate, Factor VIIa, and fresh frozen plasma prevented excess intracerebral hematoma expansion^[10]. CD1 mice were given warfarin or dabigatran by gavage, and the effects on *in vitro* coagulation assays, volume of blood loss and the bleeding time following tail transection injury were evaluated with different reversal agents. Prothrombin complex concentrates (PCC) reduced blood loss in murine coagulopathy induced by warfarin. PCC treatment prevented excess bleeding much more effectively in warfarin-induced coagulopathy than in dabigatran-induced coagulopathy^[11].

In the carotid artery occlusion model, it has been shown that γ T-S195A-IIa decreased the anticoagulant effects of dabigatran *in vitro*^[13]. The reversal of dabigatran anticoagulation by prothrombin complex concentrate (Beriplex P/N) in a rabbit model has been investigated and it has been found that the prothrombin complex concentrate showed potential as an agent for reversing the effects of dabigatran^[14].

In the liver trauma model, prothrombin complex concentrate and activated prothrombin complex concentrate were found effective in reducing the anticoagulant effects of dabigatran under different conditions^[15].

Table 1. The animal models, the species and the used Direct Oral Anticoagulant in studies

Author	Animal Model	Used DOAC	Animal Species Used
Zhou W ^[10]	Intracerebral hemorrhage	Rivaroxaban 3; 10 or 30 mg/kg	Mouse (C57BL/6)
Lambourne MD ^[11]	Tail bleeding	Dabigatran 60 mg/kg, oral (1.5 mg)	Mouse (CD1)
Van Ryn J ^[12]	Tail bleeding	Dabigatran 30 mg/kg, oral	Rat
Sheffield WP ^[13]	Tail bleeding, Carotid artery occlusion	Dabigatran 13 mg/kg and 60 mg/kg	Mouse
Pragst I ^[14]	Standardized kidney incision	Dabigatran 0.4 mg/kg, i.v.	Rabbit
Honickel M ^[16]	Blunt liver injury, Bilateral femur fractures	Dabigatran 30 mg/kg twice daily	Pigs
Perzborn E ^[17]	Mesenteric bleeding, Incision on forearm	Rivaroxaban 2 mg/kg i.v. 0.6 mg/kg i.v bolus	Rat; Baboon
Lu G ^[18]	Liver laceration	Rivaroxaban 1 mg/kg i.v. bolus	Rabbit

A novel antithrombotic agent BAY 59-7939—an oral, direct Factor Xa inhibitor has been studied and the bleeding times in rats and rabbits were not significantly affected at antithrombotic doses (3 mg kg⁻¹) p.o., AV shunt) and based on the studies results, BAY 59-7939 has been selected for clinical development [17].

In the concept of this review it is important to mention that preclinical safety and efficacy of andexanet alfa in animal models were investigated, and it has been found that, andexanet is a promising therapy for the reversal of FXa inhibitor-induced anticoagulation, supporting clinical studies in humans [19].

DEVELOPMENT OF AN ANIMAL MODEL TO INVESTIGATE THE “SAFETY AND DRUG DRUG INTERACTIONS” OF DOACS

From a pharmacological point of view, for a rational selection of an animal model to study a drug-drug interaction, the animal model should be similar to humans in terms of pharmacokinetic parameters (absorption, distribution, metabolism or excretion [ADME]) processes. In previously published animal models, we also studied with different kind of animal species and develop animal models that are closely reflect the disease scenario similar to pathologies seen in humans (diabetes model, ageing, alcoholic animal models, polymicrobial sepsis model). We characterized the effects of different drugs in terms of their effectiveness, safety and tolerability in the Balb/c mouse, Wistar albino rats and rabbit models [20-24].

As it is already known, cytochrome-p450-system and the p-glycoprotein transport system plays a key role in the DDIs of DOACs. To investigate the safety and drug-drug interaction (DDI) potential of a DOAC and understanding of the underlying mechanism for DDI of a DOAC, different animal models can be developed; especially, *in vivo* animal models might be valuable in this situation.

There are *in vitro* studies that has been done previously: the *in vitro* assessment of pharmacokinetic drug-drug interactions of DOACs has been studied and strong *in vitro* inhibition of DOAC efflux by PDE5 has been shown [25]. Animal and *in vitro* studies in a human placental perfusion model indicated that apixaban, dabigatran and rivaroxaban, respectively, exhibit placental transfer [26].

On the other hand, the literature on the safe use of DOACs includes the management in special situations such as renal impairment, overdose and bleeding risk. Role of renal function and hepatic function in co-administration of DOACs with other drugs should be taken into consideration to study on the effective animal model. It may be also important to investigate the “safety profile of DOACs” first

in *naive animals* without a pathology (renal or hepatic impairment) and then develop a pathological situation (such as bleeding, renal impairment, hepatic impairment) especially for safety studies.

NOVEL APPROACHES TO THE REVERSAL OF DIRECT ORAL ANTICOAGULANTS -THE ANTIDOTES

For the reversal of the DOACs, class and drug-specific compounds are currently in development. A class-specific drug, andexanet alfa, is being developed for the reversal of the oral factor Xa inhibitors and has also shown reversal activity against the indirect Xa inhibitor enoxaparin. A drug-specific agent, currently before the FDA for approval based on data from healthy volunteers as well as patients with active bleeding or needing urgent reversal in preparation for surgery is the idarucizumab [27].

A global universal inhibitor ciraparantag, has demonstrable activity against direct oral factor IIa and Xa inhibitors as well as the indirect Xa inhibitor enoxaparin, and UFH.

Three antidotes are currently in development for DOAC reversal. To date idarucizumab has been approved in the USA for the reversal of dabigatran. Andexanet has completed phase 3b/4 study and is pending further presentation of data. A third one is aripazine which is in phase II trial [28]. DOAC specific antidotes and the synthetic antidote is shown in Table 2.

TARGETED REVERSAL DRUGS

1. Idarucizumab (BI655075-Dabi-Fab)(Praxbind): Dabigatran Antidote

Idarucizumab (fragment antigen-binding; Fab) was licensed in 2015 as a specific reversal agent for dabigatran. It is approved for the emergent reversal of the anticoagulant effects of dabigatran [28].

It is a humanized mouse monoclonal antibody fragment. It binds dabigatran and reverses its anticoagulant effects. Idarucizumab has an extremely high affinity for dabigatran and is able to reverse dabigatrans anticoagulant effects at a 1:1 stoichiometric ration. It is generated from mouse monoclonal antibody, then humanised and reduced to a Fab fragment. It binds to the thrombin binding site

DOAC-Specific Antidotes	Synthetic Antidote
Idarucizumab	Aripazine (ciraparantag; PER977)
Andexanet Alfa (PRT 064445)	
DOAC-Specific antidotes: Idarucizumab, Andexanet Alfa (PRT 064445) Synthetic antidote: Aripazine (ciraparantag; PER977)	

of dabigatran with an affinity that is 350 times as high as thrombin.

Dabigatran almost completely inhibits fibrinopeptide A formation at the wound site and idarucizumab is aimed at restoring systemic blood coagulation and reenabling the formation of this fibrin ^[29].

Idarucizumab is currently being evaluated for the emergency reversal of dabigatran, in the ongoing RE-VERSE AD phase 3 study. An analyses of interim data from the first 90 patients enrolled in the study showed that idarucizumab completely reversed dabigatran's anticoagulant effect within minutes, paralleled by a profound reduction in unbound dabigatran concentration. The use of idarucizumab may simplify emergency management of dabigatran treated patients with life threatening bleeds and reduce perioperative complications in patients undergoing emergency surgery ^[30].

Idarucizumab is administered by intravenous infusion. The half life of Idarucizumab is 45 min. It binds both free and also thrombin bound dabigatran with a rapid on rate and slow off rate. The bound complex is eliminated predominantly by renal excretion. Idarucizumab is distributed solely within the intravascular space. It eliminates dabigatran by drawing it from the extravascular spaces into the intravascular space ^[31].

2. Andexanet Alfa: Factor Xa Inhibitor Antidote

Andexanet alfa is a reversal agent for FXa and is currently undergoing phase 3b to 4 trials. It binds FXa in a 1:1 stoichiometric ratio and restores endogenous factor Xa activity via reducing anti-factor Xa activity. It works for rivaroxaban, apixaban and edoxaban ^[28].

It is a recombinant and an inactivated form of factor Xa engineered as a universal antidote for factor Xa inhibitors. It also binds LMWH and fondaparinux activated antithrombin III, which acts as indirect Xa inhibitors. It is a small (kDa), catalytically inactive, human recombinant modified molecule that is similar to native factor Xa. Acting as a decoy receptor, it binds and sequesters direct factor Xa inhibitors, preventing them from inhibiting the activity of the native factor Xa, thus restoring the normal haemostatic processes ^[29].

3. Ciraparantag (Aripazine/PER 977)

Ciraparantag (PER 977) is a small synthetic and cationic molecule that binds direct Xa inhibitors, direct thrombin inhibitors, and unfractionated and low molecular weight heparin.

Developed by Perosphere, this is a small (500 Da), synthetic, water soluble, thermally stable, cationic D-arginine compound that has broad activity against various old (heparin, LMWH) and newer anticoagulants (Dabigatran, rivaroxaban, apixaban, edoxaban) ^[29].

Ciraparantag is another possible option for the reversal of DOACs which seems to be promising in the future, being able to inhibit dabigatran and the factor Xa inhibitors ^[32].

This is the third antidote aiming to reverse all anti-thrombotic agents including direct thrombin inhibitors and FXa. It can be difficult to monitor because it binds to drugs, and not coagulation factors. It has not exhibited procoagulant or anticoagulant properties ^[33].

CLINICAL CONCEPTS THAT SHOULD BE KNOWN-ANTICOAGULATION REVERSAL

The potential candidates for targeted reversal therapy is important in the decision to reverse anticoagulation ^[4]. It is important to consider reversal in the following patients and clinical settings:

1. Severe bleeding that results in hemodynamic compromise,
2. Organ dysfunction or a need for massive blood transfusion,
3. Patients within 24 h of receiving an anticoagulant who require emergency surgery or an invasive procedure known to be associated with a significant risk of bleeding.

Consider prior to reversal:

Other factors that must be considered prior to reversal include:

1. The time since a last dose of a DOAC,
2. Indication for anticoagulation and risk of thrombosis,
3. Drug interactions,
4. Associated renal or hepatic dysfunction and local or systemic factors,

that increase the risk of poor outcomes with bleeding, for example, advanced age. Idarucizumab, a monoclonal antibody fragment, was developed to reverse the anticoagulant effect of dabigatran. It is a multicenter, prospective, open-label study to determine whether 5 g of intravenous idarucizumab would be able to reverse the anticoagulant effect of dabigatran in patients who had uncontrolled bleeding (group A) or were about to undergo an urgent procedure (group B) in total of 503 patients. It was concluded that idarucizumab rapidly, durably, and safely reversed the anticoagulant effect of dabigatran ^[30]. Recent results from phase 3/4 studies demonstrate efficacy for idarucizumab (an antidote to dabigatran) and for andexanet alfa. Ciraparantag for many anticoagulants, including the DOACs, shows promise in results from phase 1 and 2 studies ^[34].

CONCLUSION AND FUTURE PROSPECTS

Systems related to the development of DDIs of DOACs in co-administration with other drugs are cytochrome-P450-system and the p-glycoprotein transport system. These two systems should be taken into consideration for the animal models investigating the DDIs of DOACs.

Although, there are already known animal models that are used in the reversal of DOACs that may guide the researchers on this area there is not enough data on the animal models for the "safety and drug-drug interactions of DOACs".

Although some animal models that are used in DOAC research are already summarized there are still questions that exists: which are the most promising models currently in use and how can they be refined? Also there is another discussion that still needs to be highlighted: how could findings generated by the animal models be translated clinically?

It might be concluded that to investigate the safety profile and DDIs of DOACs in co-administration with other drugs, the development and use of standardized in vivo animal models should become true in the future.

Lastly, the findings that can be generated by the animal models should be translated clinically. This review might be an introduction to the availability, use and development of animal models in terms of safety and drug-drug interactions of DOACs in pharmacological area which is a clearly lacking concept up to date.

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