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Pharmacokinetics of a microdosed cocktail of 3 direct oral anticoagulants in children with congenital heart defects: study protocol for a single centre clinical trial (DOAC-Child)

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Abstract

Introduction: Direct oral anticoagulants (DOACs) are direct inhibitors of coagulation factor Xa and are frequently used in adults for different indications such as deep vein thrombosis or non-valvular atrial fibrillation. Paediatric patients might benefit as well from DOACs because the simplicity and convenience of their use is likely to decrease physical and psychological stress related to invasive procedures associated with phenprocoumon and heparin therapy. Thus it is expected that the future use of DOACs will ultimately improve compliance and overall safety of anticoagulant therapies in paediatric populations. To assure safe and effective use the clinical pharmacology and pharmacokinetics (PK) of these drugs need to be evaluated in children.

Methods and analysis: This study is a single centre, open-label, clinical trial in a paediatric population with non-cyanotic congenital heart defects. After having obtained informed consent from the parents, each participant will receive a single oral administration of a drinkable solution of a microdose cocktail of 3 FXa inhibitors consisting of apixaban (12.5 µg), rivaroxaban (12.5 µg), edoxaban (50 µg), plus a microdose of the two probe drugs midazolam (10 µg) and yohimbine (25 µg). Serial blood samples will be collected at specified time points before and up to 25 h after cocktail administration. The primary PK endpoint will be the area under the plasma concentration time curve ($AUC_{0-\infty}$) of apixaban, rivaroxaban and edoxaban. Secondary PK outcomes will be C_{max}, t_{max}, $t_{1/2}$, Cl/F, and V_{ss}/F. Safety and tolerability of the microdose cocktail will be evaluated as well by collection of adverse events.

Ethics: This study has been approved by the responsible Ethics Committee of the Medical Faculty of Heidelberg University.

Dissemination: Study results will be presented at international scientific meetings and published in peer-reviewed journals.

What is known about the subject?

- Current anticoagulation regimes in children with congenital heart defects consist of phenprocoumon, (low molecular weight) heparin, or antiplatelet agents. Disadvantages of these regimes comprise invasive monitoring (phenprocoumon) or invasive administration techniques (heparin).
- Direct oral anticoagulants (DOAC) are a relatively new class of anticoagulants which have been approved and are widely used in adults.
- There are no data available regarding the pharmacokinetics of DOACs in pediatric heart patients.
- The expression of drug metabolising enzymes involved in the eliminations of DOACs varies during human development and growth.

What this study hopes to add:

- Evaluation of the pharmacokinetics of three DOACs: rivaroxaban, edoxaban, apixaban simultaneously in children with congenital heart disease aged 6 months to 6 years using a microdose-cocktail approach.
- Evaluation of CYP3A and CYP2D6 metaboliser status using the microdosed probe drugs midazolam and yohimbine in pediatric heart patients.

How this study might affect research, practice and/ or policy.

- The pharmacokinetics of rivaroxaban, edoxaban and apixaban will be evaluated regarding their interindividual variability and their potential for drug-drug interactions.
- The DOAC with the most favorable profile will be selected and a subsequent study using therapeutic doses will be conducted.
- The overall goal of this study is to pave the way for less painful pediatric anticoagulation.

Introduction

Congenital heart disease (CHD) is the most common form of major congenital anomalies. Congenital heart defects are defined as a structural abnormality of the heart and/or great vessels that is present at birth. Reported birth prevalence of CHD varies between 8-12 per 1000 live births[1 2]. The prevalence of complex CHD diagnosis is reported to be 0.8 to 2.2 per 1000 live births depending on the demographics of the study population, the inclusion criteria, and the study era. Severe CHD accounts for most of the morbidity and mortality attributable to CHD[3].

A recent German analysis which focused on patients with CHD born between 1996 and 2015 showed that the number of patients with severe CHD had increased significantly since 2008/2009. About 9.4% of patients in this cohort had complex CHD classified as univentricular heart[4]. Certain patients with severe congenital heart disease require temporary or lifelong anticoagulation. This applies especially to patients after biological (temporary anticoagulation) or mechanical (lifelong anticoagulation) heart valve replacement, or patients after Glenn or Fontan palliation, when the blood flows passively through the pulmonary circulation at low velocity.

The currently used anticoagulants in children are either oral phenprocoumon or warfarin, heparin continuous infusion, and subcutaneously injected low molecular weight heparins, the latter often limited to short-term therapy.

Since 2008, direct oral anticoagulants (DOAC) including the drugs dabigatran, rivaroxaban, apixaban and edoxaban have been approved for adults in Europe and the US, and efficacy, safety and pharmacokinetic data have been obtained in adults. These drugs so far have not yet been adequately studied in children and are currently not approved for the use in paediatric heart patients. For children and adolescents, there are ongoing studies on rivaroxaban and apixaban, but most of them are not yet completed or published[5].

There are body-weight adjusted treatment recommendations available for rivaroxaban in children with venous thromboembolism. Children with a body weight 10 kg or more should be

treated with a daily dose of 9 mg or higher, depending on the actual body weight [6]. Assessment of 92 children aged < 6 month – 17 years with weight-adjusted rivaroxaban after previous therapeutic treatment with common medication such as low-molecular weight heparin because of venous thromboembolism was performed. No major bleeding was observed during the clinical trial in 92 children aged < 6 months to 17 years.

An oral microdose DOAC cocktail (µ-FXaI) containing apixaban (25 µg), edoxaban (50 µg) and rivaroxaban (25 µg) has been successfully used in adults to study drug-drug interactions[7 8]. The pharmacokinetics (PK) of these 3 FXaI, obtained after simultaneous administration, correspond well with the PK characteristics after therapeutic FXaI doses (dose-proportional pharmacokinetics). In one study in18 healthy adults (> 18 years) the effect of ketoconazole on µ-FXaI revealed the quantitative drug-interaction effects ketoconazole on the three FXaI was reproducible with the clearances of µ-FXaI comparable to already published data of therapeutic doses[7].

In children with certain heart defects long-term anticoagulation is required, which is usually achieved by vitamin K-antagonists. Only few studies focusing on direct oral anticoagulants (DOAC) in children have been carried out so far, or are still ongoing[5 9]. Currently, there are only limited data published for edoxaban or apixaban in children[10]. The few results from studies of rivaroxaban PK report a favorable safety profile and pharmacokinetics comparable to those already known for adults[9]. Therefore, it is important to generate pharmacokinetic data of DOAC in children of this young age with congenital heart disease without an increased risk of bleeding, which would be harmful to this vulnerable group, simultaneously for all three DOAC. Therefore, this study will use the simultaneous administration of microdosed drugs only to assess the pharmacokinetics, safety, and tolerability.

In order to obtain data of CYP3A and CYP2D6 activity in young children, microdose co-administration of the CYP3A substrate midazolam (MDZ; 10 µg) and the CYPD6 substrate

yohimbine (YOH; 25 µg) as probe drugs along with the three FXaI products will be carried out. Thus, the microdose cocktail to be used in the study contains a total of 5 different drugs which will be administered simultaneously by oral route.

Methods and analysis

Study Design

This is an open-label, single-centre, single dose clinical trial in a paediatric population with non-cyanotic congenital heart defects at the Department of Paediatric and Congenital Cardiology of the Heidelberg University Hospital in cooperation with the paediatric Clinical Pharmacological Study Centre (paedKliPS). This protocol was designed following the SPIRIT guidelines[11].

Study population

Infants and children aged 6 months up to 6 years with a body weight >7 kg who are admitted to the paediatric cardiology wards for the surgical correction on a non-cyanotic congenital heart defect are included. Children who are treated with anticoagulants, who have a coagulopathy or lesions or conditions which are associated with a significant risk for major bleeding are excluded, as well as children who have kidney or liver insufficiency, or are treated with drugs known to be relevant inducers or inhibitors of drug metabolizing enzymes. Detailed inclusion and exclusion criteria are listed in Supplemental Table 1.

For the purpose of surgery all patients are under routine continuous cardiorespiratory monitoring (Intellivue, Philips Healthcare, Eindhoven, The Netherlands), have central vascular access, and a feeding tube, depending on the respective age and the clinical condition.

Primary objective

Assessment of the pharmacokinetics of rivaroxaban, apixaban and edoxaban, when co-administered as a microdose cocktail (apixaban 12.5 µg, rivaroxaban 12.5 µg, edoxaban 50 µg) in children with congenital heart defects, aged 0.5-6 years.

Secondary objectives

- To compare the pharmacokinetics of the FXaI in children with those reported in healthy adults and patients from literature.
- To characterise the CYP3A activity by means of a midazolam microdose in children.
- To characterise the CYP2D6 activity by means of a yohimbine microdose in children.
- To evaluate tolerability and safety of this microdose cocktail in children.

Study Outcome

Primary outcome is the area under the concentration-time curve ($AUC_{0-\infty}$) of the three DOACs apixaban, edoxaban, and rivaroxaban. Secondary outcomes include standard PK parameters (C_{max}, t_{max}, $t_{1/2}$, Cl/F, and Vss/F) of each FXaI and yohimbine, as well as the metabolic clearance of midazolam by means of AUC_{2-4h}[12].

Recruitment

Recruitment takes place at the Department of Paediatric and Congenital Cardiology. Prior to the surgical intervention parents of potential participants are addressed regarding the study which serves as short information about the clinical trial. After the surgery detailed information is given and discussed with both parents. After full explanation of the purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort of the clinical trial both parents sign the informed consent form before any study procedures are carried out.

Screening

The screening visit is defined as accurate check of all diagnostic findings which were made in course of the pre-operative assessment to evaluate eligibility. This is done after the surgery by a trial team physician in order to check inclusion and exclusion criteria.

Interventions

The intervention may begin on postoperative day 3 (about 72 hours after surgery) with the oral administration of the study drugs. They are dissolved in 110 ml tap water to ensure comfortable oral application. If the patient has a feeding tube, this will be used for administration. All patients have as a clinical routine measure prior to cardiac surgery a central venous line. Vital parameters and other clinical or laboratory which are necessary to ensure patients safety are extracted from the daily documentation during clinical routine.

Sample collection

PK blood samplings will be collected via a central venous line and time points are dependent on bodyweight (Figure 1). The total amount of blood drawn will be 25 mL over 25 hours for children of at least 10 kg. This includes 17 samples to evaluate PK parameters (S-Monovette® 1.2 mL lithium heparin collection tubes, Sarstedt, Nümbrecht, Germany) and 3 safety checks in which the coagulation parameters will be observed (S-Monovette® 1.4 mL citrate collection tubes, Sarstedt, Nümbrecht, Germany). This is equivalent to a loss of blood volume of 3.28 % of a child weighing 10 kg. For children weighing 7 to 9.9 kg a reduced sampling scheme (12 PK samples and 3 coagulation samples) will be applied to limit the blood loss (18.6 mL in 24 hours equivalent to 3.54 % for a child weighing 7 kg)[13]. All PK samples will be analysed in the Clinical Analytical Laboratory of the Department of Clinical Pharmacology and Pharmacoepidemiology at the University Hospital Heidelberg[14].

Midazolam will be measured in 5 samples (before and 2, 2.5, 3, and 4 h after administration) using an established limited sampling strategy[12] and yohimbine[15] in the samples taken up to 12 hours after administration.

Three coagulation checks (quick, INR, aPTT) are obtained before, 2 and 24 or 25 h after drug administration.

End of trial (EOT)

After the last blood sample the EOT visit includes reviewing the last clinical examination and laboratory assessment, which both are performed at least once during 24 hours by clinical routine.

Concomitant medication

Any necessary medication (judged by a responsible physician) for the best clinical care and/or emergency treatment is permitted. Concomitant medication will be documented (date, time, dose, route of administration) by a member of the trial team. For safety reasons simultaneous administration of anticoagulants, thrombolytics, platelet inhibitors such as GPIIb/IIIa-inhibitors and $P2Y_{12}$ inhibitors, and thienopyridine (clopidogrel) are prohibited for the duration of the trial.

Sample size calculation

Since no PK data in young children are available for the microdosed FXaI cocktail, no formal sample size calculation could be performed. It is planned to enroll up to 20 children with congenital heart defects. This number of patients is consistent with sample sizes of similar exploratory PK studies, which is expected to provide sufficient information on PK, safety, and tolerability of the µ-FXaI cocktail and the suitability of this methodology in future clinical paediatric drug development. In addition, it is expected that robust single-dose PK data of 3 FXaI in a young paediatric population will be generated to provide first guidance for dose adjustment in clinical use. An outstanding feature of our methodology is the simultaneous administration of 3 substances, so that each substance does not have to be examined in separate studies.

Data management

Clinical routine data are also documented for this clinical trial. Vital signs (i.e. blood pressure, heart rate) are documented at screening, prior to the drug administration, at any specific PK

timepoint, and at EOT. Concomitant medication and adverse events are also recorded. Printed source data sheets are used for the primary data collection and documentation. All source data information are timely transferred to the clinical trial management database (Promasys, OmniComm Systems, Fort Lauderdale, USA).

Data analysis and missing data

Standard PK parameters (C_{max}, t_{max}, $AUC_{0-\infty}$, $t_{1/2}$, Cl/F, and Vss/F) of each FXaI and yohimbine will be calculated by non-compartmental analyses (Monolix 2021R2, Lixoft SAS, Antony, France) and its results will be presented by descriptive statistics (GraphPad Prism 8.0.0 for Windows, GraphPad Software, San Diego, California USA). CYP3A activity will be quantified using the estimated metabolic clearance of midazolam by means of AUC_{2-4h}[12].

Individual missing or inconsistent data will be subject to a simple edit query process. Eventually missing data will not be imputed.

Patient and Public Involvement

Patients and/ or the public were not involved in any stages of this clinical trial: study design/ conduction/ analysis.

Ethics and dissemination

Ethics approval

The study protocol (Version 4.0), patient information and informed consent form were approved by the ethics committee of the Medical Faculty of Heidelberg University (AFmo-606/2019) and the German competent authority (BfArM).

To enable the participation for children younger than 2 years and with a weight lower than 10 kg a protocol amendment was submitted and approved (Protocol Version 5.1). For these patients, a reduced sampling scheme was introduced to keep the relative loss of blood volume below 5 % in 24 hours[13].

Study monitoring

A trained clinical trial monitor is assigned from the Department of Clinical Pharmacology and Pharmacoepidemiology at the University Hospital Heidelberg to perform the monitoring of this clinical trial.

Safety considerations

All medications will be administered as a subtherapeutic microdose. Although it is unlikely that the achieved concentrations of the administered trial medications confer any pharmacological effect or adverse drug reaction, a maximum degree of safety is essential in this vulnerable population.

Benefit and Risk assessment

There is no direct benefit to the patients or their parents. Because of the clinical setting in which the study will be carried out, no additional invasive procedures have to be performed. All participants are admitted as inpatient to the paediatric cardiac wards, where continuous cardiorespiratory monitoring and central vascular access, etc. are already provided.

In order to reduce additional burden of study procedures to the patient in the postoperative phase, after having signed informed consent, the patients' medical history, findings during the clinical examination and other parameters routinely assessed and documented during the treatment for these patients will also be completely recorded for this clinical trial. This is a common approach in clinical studies. Due to the sensitive drug assay, the amount of blood taken for the PK analyses is minimised.

All study drugs will be given as single microdoses only which does not elicit any pharmacological effects or adverse events[7]. The combination of microdoses of apixaban, rivaroxaban, edoxaban has been used in adults and at FXaI peak concentrations, International normalised ratio (INR) increased just by 3.9% and activated partial thromboplastin time (aPTT) by 3.1% which was clinically not relevant[7]. No bleeding complications occurred. Clinically relevant non-major bleeding (menorrhagia and gingival bleeding) has been observed in four children with venous thromboembolism taking therapeutic rivaroxaban within the Einstein-Jr phase III study[16].

Due to very sensitive analytical assay for apixaban and rivaroxaban, the dose of apixaban and rivaroxaban is halved to 12.5 µg each compared to the adult study. MDZ and yohimbine were used frequently in adults and no relevant AEs were observed at all.

Thus, there is no risk of drug accumulation and the risk for adverse events (AE) or toxicity is minimal. We do not expect any bleeding complications by µ-FXaI. If a case of bleeding complications the effects of rivaroxaban and apixaban can be reversed by Andexanet alpha. It also inhibits the function of all heparins[17]. We have summarised the case reports where children were exposed to rivaroxaban[18-21] or edoxaban[22], intentionally or unintentionally in the supplemental material (Supplemental Table 2).

In summary, potential risks of participation in this clinical trial are small and predictable. The trial drugs are administered at sub-therapeutic microdoses on one occasion, so that no adverse

drug reactions are to be expected. The participant's suitability will be carefully evaluated, and their health will be closely monitored during the trial.

Publications and Data access

The results of this investigation will be published in an international scientific peer-reviewed journal. It is not planned to make the data publicly available.

Figure 1: Study design assessing the pharmacokinetics of rivaroxaban, apixaban, edoxaban as well as the CYP3A and CYP2D6 enzyme activity in children with congenital heart defects. Upper timeline: Sampling schedule for children weighing 10 kg and more, lower timeline: sampling schedule for children weighing 7-9.9 kg.

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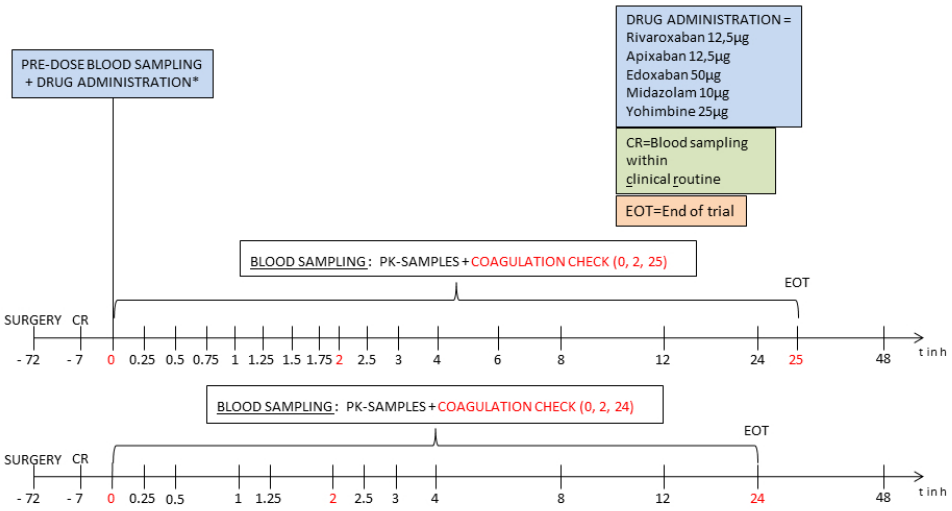

Study design assessing the pharmacokinetics of rivaroxaban, apixaban, edoxaban as well as the CYP3A and CYP2D6 enzyme activity in children with congenital heart defects. Upper timeline: Sampling schedule for children weighing 10 kg and more, lower timeline: sampling schedule for children weighing 7-9.9 kg.

254x190mm (96 x 96 DPI)

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Supplemental Table 1:

Detailed inclusion and exclusion criteria of DOAC-Child

Inclusion criteria	*Exclusion criteria*
• Infants and children aged 6 month up to 6 years (inclusive) • Body weight ≥ 7 kg • Admitted as inpatient to the paediatric cardiac wards after congenital cardiac surgery, • Cardiac defects: non-cyanotic congenital heart defects such as e.g. atrial septal defect, ventricular septal defect • Availability of a central vascular access • Otherwise healthy children as determined by medical assessment consisting of a medical history, physical examination, an ECG, and a laboratory evaluation, all performed within the clinical routine, that all must show no clinically relevant abnormalities • Minor deviations of laboratory values from the normal range may be acceptable in the pre-operative assessment, if judged by the	• Intake of a substance known to induce or inhibit drug metabolizing enzymes or drug transporters within a period of less than 10 times the respective elimination half-life or two weeks, whatever is longer • Simultaneous treatment with anticoagulants (i.e. phenprocoumon, warfarin, heparin - prior intake of heparin is allowed if more than 24 h prior to the start of study) • Active, clinical relevant bleeding • Any hepatic disease which could lead to coagulopathy or clinical relevant risk of bleeding • Lesion or condition, if considered to be a significant risk for major bleeding. This may include current or recent gastrointestinal ulceration, presence of malignant neoplasms at high risk of bleeding, recent brain or spinal injury, recent brain, spinal or ophthalmic surgery, recent intracranial haemorrhage, known or suspected oesophageal varices, arteriovenous

investigator to be of no clinical relevance for this trial. Criteria include, but are not limited to • ALT ≤ ULN x 1.1 • AST ≤ ULN x 1.2 • Bilirubin ≤ ULN x 1.2 (this does not apply to patients with Gilbert's syndrome) • Creatinine ≤ ULN + 0.1 mg/dl • Haemoglobin > 10 g/dl (pre- and postoperatively) • Both parents (or legal representatives) have to be able to communicate well with the investigator, to understand and comply with the requirements of the trial • Voluntarily signed informed consent after full explanation of the objectives, meaning and consequences of the trial to both parents (or legal representatives) of the participant. The informed consent will be obtained after the surgery but before any specific study procedures will be carried out	malformations, vascular aneurysms or major intraspinal or intracerebral vascular abnormalities • Uncontrolled severe hypertension/hypotension • Severe respiratory insufficiency • Tachycardic arrhythmias • Renal/Liver insufficiency • Glaucoma • Gastrointestinal ulceration • Clinical relevant mental disorder • Any physical disorder that could interfere with the participant's safety during the clinical trial or with the trial objectives • Body weight lower than 7 kg • Allergies (except for mild forms of hay fever) or history of hypersensitivity reactions/ intolerance to the study drugs • Any acute or chronic illness or clinically relevant finding during the clinical course known or expected to modify absorption, distribution, metabolism, or excretion of the drug under investigation • Any participation in an interventional clinical trial within 30 days before inclusion.

Hermann S et al.: Pharmacokinetics of a microdosed cocktail of 3 direct oral anticoagulants in children with congenital heart defects: study protocol for a single centre clinical trial, BMJ Pediatrics Open 2022.

	• Specific exclusion criteria for Midazolam: Administration of midazolam less than 48 h prior to the start of study

Supplemental Table 2: Case reports about children who were exposed to direct oral anticoagulants

DOAC	Patient	Indication	Dose	Result
Rivaroxaban[1]	12 year old girl	DVT	20 mg once daily for 5 days	Rivaroxaban trough level after 2 days 109 ng/mL and after 5 days 100 ng/mL. No bleeding.
Rivaroxaban[2]	35 month old boy	Accidental intake	200 mg (16 mg/kg)	Activated charcoal (2 g/kg) p.o. within 45 minutes of ingestion. Plasma anit-FXa level about four hours after ingestion > 4.00 IU/mL. At 13.5 hours after ingestion was 1.51 IU/mL. No adverse effects reported. No bleeding complication.
Rivaroxaban[3]	6 year old girl	Severe Protein S deficiency with repetitive skin necrosis during warfarin treatment.	Stepwise increase up to 40 mg daily	No adverse effects reported at 1 year follow-up. No bleeding episodes.
Rivaroxaban[4]	4 year old boy	Thrombotic storm	Daily dose unknown. Longterm treatment.	No bleeding or other adverse effects after 1 year of continues administration.
Edoxaban[5]	4 year old boy	Protein C deficiency	2 mg/kg/dose, four times a day	Two years follow-up did not result in adverse events.
Apixaban[6]	3 year old girl, 2 & 6 year old boys	Intracardiac thrombosis	2.5 mg twice a day	No bleeding events, complete resolution of the thrombi (n=2), substantial reduction in clot size (n=1) (follow up 6 days – 4 weeks)

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Pharmacokinetics of a microdosed cocktail of 3 direct oral anticoagulants in children with congenital heart defects: study protocol for a single centre clinical trial (DOAC-Child)

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Abstract

Introduction: Direct oral anticoagulants (DOACs) are direct inhibitors of coagulation factor Xa and are frequently used in adults for different indications such as deep vein thrombosis or non-valvular atrial fibrillation. Paediatric patients might benefit as well from DOACs because the simplicity and convenience of their use is likely to decrease physical and psychological stress related to invasive procedures associated with phenprocoumon and heparin therapy. Thus it is expected that the future use of DOACs will ultimately improve compliance and overall safety of anticoagulant therapies in paediatric populations. To assure safe and effective use the clinical pharmacology and pharmacokinetics (PK) of these drugs need to be evaluated in children.

Methods and analysis: This study is a single centre, open-label, clinical trial in a paediatric population with non-cyanotic congenital heart defects. After having obtained informed consent from the parents, each participant will receive a single oral administration of a drinkable solution of a microdose cocktail of 3 FXa inhibitors consisting of apixaban (12.5 µg), rivaroxaban (12.5 µg), edoxaban (50 µg), plus a microdose of the two probe drugs midazolam (10 µg) and yohimbine (25 µg). Serial blood samples (n=up to 20) will be collected at specified time points before and up to 25 h after cocktail administration. The primary PK endpoint will be the area under the plasma concentration time curve ($AUC_{0-\infty}$) of apixaban, rivaroxaban and edoxaban. Secondary PK outcomes will be C_{max}, t_{max}, $t_{1/2}$, Cl/F, and V_{ss}/F. Safety and tolerability of the microdose cocktail will be evaluated as well by collection of adverse events.

Ethics: This study has been approved by the responsible Ethics Committee of the Medical Faculty of Heidelberg University.

Dissemination: Study results will be presented at international scientific meetings and published in peer-reviewed journals.

What is known about the subject?

- Current anticoagulation regimes in children with congenital heart defects consist of phenprocoumon, (low molecular weight) heparin, or antiplatelet agents. Disadvantages of these regimes comprise invasive monitoring (phenprocoumon) or invasive administration techniques (heparin).
- Direct oral anticoagulants (DOAC) are a relatively new class of anticoagulants which have been approved and are widely used in adults.
- There are no data available regarding the pharmacokinetics of DOACs in pediatric heart patients.
- The expression of drug metabolising enzymes involved in the eliminations of DOACs varies during human development and growth, but might be influenced by genetic polymorphisms of drug metabolising enzymes as well.

What this study hopes to add:

- Evaluation of the pharmacokinetics of three DOACs: rivaroxaban, edoxaban, apixaban simultaneously in children with congenital heart defects aged 6 months to 6 years using a microdose-cocktail approach.
- Evaluation of CYP3A and CYP2D6 metaboliser status using the microdosed probe drugs midazolam and yohimbine in pediatric heart patients.

How this study might affect research, practice and/ or policy.

- The pharmacokinetics of rivaroxaban, edoxaban and apixaban will be evaluated regarding their interindividual variability and their potential for drug-drug interactions.
- The DOAC with the most favorable profile will be selected and a subsequent study using therapeutic doses will be conducted.

- The overall goal of this study is to pave the way for less painful pediatric anticoagulation.

Introduction

Congenital heart defects (CHD)are the most common major congenital anomalies. Congenital heart defects are defined as a structural abnormality of the heart and/or great vessels that is present at birth. Reported birth prevalence of CHD varies between 8-12 per 1000 live births[1 2]. The prevalence of complex CHD is reported to be 0.8 to 2.2 per 1000 live births depending on the demographics of the study population, the inclusion criteria, and the study era. Severe CHD account for most of the morbidity and mortality attributable to CHD[3].

A recent German analysis which focused on patients with CHD born between 1996 and 2015 showed that the number of patients with severe CHD had increased significantly since 2008/2009. About 9.4% of patients in this cohort had complex CHD classified as univentricular heart[4]. Certain patients with severe congenital heart defects require temporary or lifelong anticoagulation. This applies especially to patients after biological (temporary anticoagulation) or mechanical (lifelong anticoagulation) heart valve replacement, or patients after Glenn or Fontan palliation, when the blood flows passively through the pulmonary circulation at low velocity.

The currently used anticoagulants in children are either oral phenprocoumon or warfarin, heparin continuous infusion, and subcutaneously injected low molecular weight heparins, the latter often limited to short-term therapy.

Since 2008, direct oral anticoagulants (DOAC) including the drugs dabigatran, rivaroxaban, apixaban and edoxaban have been approved for adults in Europe and the US, and efficacy, safety and pharmacokinetic data have been obtained in adults.

An oral microdose DOAC cocktail (µ-FXaI) containing apixaban (25 µg), edoxaban (50 µg) and rivaroxaban (25 µg) has been successfully used in adults to study drug-drug interactions[5 6]. Comparing the pharmacokinetics (PK) of these 3 µ-FXaI, obtained after simultaneous administration[8] with the PK characteristics after therapeutic DOAC doses from literature showed DOAC clearances always in the same range (dose-proportional pharmacokinetics)[7-9].

In addition, comparable to already published drug interaction data of therapeutic doses a drug-drug interaction study using ketoconazole in18 healthy adults (> 18 years) and µ-FXaI revealed quantitative comparable effects on the clearances of µ-FXaI[5].

These drugs so far have not yet been adequately studied in children and are currently not approved for the use in children with congenital heart defects. For children and adolescents, there are ongoing studies on rivaroxaban and apixaban, but most of them are not yet completed or published[10 11] (in addition, see supplement 1).

Only few studies focusing on DOACs in children have been carried out so far, or are still ongoing[10 12]. Currently, there are only limited data published for edoxaban or apixaban in children[13]. The few results from studies of rivaroxaban PK report a favorable safety profile and PK comparable to those already known for adults[12].

There are body-weight adjusted treatment recommendations available for rivaroxaban in children with venous thromboembolism. Children with a body weight 10 kg or more should be treated with a daily dose of 9 mg or higher, depending on the actual body weight [14]. Assessment of 92 children aged < 6 month – 17 years with weight-adjusted rivaroxaban after previous therapeutic treatment with common medication such as low-molecular weight heparin because of venous thromboembolism was performed. No major bleeding was observed during the clinical trial in 92 children aged < 6 months to 17 years.

Therefore, it is important to generate pharmacokinetic data of DOAC in children of young age with congenital heart defects without an increased risk of bleeding, which would be harmful to this vulnerable group. The simultaneous pharmacokinetic assessment of three molecules within the same study is an elegant, modern, ethically-adequate design, allowing to limit discomfort to both participants and their families.

In order to obtain data of CYP3A and CYP2D6 activity in young children, microdosed co-administration of the CYP3A substrate midazolam (MDZ; 10 µg) and the CYP2D6 substrate

yohimbine (YOH; 25 µg) as probe drugs along with the µ-FXaI will be carried out. These both drugs where proven to function as microdosed probe drugs for phenotyping these enzymes by showing dose linearity [15 16]. Pharmacogenomics of further enzymes, transporters, etc. could influence the PK of the respective drugs as well, however including more substrates into this first microdose approach in children seems not feasible yet. . Thus, the microdose cocktail to be used in the study contains a total of 5 different drugs which will be administered simultaneously by oral route.

Methods and analysis

Study Design

This is an open-label, single-centre, single dose clinical trial in a paediatric population with non-cyanotic congenital heart defects at the Department of Paediatric and Congenital Cardiology of the Heidelberg University Hospital in cooperation with the paediatric Clinical Pharmacological Trial Centre (paedKliPS). This protocol was designed following the SPIRIT guidelines[17].

Study population

Infants and children aged 6 months up to 6 years with a body weight >7 kg who are admitted to the paediatric cardiology wards for the surgical correction on a non-cyanotic congenital heart defect will be eligible for study inclusion. Children who are treated with anticoagulants, who have a coagulopathy or lesions or conditions which are associated with a significant risk for major bleeding are excluded, as well as children who have kidney or liver insufficiency, or are treated with drugs known to be relevant inducers or inhibitors of drug metabolizing enzymes. Detailed inclusion and exclusion criteria are listed in Table 1.

For the purpose of surgery all patients are under routine continuous cardiorespiratory monitoring (Intellivue, Philips Healthcare, Eindhoven, The Netherlands) and have central vascular access. Depending on the age and the clinical condition, most patients have a feeding tube as well.

Primary objective

Assessment of the pharmacokinetics of rivaroxaban, apixaban and edoxaban, when co-administered as a microdose cocktail (apixaban 12.5 µg, rivaroxaban 12.5 µg, edoxaban 50 µg) in children with congenital heart defects, aged 6 months to 6 years.

Secondary objectives

- To compare the pharmacokinetics of the FXaI in children with those reported in healthy adults and patients from literature.
- To characterise the CYP3A activity by means of a midazolam microdose in children.
- To characterise the CYP2D6 activity by means of a yohimbine microdose in children.
- To evaluate tolerability (and safety regarding laboratory parameters) of this microdose cocktail in children.

Study Outcome

Primary outcome is the area under the concentration-time curve ($AUC_{0-\infty}$) of the three DOACs apixaban, edoxaban, and rivaroxaban. Secondary outcomes include standard PK parameters (C_{max}, t_{max}, $t_{1/2}$, Cl/F, and Vss/F) of each FXaI and yohimbine, as well as the metabolic clearance of midazolam by means of AUC_{2-4h} [18].

Recruitment

Recruitment takes place at the Department of Paediatric and Congenital Cardiology. Prior to the surgical intervention parents of potential participants will be addressed by a study physician regarding the study which serves as short information about the clinical trial. After the surgery detailed information will be given and discussed with both parents. After full explanation of the purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort of the clinical trial both parents will be asked whether they agree to participate and, in that case, to sign the informed consent form before any study procedures are carried out.

Screening

The screening visit is defined as accurate check of all diagnostic findings which were made in course of the pre-operative assessment to evaluate eligibility. This is done after the surgery by a trial team physician in order to check inclusion and exclusion criteria.

Interventions

The intervention begins as early as on postoperative day 3 (about 72 hours after surgery) with the oral administration of the study drugs. They are dissolved in 110 ml drinking water to ensure comfortable oral application. If the patient has a feeding tube, this will be used for administration and will afterwards be flushed with 5mL drinking water. All patients have as a clinical routine measure prior to cardiac surgery a central venous line. Vital parameters and other clinical or laboratory which are necessary to ensure patients safety are extracted from the daily documentation during clinical routine.

Sample collection

PK blood samplings will be collected via a central venous line and time points are dependent on bodyweight (Figure 1). The total amount of blood drawn will be 25 mL over 25 hours for children of at least 10 kg. This includes 17 samples to evaluate PK parameters (S-Monovette® 1.2 mL lithium heparin collection tubes, Sarstedt, Nümbrecht, Germany) and 3 safety checks in which the coagulation parameters will be observed (S-Monovette® 1.4 mL citrate collection tubes, Sarstedt, Nümbrecht, Germany). Blood samples for the DOAC PK-analysis (Li-Hep) will be obtained before and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, and 25 h after intake of the oral solution. This is equivalent to a loss of blood volume of 3.28 % of a child weighing 10 kg. For children weighing 7 to 9.9 kg a reduced sampling scheme (12 PK samples and 3 coagulation samples, specific timepoints are before and 0.25, 0.5, 1, 1.25, 2, 2.5, 3, 4, 8, 12, and 24 h after intake of the oral solution.) will be performed to limit the blood loss (18.6 mL in 24 hours equivalent to 3.54 % for a child weighing 7 kg)[19]. All PK samples will be quantified

by ultra-performance liquid chromatography – tandem mass spectrometry methods in the Clinical Analytical Laboratory of the Department of Clinical Pharmacology and Pharmacoepidemiology at the University Hospital Heidelberg.

Midazolam will be measured in 5 samples (before and 2, 2.5, 3, and 4 h after administration) using an established limited sampling strategy[18] and yohimbine[20] in the samples taken up to 12 hours after administration.

Three coagulation checks (quick, INR, aPTT) are obtained before, 2 and 2425 h after drug administration.

End of trial

After the last blood sample the end of trial visit includes reviewing the last clinical examination and laboratory assessment, which both are performed at least once during 24 hours by clinical routine.

Concomitant medication

Any necessary medication (judged by a responsible physician) for the best clinical care and/or emergency treatment is permitted. Concomitant medication will be documented (date, time, dose, route of administration) by a member of the trial team. For safety reasons simultaneous administration of anticoagulants, thrombolytics, platelet inhibitors such as GPIIb/IIIa-inhibitors and $P2Y_{12}$ inhibitors, and thienopyridine (clopidogrel) are prohibited for the duration of the trial.

Sample size calculation

Since no PK data in young children are available for the microdosed FXaI cocktail, no formal sample size calculation could be performed. It is planned to enroll up to 20 children with congenital heart defects. This number of patients is consistent with sample sizes of similar

exploratory PK studies, which is expected to provide sufficient information on PK, safety regarding laboratory parameters, and tolerability of the µ-FXaI cocktail and the suitability of this methodology in future clinical paediatric drug development. In addition, it is expected that robust single-dose PK data of 3 FXaI in a young paediatric population will be generated to provide first guidance for dose adjustment in clinical use.

Data management

Clinical routine data are also documented for this clinical trial. Vital signs (i.e. blood pressure, heart rate) are documented at screening, prior to the drug administration, at any specific PK timepoint, and at end of trial. Concomitant medication and adverse events are also recorded. Printed source data sheets are used for the primary data collection and documentation. All source data information are timely transferred to the clinical trial management database (Promasys, OmniComm Systems, Fort Lauderdale, USA).

Data analysis and missing data

Standard PK parameters (C_{max}, t_{max}, $AUC_{0-\infty}$, $t_{1/2}$, Cl/F, and Vss/F) of each FXaI and yohimbine will be calculated by non-compartmental analyses (Monolix 2021R2, Lixoft SAS, Antony, France) and its results will be presented by descriptive statistics (GraphPad Prism 8.0.0 for Windows, GraphPad Software, San Diego, California USA). CYP3A activity will be quantified using the estimated metabolic clearance of midazolam by means of AUC_{2-4h}.

Individual missing or inconsistent data will be subject to a simple edit query process. Eventually missing data will not be imputed.

Patient and Public Involvement

Patients and/ or the public were not involved in any stages of this clinical trial: study design/ conduction/ analysis.

Ethics and dissemination

Ethics approval

The study protocol (Version 4.0), patient information and informed consent form were approved by the ethics committee of the Medical Faculty of Heidelberg University (AFmo-606/2019) and the German competent authority (BfArM).

To enable the participation for children younger than 2 years and with a weight lower than 10 kg a protocol amendment was submitted and approved (Protocol Version 5.1). For these patients, a reduced sampling scheme was introduced to keep the relative loss of blood volume below 5 % in 24 hours[19].

Study monitoring

A trained clinical trial monitor is assigned from the Department of Clinical Pharmacology and Pharmacoepidemiology at the University Hospital Heidelberg to perform the monitoring of this clinical trial.

Safety considerations

All medications will be administered as a subtherapeutic microdose. Although it is unlikely that the achieved concentrations of the administered trial medications confer any pharmacological effect or adverse drug reaction, a maximum degree of safety is essential in this vulnerable population. Clinical monitoring as per standard of care after cardiac surgery will be used.

Benefit and Risk assessment

There is no direct benefit to the patients or their parents. Because of the clinical setting in which the study will be carried out, no additional invasive procedures have to be performed. All participants are admitted as inpatient to the paediatric cardiac wards, where continuous cardiorespiratory monitoring and central vascular access, etc. are already provided.

In order to reduce additional burden of study procedures to the patient in the postoperative phase, after having signed informed consent, the patients' medical history, findings during the clinical examination and other parameters routinely assessed and documented during the treatment for these patients will also be completely recorded for this clinical trial. Due to the sensitive drug assay, the amount of blood taken for the PK analyses is minimised.

All study drugs will be given as single microdoses only which does not elicit any pharmacological effects or adverse events[5]. The combination of microdoses of apixaban, rivaroxaban, edoxaban has been used in adults and at FXaI peak concentrations, International normalised ratio (INR) increased just by 3.9% and activated partial thromboplastin time (aPTT) by 3.1% which was clinically not relevant[5]. No bleeding complications occurred. Clinically relevant non-major bleeding (menorrhagia and gingival bleeding) has been observed in four children with venous thromboembolism taking therapeutic rivaroxaban within the Einstein-Jr phase III study[21].

Due to very sensitive analytical assay for apixaban and rivaroxaban, the dose of apixaban and rivaroxaban is halved to 12.5 µg each compared to the adult study. MDZ and yohimbine were used frequently in adults and no relevant AEs were observed at all.

Thus, the risk for adverse events (AE) or toxicity is minimal. We do not expect any bleeding complications by µ-FXaI. If a case of bleeding complications the effects of rivaroxaban and apixaban can be reversed by Andexanet alpha. It also inhibits the function of all heparins[22]. We have summarised the case reports where children were exposed to rivaroxaban or edoxaban[23], intentionally or unintentionally in the Table 2.

In summary, potential risks of participation in this clinical trial are small and predictable. The trial drugs are administered at sub-therapeutic microdoses on one occasion, so that no Type A adverse drug reactions are expected. The participant's suitability will be carefully evaluated, and their health will be closely monitored during the trial.

Publications and Data access

The results of this investigation will be published in an international scientific peer-reviewed journal. It is not planned to make the data publicly available.

Figure 1: Study design assessing the pharmacokinetics of rivaroxaban, apixaban, edoxaban as well as the CYP3A and CYP2D6 enzyme activity in children with congenital heart defects. Upper timeline: Sampling schedule for children weighing 10 kg and more, lower timeline: sampling schedule for children weighing 7-9.9 kg. CR = Blood sampling within clinical routine. EOT = End of trial

Table 1: Detailed inclusion and exclusion criteria of DOAC-Child

Inclusion criteria	*Exclusion criteria*

• Infants and children aged 6 months up to 6 years (inclusive)
• Body weight ≥ 7 kg
• Admitted as inpatient to the paediatric cardiac wards after congenital cardiac surgery
• Cardiac defects: non-cyanotic congenital heart defects such as e.g. atrial septal defect, ventricular septal defect
• Availability of a central vascular access
• Otherwise healthy children as determined by medical assessment consisting of a medical history, physical examination, an ECG, and a laboratory evaluation, all performed within the clinical routine, that all must show no clinically relevant abnormalities
• Minor deviations of laboratory values from the normal range may be acceptable in the pre-operative assessment, if judged by the investigator to be of no clinical relevance for this trial.
Criteria include, but are not limited to
  • ALT ≤ ULN x 1.1
  • AST ≤ ULN x 1.2
	• Intake of a substance known to induce or inhibit drug metabolizing enzymes or drug transporters within a period of less than 10 times the respective elimination half-life or two weeks, whatever is longer
	• Simultaneous treatment with anticoagulants (i.e. phenprocoumon, warfarin, heparin - prior intake of heparin is allowed if more than 24 h prior to the start of study)
	• Active, clinically relevant bleeding
	• Any hepatic disease which could lead to coagulopathy or clinically relevant risk of bleeding
	• Lesion or condition, if considered to be a significant risk for major bleeding. This may include current or recent gastrointestinal ulceration, presence of malignant neoplasms at high risk of bleeding, recent brain or spinal injury, recent brain, spinal or ophthalmic surgery, recent intracranial haemorrhage, known or suspected oesophageal varices, arteriovenous malformations, vascular aneurysms or major intraspinal or intracerebral vascular abnormalities
	• Uncontrolled severe hypertension/hypotension
	• Severe respiratory insufficiency

• Bilirubin ≤ ULN x 1.2 (this does not apply to patients with Gilbert's syndrome) • Creatinine ≤ ULN + 0.1 mg/dl (=ULN + 8.8 µmol/L) • Haemoglobin > 10 g/dl (pre- and postoperatively) • Both parents (or legal representatives) have to be able to communicate well with the investigator, to understand and comply with the requirements of the trial • Voluntarily signed informed consent after full explanation of the objectives, meaning and consequences of the trial to both parents (or legal representatives) of the participant. The informed consent will be obtained after the surgery but before any specific study procedures will be carried out	• Tachycardic arrhythmias • Renal/Liver insufficiency • Glaucoma • Gastrointestinal ulceration • Clinically relevant mental disorder • Any physical disorder that could interfere with the participant's safety during the clinical trial or with the trial objectives • Body weight lower than 7 kg • Allergies (except for mild forms of hay fever) or history of hypersensitivity reactions/ intolerance to the study drugs • Any acute or chronic illness or clinically relevant finding during the clinical course known or expected to modify absorption, distribution, metabolism, or excretion of the drug under investigation • Any participation in an interventional clinical trial within 30 days before inclusion. • Specific exclusion criteria for Midazolam: Administration of midazolam less than 48 h prior to the start of study

Table 2: Case reports about children who were exposed to direct oral anticoagulants

DOAC	Patient	Indication	Dose	Result
Rivaroxaban[24]	12 year old girl	DVT	20 mg once daily for 5 days	Rivaroxaban trough level after 2 days 109 ng/mL and after 5 days 100 ng/mL. No bleeding.
Rivaroxaban[25]	35 month old boy	Accidental intake	200 mg (16 mg/kg)	Activated charcoal (2 g/kg) p.o. within 45 minutes of ingestion. Plasma anit-FXa level about four hours after ingestion > 4.00 IU/mL. At 13.5 hours after ingestion was 1.51 IU/mL. No adverse effects reported. No bleeding complication.
Rivaroxaban[26]	6 year old girl	Severe Protein S deficiency with repetitive skin necrosis during warfarin treatment.	Stepwise increase up to 40 mg daily	No adverse effects reported at 1 year follow-up. No bleeding episodes.
Rivaroxaban[27]	4 year old boy	Thrombotic storm	Daily dose unknown. Longterm treatment.	No bleeding or other adverse effects after 1 year of continued administration.
Edoxaban[23]	4 year old boy	Protein C deficiency	2 mg/kg/dose, four times a day	Two years follow-up did not result in adverse events.
Apixaban[13]	3 year old girl, 2 & 6 year old boys	Intracardiac thrombosis	2.5 mg twice a day	No bleeding events, complete resolution of the thrombi (n=2), substantial reduction in clot size (n=1) (follow up 6 days – 4 weeks)

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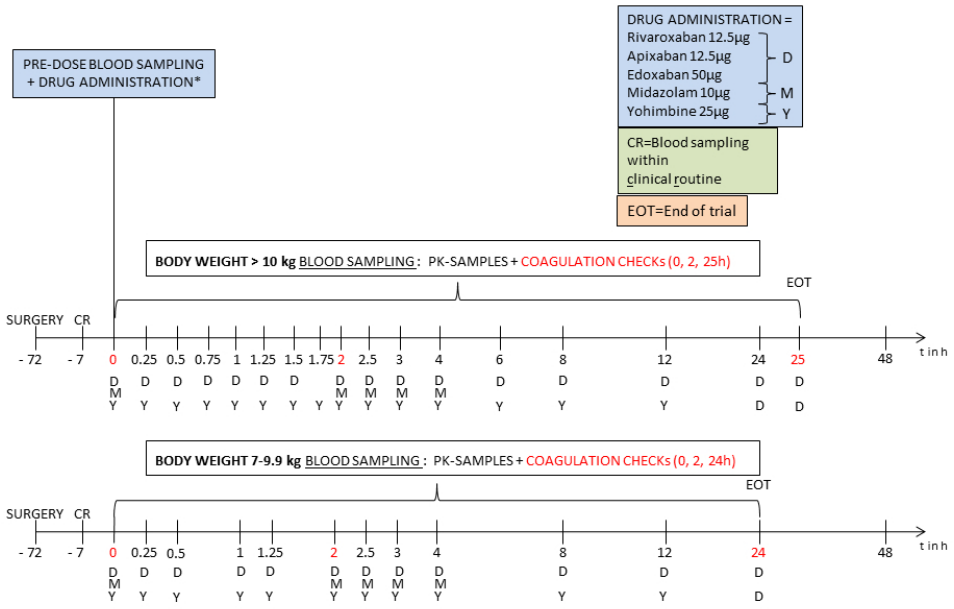

Study design assessing the pharmacokinetics of rivaroxaban, apixaban, edoxaban as well as the CYP3A and CYP2D6 enzyme activity in children with congenital heart defects. Upper timeline: Sampling schedule for children weighing 10 kg and more, lower timeline: sampling schedule for children weighing 7-9.9 kg. CR = Blood sampling within clinical routine. EOT = End of trial.

254x190mm (96 x 96 DPI)

Hermann S et al.: Pharmacokinetics of a microdosed cocktail of 3 direct oral anticoagulants in children with congenital heart defects: study protocol for a single centre clinical trial, 2022.

Supplementary Material

Rivaroxaban

NCT number	Anticipated end date	Current status	Results
01684423	January 2016	completed	https://clinicaltrials.gov/ct2/show/results/NCT01684423?term=Rivaroxaban%2C+Children&draw=2&rank=2
02497716	April 2016	completed	
02564718	October 2016	completed	https://clinicaltrials.gov/ct2/show/results/NCT02564718?term=Rivaroxaban%2C+Children&draw=2&rank=5
02309411	March 2017	completed	https://clinicaltrials.gov/ct2/show/results/NCT02309411?term=Rivaroxaban%2C+Children&draw=2&rank=1
02234843	November 2018	completed	https://clinicaltrials.gov/ct2/show/results/NCT02234843?term=Rivaroxaban%2C+Children&draw=2&rank=3

Apixaban

NCT number	Anticipated end date	Current status	Results
01707394	August 2016	completed	
02369653	May 2019	completed	https://clinicaltrials.gov/ct2/show/results/NCT02369653?term=Apixaban%2C+Children&draw=2&rank=1
02464969	October 2020	recruiting	

Edoxaban

NCT number	Anticipated end date	Current status	Results
02303431	December 2016	completed	

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Dabigatran

NCT number	Anticipated end date	Current status	Results
01083732	November 2015	completed	https://clinicaltrials.gov/ct2/show/results/NCT01083732?term=Dabigatran%2C+Children&draw=2&rank=2
01773174	December 2015	withdrawn	
02223260	April 2016	completed	https://clinicaltrials.gov/ct2/show/results/NCT02223260?term=Dabigatran%2C+Children&draw=2&rank=1
01895777	March 2018	completed	https://clinicaltrials.gov/ct2/show/results/NCT01895777?term=Dabigatran%2C+Children&draw=2&rank=6
02197416	June 2018	completed	https://clinicaltrials.gov/ct2/show/results/NCT02197416?term=Dabigatran%2C+Children&draw=2&rank=3