ANESTHESIOLOGY

Intravenous Dabigatran Provides Adequate Anticoagulation for Cardiopulmonary Bypass Using a Rabbit Model

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Heparin is used to provide anticoagulation during cardiopulmonary bypass but it has disadvantages, including potential development of antiplatelet factor 4 heparin antibodies
- A recommended alternative to heparin, bivalirudin, has its own disadvantages, including the lack of a reversal agent
- Dabigatran may be a better alternative in patients with contraindications to the use of heparin, because it has an approved reversal agent

What This Article Tells Us That Is New

- The hypothesis that dabigatran would provide sufficient anticoagulation for cardiopulmonary bypass was tested in a first-use, proof-of-concept study using a rabbit model of cardiopulmonary bypass that included a comparison group receiving heparin
- The dabigatran loading dose and maintenance infusions were designed after first determining its pharmacokinetics in rabbits and the target concentration in an *in vitro* simulation of cardiopulmonary bypass
- Dabigatran provided acceptable anticoagulation to prevent thrombosis during 2 h of cardiopulmonary bypass in rabbits that was similar to that provided by heparin and was well tolerated despite accumulating well above the target concentration during the period of anesthesia and cardiopulmonary bypass

ABSTRACT

Background: Heparin anticoagulation has been used successfully for cardiopulmonary bypass (CPB). However, an alternative anticoagulant approach is desirable due to the cases of heparin-induced thrombocytopenia. Dabigatran provides anticoagulation for an *in vitro* model of simulated CPB. The current analysis tests the hypothesis that dabigatran provides sufficient anticoagulation for CPB in intact rabbits.

Methods: Nonlinear mixed effects models were used to estimate dabigatran parameters for a two-compartment pharmacokinetic model in 10 New Zealand White rabbits. A dabigatran infusion designed to maintain a plasma concentration of 90 μg/ml was run throughout CPB based on the pharmacokinetics. Animals were subjected to sternotomy and anticoagulated with IV dabigatran (six animals) or heparin (four animals). Rabbits were cannulated centrally using the right atrium and ascending aorta and CPB was maintained for 120 min. Measurement of activated clotting time, thromboelastometric reaction time, and blood gases were performed during CPB. Then, the animals were euthanized, and the brain and one kidney were removed for histology. Sections of the arterial filters were inspected using electron microscopy.

Results: The observed dabigatran concentrations during CPB were greater than the target concentration, ranging from 137 ± 40 μg/ml at 5 min of CPB to 428 ± 150 μg/ml at 60 min, and 295 ± 35 μg/ml at 120 min. All rabbits completed 2 h of CPB without visible thrombosis. In the two groups, reaction time values were elevated, reaching 10,262 ± 4,198 s (dabigatran group) and 354 ± 141 s (heparin group) at 120 min of CPB. Brains and kidneys showed no evidence of thrombosis or ultrastructural damage. Sections of the arterial line filter showed minimal or no fibrin. There was no significant difference in outcomes between dabigatran- and heparin-treated animals.

Conclusions: In this first-use, proof-of-concept study, the authors have shown that dabigatran provides acceptable anticoagulation similar to heparin to prevent thrombosis using a rabbit CPB model.

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Since the inception of cardiopulmonary bypass (CPB), anticoagulation has been accomplished with heparin. However, heparin has shortcomings in this indication, particularly the necessity for sufficient intrinsic antithrombin, and also its antigenic potential.[1] Although only 1% of cardiac surgical patients may develop heparin-induced thrombocytopenia after CPB,[2] as many as 50% of patients will develop anti–heparin-platelet factor 4 antibodies that are associated with an increased risk of adverse outcomes.[3] Bivalirudin has been recommended as an alternative to heparin for this indication,[4] but there are problems with its use, including the lack of a reversal agent, poor sensitivity of the commonly used activated clotting time, and a high incidence of bleeding and thrombotic complications.[5] Thus,

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a continued search for an ideal alternative that can be safely used during CPB is warranted.

Dabigatran is a direct thrombin inhibitor that is approved by the Food and Drug Administration (Silver Spring, Maryland) for oral administration in humans for thromboprophylaxis and treatment.[6,7] A reversal agent for dabigatran, idarucizumab, has been developed and approved for human use.[8] Establishing dabigatran anticoagulation as effective and safe for anticoagulation for CPB may provide a better approach, particularly for patients with heparin contraindications.

We have previously shown that dabigatran can provide adequate anticoagulation for an *in vitro* model of simulated CPB.[9] The critical next step is to evaluate dabigatran within an animal model using CPB. In this proof-of-concept study we established the pharmacokinetics and pharmacodynamics of dabigatran in rabbits, and then we used the consequent parameter estimates to employ dabigatran as an anticoagulant for CPB in a rabbit model. Our hypothesis is that dabigatran anticoagulation will maintain blood fluidity for 120 min of CPB in this animal model.

Materials and Methods

Animals and Materials

Thirty-five male adult New Zealand White rabbits 4.0 to 5.1 kg (median 4.7) were used in this study. All rabbits were maintained in a specific pathogen-free vivarium in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility under recommendations of the National Institutes of Health (Bethesda, Maryland) Guide for the Care and Use of Laboratory Animals, with a 12:12-h, light-dark cycle and food and water available *ad libitum*. All experimental protocols were approved by the University of Rochester (Rochester, New York) Committee on Animal Resources.

Dabigatran Solutions

One milligram of dabigatran (Clearsynth, Ontario, Canada) was dissolved in 60 μl of 0.075 M HCl, and then the solution was added into 0.2 ml of 20% *N*,*N*-dimethylacetamide. Based on this formulation, *in vivo* injection of 15 mg/kg dabigatran into a 4-kg rabbit required 60 mg of dabigatran dissolved in 3.6 ml of 0.075 M HCl and diluted in 12 ml of 20% *N*,*N*-dimethylacetamide, totaling 15.6 ml of injection volume.

Pharmacokinetics and Pharmacodynamics Determination

The central ear artery and a marginal ear vein of 10 rabbits were cannulated with a 24 g IV catheter (Jelco, ICU Medical, Inc., USA) after aseptic preparation. Dabigatran (15 mg/kg) was injected *via* the IV cannula manually for 15 s, followed by a normal saline flush (3 ml). Samples (2 ml) were collected from the arterial catheter into citrate tubes at baseline (before injection), 5, 15, 30, and 60 min, while the rabbits were restrained. Rabbits were returned to their cages with food and water *ad libitum*, and the remaining blood samples at 120, 180, 300 and 420 min were collected under manual restraint. The total amount of collected blood was 18 ml/rabbit. Rabbits were monitored for 24 h for adverse reactions, and then housed for up to 1 yr until terminal blood collection as donors for either Chandler loop or CPB experiments.

Laboratory Measurements

Reaction time (time in seconds to clot initiation), activated clotting time, hemoglobin concentration, and dabigatran concentration were measured in all blood samples. Kaolin/tissue-factor-activated thromboelastography measurements were performed using a Thromboelastograph Analyzer 5000 (Haemoscope Corp., USA). The kaolin/tissue-factor-activated thromboelastography was initiated when 340 μl of blood was mixed with 10 μl of kaolin/tissue-factor-activated thromboelastography reagents and 20 μl of 0.2 M $CaCl_2$. Reaction was monitored until reaction time was established. Activated clotting time and hemoglobin concentration were measured using the Hemochron Signature Elite (Accriva Diagnostics, Inc., USA) and HemoCue (HemoCue Company, Ängelholm, Sweden) according to the manufacturer instructions. One milliliter

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of blood was centrifuged at 700*g* for 15 min to obtain plasma for determination of dabigatran concentrations as previously reported,[10] using liquid chromatography/mass spectrometry with a Dionex Ultimate 3000 UHPLC coupled to a Q Exactive Plus mass spectrometer (Thermo Scientific, USA).

Chandler Loop

The Chandler loop is a simplified *in vitro* simulation of CPB.[9,10] For the purpose of Chandler loop experiments, terminal blood collection was performed on donor rabbits after the induction of general anesthesia with 15 to 30 mg/kg ketamine HCl and 3 mg/kg xylazine subcutaneously. If needed to achieve a deep plane of anesthesia, IV propofol (2 to 8 mg/kg) was administered. Blood was collected *via* cardiac puncture with the blood collected in sterile 250-ml bags containing 35 ml of citrate phosphate dextrose. A 54-cm length of 3/8 inch polyvinyl chloride pump tubing was connected end-to-end using a t-connector with a side port used for sampling. The loop was filled with the mixture of whole blood with PlasmaLyte-A (Baxter Healthcare Corporation, USA) at 2:1 ratio. The loop was mounted into the apparatus (Ebo Kunze, Neuffen, Germany) and was rotated at 6 revolutions per minute in a water bath at 37°C. The mixtures of blood and PlasmaLyte-A inside the Chandler loops were treated with 2.5, 5, 10, 20, 40, 60, and 80 μg/ml of dabigatran. Five minutes later $CaCl_2$ (5 mM) was added in the loops, and the blood fluidity was monitored for 120 min.

Dabigatran Pharmacokinetics

Population Parameter Estimations

A two-compartment (central and peripheral) pharmacokinetic model was used to fit data obtained from the *in vivo* animal experiments. The model was parameterized in terms of elimination clearance, intercompartmental clearance, central volume, and peripheral volume of distribution. Population parameter estimates were obtained using nonlinear mixed effects models (NONMEM 7.4, ICON Development Solutions, USA). This model accounts for population parameter variability (between subjects) and residual variability (random effects) as well as parameter differences predicted by covariate (fixed) effects. Population parameter variability was described using exponential models, which is equivalent to assuming a log-normal distribution and avoids biologically inappropriate parameter values of zero or less. Residual unidentified variability was modeled using additive residual errors. These population mean parameters, between-subject variance, and residual variance were estimated using the first-order conditional interaction estimate method using ADVAN3 TRANS4 of NONMEM VII. Convergence criterion was three significant digits. The nonlinear mixed effects model translator (NMTran) code has been published.[11] The population parameter variability is modeled in terms of random effect (η) variables. Each of these variables is assumed to have mean 0 and a variance denoted by ω^2, which is estimated.

The covariance between two elements of η (*e.g.*, elimination clearance [CL] and volume [*V*]) is a measure of statistical association between these two variables. Their covariance is related to their correlation (R), *i.e.,*

$$R = \frac{\text{cov}\,ariance}{\sqrt{(\omega^2{}_{CL} \times \omega^2{}_{Y})}}$$

The covariance of clearance and distribution volume variability was incorporated into the model.

Covariate Analysis

The parameter values were expressed as per kilogram using an allometric model.[12,13]

$$P_i = P_{std} \times \left({}^{W_i}\!/_{W_{std}}\right)^{EXP}$$

where P_i is the parameter of the *i*th subject, W_i is the weight of the *i*th subject, and P_{std} is the parameter of a rabbit with a weight W_{std} of 1 kg (*i.e.*, expressed as per kilogram). The EXP exponent was 0.75 for clearances and 1 for distribution volumes.[14]

Quality of Fit

Model selection required a statistically significant improvement in the nonlinear mixed effects models objective function between nested models, equating to a reduction of more than 3.84 based on a chi-square distribution ($\alpha < 0.05$). Bootstrap methods, incorporated within the Wings for nonlinear mixed effects models program, provided a means to evaluate parameter uncertainty. The quality of fit during model building was assessed by visual inspection of diagnostic plots, *e.g.*, observed *versus* predicted concentrations and residual plots.

Simulation

Parameter estimates were used to simulate concentration *versus* time profiles using differential equations in Berkeley Madonna modeling and simulation software (Robert Macey and George Oster, University of California Berkeley, USA). Loading doses and maintenance infusions that achieve steady-state concentrations of 60, 90, and 120 μg/ml.

Cardiopulmonary Bypass

Twenty rabbits were used for the CPB segment, 10 undergoing CPB and 10 as blood donors. Six CPB rabbits

received dabigatran and four control animals received heparin. Animals were randomly assigned to the groups, and within the group were tested in sequential order. Study drug assignment was not randomized or blinded. In donor animals, terminal blood collection was performed as described earlier for Chandler loops.

For CPB animals, buprenorphine sustained-release (0.12 mg/kg subcutaneously once before surgery) was administered preoperatively to provide analgesia. For CPB animals, sedation was achieved using one of two protocols: ketamine (15 mg/kg) with dexmedetomidine (0.05 to 0.1 mg/kg) or ketamine (35 to 55 mg/kg) with midazolam (5 mg/kg) administered subcutaneously. The sedation and maintenance anesthesia protocol was modified after the first three surgeries were completed in an effort to improve hemodynamic stability. Each rabbit was intubated or had a supraglottic airway placed for airway maintenance and was artificially ventilated. Anesthesia was maintained with 1 to 3% isoflurane balanced with an IV infusion of propofol (0.2 $mg \cdot kg^{-1} \cdot min^{-1}$) or sufentanil-midazolam (0.3 $ml \cdot kg^{-1} \cdot h^{-1}$ infusion of a solution containing 2.3 μg/ml sufentanil and 450 μg/ml midazolam). During anesthesia, rabbits continuously received lactate Ringer's solution with 5% dextrose at a rate of 5 to 10 $ml \cdot kg^{-1} \cdot h^{-1}$ and monitored for heart rate and rhythm (electrocardiogram), oxygen saturation (pulse oximetry), expired carbon dioxide (capnography), blood pressure (*via* arterial catheter or transducer), temperature (rectal), jaw tone, and absence of reflexes.

After placement of femoral arterial and external jugular catheters, the mediastinum was entered *via* a midline sternotomy. After placement of purse-string sutures in the aorta and right atrium, the study anticoagulant was administered intravenously; either dabigatran 17 mg/kg (6 animals) or heparin 400 U/kg (4 animals) was injected over 30 s. To provide the target dabigatran concentration of 90 μg/ml (in plasma) throughout CPB, dabigatran animals received an IV infusion of 0.9 $mg \cdot kg^{-1} \cdot min^{-1}$ for 40 min followed by 0.55 $mg \cdot kg^{-1} \cdot min^{-1}$ until the end of bypass. We targeted a dabigatran concentration of 90 μg/ml, slightly greater than the minimum effective concentration found in the Chandler loop experiment.

The ascending aorta was cannulated with 8-French arterial cannula (Biomedicus, Medtronic, Inc., USA). The right atrium was cannulated with 12-French right-angle single-stage venous cannula (Edwards Thin Flex, Edwards Lifesciences, USA). The cannulae were connected to tubing from a CPB circuit as per standard practice, and bypass was initiated.

The bypass circuit consisted of a Custom Heart Lung Pack (Medtronic, Inc., USA) with uncoated 3/16 inch arterial and 1/4 inch venous tubing, and a 1/4 inch pump boot. The oxygenator used was a Capiox FX05 advance oxygenator with poly(2-methoxyethyl acrylate) coating (Terumo Cardiovascular, USA). The bypass machine used was a Medtronic Century Heart Lung Machine (SOMA Technology, USA). The bypass prime (120 ml) consisted of the donor rabbit blood, PlasmaLyte-A (Baxter Healthcare Corporation, USA), 25% human albumin (Flexbumin, Bexalta US Inc., USA), and Sodium Bicarbonate 8.4% (IMS Limited, So. USA). For dabigatran animals, the infusion was initiated; for heparin animals, 1,000 U/ml was added to the prime. Calcium chloride ($CaCl_2$ 5 mM) was added to the prime. In the event of acid-base derangement, the prime was adjusted with additional sodium bicarbonate. Bypass was instituted at blood flow rates of approximately 100 $ml \cdot kg^{-1} \cdot min^{-1}$ and were maintained at that level throughout the experiment. Blood gas exchange was achieved with sweep gas rates of approximately 100 to 200 ml/min and a F_{IO_2} of 100%. Acid-base status was monitored throughout the bypass run using the I-Stat (Abbott Point of Care, USA) blood gas analyzer. Normothermic CPB was maintained for 120 min, with measurement of dabigatran concentrations, activated clotting time, kaolin/tissue-factor-activated thromboelastography, blood gases, and electrolytes at 5, 15, 30, 60, 90, and 120 min after onset of CPB. After CPB, the animals were euthanized by exsanguination into the pump, and the brain and one kidney were removed for histologic examination for evidence of thrombus or thromboembolism. Two 2.5-cm sections of each arterial filter were inspected for fibrin deposition using a Scanning Electron Microscope.

Statistical Analysis

The primary outcome of the study was to determine whether dabigatran would prevent clot for more than 120 min CPB duration in rabbits.

For the *in situ* experiments, reaction time, activated clotting time, and hemoglobin concentration were measured. The linear mixed effects model was used to analyze data in figure 1, figure 2, table 1, and figures S2 and S3 (https://links.lww.com/ALN/D96). In the linear mixed effects model, we use the random intercepts for subjects as random effects, the time or the dosage concentration (up to some higher orders) as the main effects. The R^2 proposed by Snijders *et al.*[15] was used for the coefficient of determination. Statistical analyses were implemented with SAS 9.4 (SAS Institute Inc., USA). The significance level was set at 0.05 for each analysis. Ten animals (to minimize animal use) were required to achieve a good fit with nonlinear mixed effects models software. No formal power analysis was done for the CPB portion of the study because the outcome was simply the demonstration of fluidity for 2 h and no comparison statistics were applied. The sample size of six with four controls was felt to be a balance with sufficient N to demonstrate repeatability while minimizing animal use in each group in a manner acceptable to the animal use committee. Data are presented as mean ± SD, except as otherwise noted.

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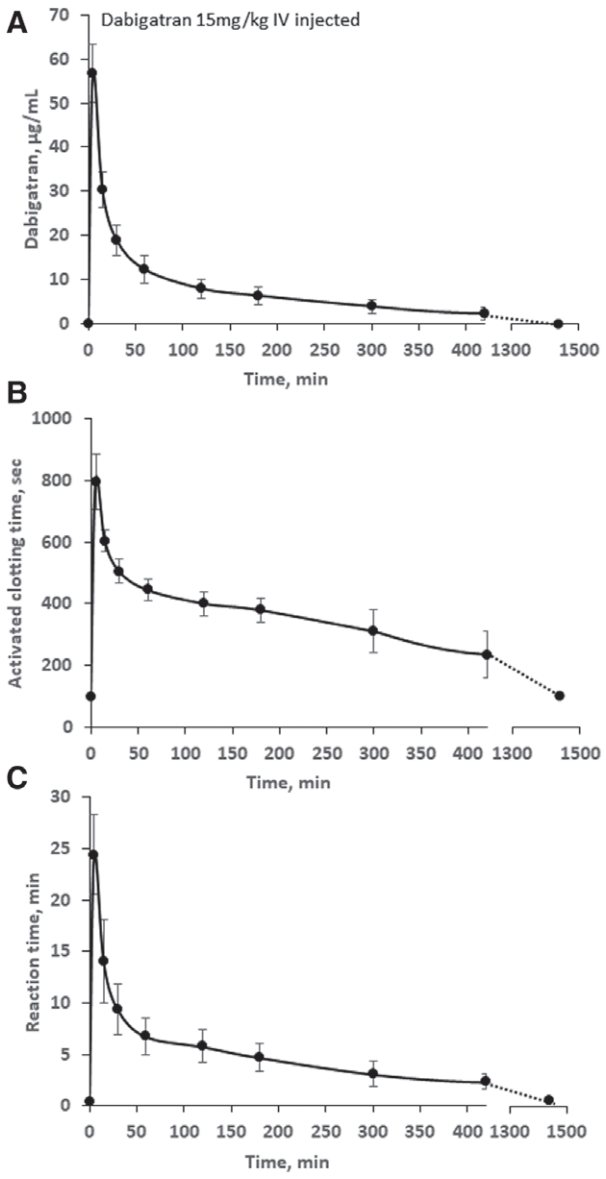

Fig. 1. Plasma dabigatran concentrations, activated clotting time, and thromboelastography reaction time. Dabigatran (15 mg/kg) was injected IV. Blood samples were collected at 5, 15, 30, 60, 120, 180, 300, and 420 min. (*A*) Dabigatran concentrations; (*B*) reaction time; and (*C*) activated clotting time were measured in all collected blood samples. The linear mixed effects (with random intercept and time as the main effect) was used to study the time trend of the outcome. Values for dabigatran concentrations, reaction time, and activated clotting time were significantly decreased with time ($P < 0.0001$). Data are shown as mean ± SD, n = 10 individual subjects. Note that activated clotting time values measured in the blood samples collected before dabigatran injection and measured in the several samples collected at 420 min after injection, were below the level of detection ("out of range low"), and thus were expressed as 100.

Results

Dabigatran Pharmacology in Rabbits

Upon injection, plasma dabigatran concentration reached the maximum value of 56.8 ± 6.6 μg/ml at 5 min (fig. 1A, n = 10), followed by a rapid decline to 30.4 ± 4.0 μg/ml at 15 min. Plasma dabigatran concentration thereafter gradually decreased and was undetectable at 24 h (1,440 min) after injection. To find out whether *in vivo* decline of dabigatran was organ-dependent, in the pilot sets of experiments the drug (80 and 40 μg/ml) was added into the blood samples *in vitro* followed by circulation of these blood samples in the Chandler loop system. Dabigatran concentrations remained stable in the samples collected at 1, 30, and 60 min of circulation (Supplemental Digital Content, fig. S1, https://links.lww.com/ALN/D96), indicating the organ-dependent decline of *in vivo* dabigatran (fig. 1A).

Dabigatran bolus increased activated clotting time and reaction time, exhibiting the highest levels at 5 min (fig. 1B and 1C) followed by a rapid decline. At 24 h (1,440 min), the values were not significantly different from baseline (before dabigatran injection). For activated clotting time, the observed values at 24 h are exactly the same as that of baseline; for reaction time, the *P* value of the paired *t* test is 0.272. Both activated clotting time and reaction time values correlated with plasma dabigatran concentrations, demonstrating the correspondent correlation coefficients of 0.94 and 0.96 (Supplemental Digital Content, fig. S2A and S2B, https://links.lww.com/ALN/D96). In addition, hemoglobin concentration was stable during the experiment (Supplemental Digital Content, fig. S3, https://links.lww.com/ALN/D96) which indicated that rabbits were not dehydrated or losing blood volume.

To define the minimum concentration required to maintain the blood fluidity for 120 min, the Chandler loops were loaded with the mixtures of blood and PlasmaLyte-A treated with 2.5, 5, 10, 20, 40, 60, and 80 μg/ml of the drug. The Chandler loop experiments revealed that 80 μg/ml of dabigatran was effective in preventing clot formation for 120 min (table S1, https://links.lww.com/ALN/D96).

Dabigatran Pharmacokinetics

Population pharmacokinetic parameter estimates were elimination clearance (5.6 $ml \cdot min^{-1} \cdot kg^{-1}$; coefficient of variation 25%; 95% CI 4.6 to 6.5 $ml \cdot min^{-1} \cdot kg^{-1}$), intercompartment clearance (13.7; coefficient of variation 7.9%; 95% CI 12.3 to 14.9 $ml \cdot min^{-1} \cdot kg^{-1}$), central volume of distribution (176; coefficient of variation 12.3%,; 95% CI 158 to 202 ml/kg), and peripheral volume of distribution (428; coefficient of variation 9.8%; 95% CI 372 to 525 ml/kg). Residual additive error was small (1.32 μg/ml). The correlations between pharmacokinetic subject variability for elimination clearance, central volume of distribution, intercompartment

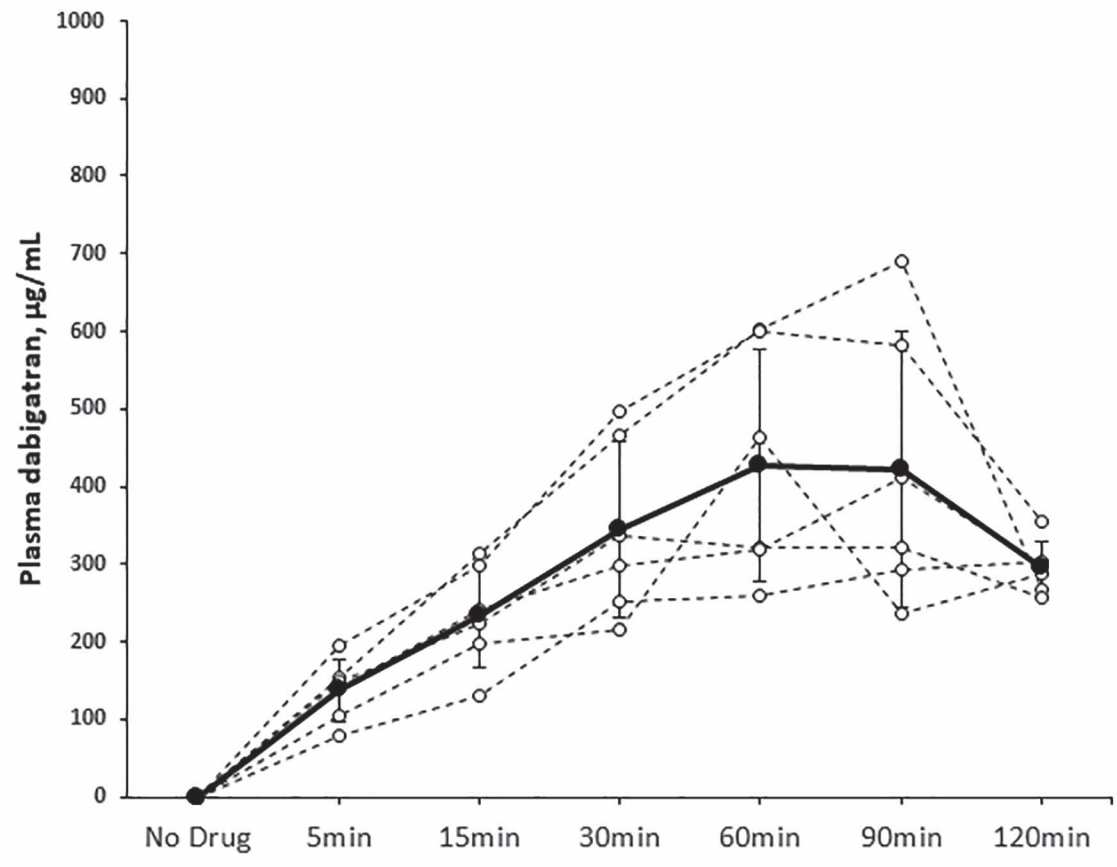

Fig. 2. Plasma concentrations of dabigatran during cardiopulmonary bypass. Dashed lines represent individual animals and the group average is presented by thickened black trace. Data are shown as mean ± SD, n = 6 individual subjects. The linear mixed effects (with random intercepts for subjects and time as the main effect) was used to study the time trend of the outcome.

clearance, and peripheral volume of distribution are shown in table S2 (https://links.lww.com/ALN/D96). Individual Bayesian estimates are shown in table S2 (https://links.lww.com/ALN/D96**);** these are estimates without per kilogram scaling. The observed *versus* predicted plots (fig. S4, https://links.lww.com/ALN/D96) for the pooled analysis confirms the adequacy of model predictions, showing no apparent deviations between model and data. Predicted doses to achieve a plasma dabigatran concentration of 60, 90, and 120 µg/ml are shown in table S3 (https://links.lww.com/ALN/D96).

Bypass

Ten animals, six dabigatran-treated and four heparin-treated rabbits ("dabigatran" and "heparin" groups), were subjected to CPB. To ensure a proper anticoagulant effectiveness during CPB, dabigatran was administrated according to the protocol calculated to provide a 90 µg/ml plasma concentration throughout CPB (table S4 and fig. S5, https://links.lww.com/ALN/D96). Rabbits in the "heparin" group received 400 U/kg IV heparin. All 10 animals completed 2 h of CPB without visible thrombosis. Blood gases and electrolytes varied within the acceptable ranges (table 1). In the dabigatran-treated rabbits, reaction times were profoundly elevated, and activated clotting time values were "out of range" at every measuring point during CPB (table 1). Dabigatran concentrations after the loading dose were at or above the target concentration in all except one animal (table 1 and fig. 2). There was substantial accumulation in all animals during bypass with concentrations significantly higher than expected. In the "heparin" group both reaction time and activated clotting time were elevated, although less than the dabigatran group (table 1). Activated clotting time values remained near 300 s or less despite rebolusing of heparin to a mean total dose of 6,673 ± 795 U. Upon completion of CPB, sections of the arterial line filters were examined, and the electron micrographs showed minimal or no fibrin depositions in both "dabigatran" and "heparin" groups (fig. 3). Finally, histopathologic examination of brains and kidneys showed no evidence of thrombosis or other abnormalities (fig. S6, https://links.lww.com/ALN/D96).

Table 1. Parameters Measured During 120 min in Rabbit Cardiopulmonary Bypass with Dabigatran and Heparin

	No Drug	1 min	15 min	30 min	60 min	90 min	120 min
CPB dabigatran (n = 6)							
pH	7.35 ± 0.09	7.19 ± 0.21	7.22 ± 0.19	7.27 ± 0.11	7.29 ± 0.06	7.30 ± 0.06	7.31 ± 0.09
pco_2, mmHg	53 ± 17	67 ± 29	63 ± 27	55 ± 11	47 ± 12	45 ± 10	39 ± 16
po_2, mmHg	291 ± 140	454 ± 103	495 ± 56	502 ± 48	517 ± 45	504 ± 55	479 ± 96
HCO_3, mmol/l	28 ± 6	24 ± 4	24 ± 3	25 ± 7	23 ± 7	22 ± 5	19 ± 6
Hematocrit, %	31 ± 7	22 ± 3	21 ± 3	22 ± 3	22 ± 4	21 ± 4	21 ± 2
Na^+, mmol/l	143 ± 4	141 ± 4	142 ± 4	142 ± 3	141 ± 3	141 ± 3	141 ± 4
K^+, mmol/l	3.5 ± 0.4	3.8 ± 0.9	3.8 ± 0.7	3.8 ± 0.7	3.8 ± 0.7	3.8 ± 0.8	3.9 ± 0.8
Ca^{2+}, mmol/l	1.7 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
[Dabigatran], μg/ml	0.06 ± 0.07	137 ± 40	234 ± 67	344 ± 114	428 ± 150	422 ± 178	295 ± 35
Reaction time, s	34 ± 9	8,408 ± 3,235	9,433 ± 2,616	11,816 ± 2,813	9,392 ± 2,413	11,482 ± 4,232	10,262 ± 4,198
Activated clotted time, s	Out of range low	Out of range high	Out of range high	Out of range high	Out of range high	Out of range high	Out of range high
CPB heparin (n = 4)							
pH	7.48 ± 0.09	7.39 ± 0.07	7.42 ± 0.03	7.42 ± 0.05	7.45 ± 0.05	7.44 ± 0.05	7.44 ± 0.05
pco_2, mmHg	43 ± 13	52 ± 8	49 ± 4	53 ± 10	49 ± 5	49 ± 7	50 ± 8
po_2, mmHg	461 ± 85	503 ± 24	508 ± 36	521 ± 46	520 ± 11	516 ± 35	538 ± 31
HCO_3, mmol/l	32 ± 4	31 ± 1	32 ± 2	34 ± 4	34 ± 2	33 ± 2	33 ± 2
Hematocrit, %	31 ± 7	21 ± 3	20 ± 3	19 ± 4	18 ± 4	18 ± 4	18 ± 4
Na^+, mmol/l	139 ± 3	141 ± 4	140 ± 4	141 ± 4	140 ± 4	139 ± 4	140 ± 4
K^+, mmol/l	3.3 ± 0.5	3.2 ± 0.4	3.2 ± 0.2	3.1 ± 0.1	3.1 ± 0.3	3.2 ± 0.2	3.3 ± 0.3
Ca^{2+}, mmol/l	1.6 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.6 ± 0.1
Reaction time, s	26 ± 2	1,344 ± 1,838	952 ± 1,244	596 ± 370	671 ± 579	342 ± 89	354 ± 141
Activated clotted time, s	Out of range low	258 ± 96	249 ± 116	304 ± 80	277 ± 49	231 ± 55	216 ± 34

All data are means ± SD. For reaction times, the linear mixed effects model with random intercepts for subjects and time as the main effect showed significant difference between "No Drug" and every time point during CPB ($P < 0.0001$), and no significant changes between time points within CPB ($P = 0.1632$ in the dabigatran group and $P = 0.1582$ in heparin group). Note: in the first experimental rabbit of the CPB dabigatran group, blood gases and electrolytes are missing at 5 and 15 min.

CPB, cardiopulmonary bypass

Discussion

We demonstrated that dabigatran provides acceptable anticoagulation to prevent thrombosis during 2 h of CPB in a rabbit model similar to heparin in this first-use, proof-of-concept study. Pharmacokinetic parameters were estimated in rabbits, allowing dose estimation for CPB use. The dabigatran dose was well tolerated by the rabbits in two phases of this study, without apparent sequelae despite very high level of anticoagulation as measured by activated clotting time and reaction time.

The choice of a direct thrombin inhibitors as a heparin alternative for CPB is consistent with published studies showing that the direct thrombin inhibitors hirudin, argatroban, and bivalirudin, have all been used successfully in human CPB.[16–18] Bivalirudin is currently recommended as a heparin alternative for patients with heparin-induced thrombocytopenia in published guidelines,[19] and it is the most commonly employed in practice.[20] However, bivalirudin has no reversal agent and is associated with both excessive bleeding and intraoperative thrombosis.[21,22] Although the Food and Drug Administration approved formulation of dabigatran as the etexilate mesylate salt for oral use, we have previously demonstrated that it can be prepared in a solution appropriate for IV administration.[9] This formulation was well tolerated by the rabbits in two phases of our study, without apparent sequelae despite very high doses. The effects of dabigatran can be rapidly and completely reversed with the monoclonal antibody idarucizumab, an IV agent the Food and Drug Administration approved for this purpose.[23]

Our study has several limitations. First, we did not do any direct toxicity testing of the very high doses of dabigatran required for CPB. Although many of the rabbits used for the pharmacokinetic portion of the study survived for months, we did not do any testing to evaluate for organ toxicity that was not clinically apparent. In this regard, one mitigation strategy might be the administration of both dabigatran with an anti-Xa drug agent for CPB. Indeed, we have reported that, in the simulated CPB model, dabigatran dose can be reduced fourfold if rivaroxaban is added into the circuit.[10]

Second, although the therapeutic concentration of dabigatran found in patients treated according to the Food and Drug Administration indication is easily reversed with idarucizumab, further work will determine the reversibility of high-concentration dabigatran, and survivability for the subject animals. Given that dabigatran is distributed in extracellular fluid and idarucizumab is distributed primarily in the intravascular space, reversal of high-concentration dabigatran will likely be more complex, time consuming, and costly than protamine reversal of heparin.

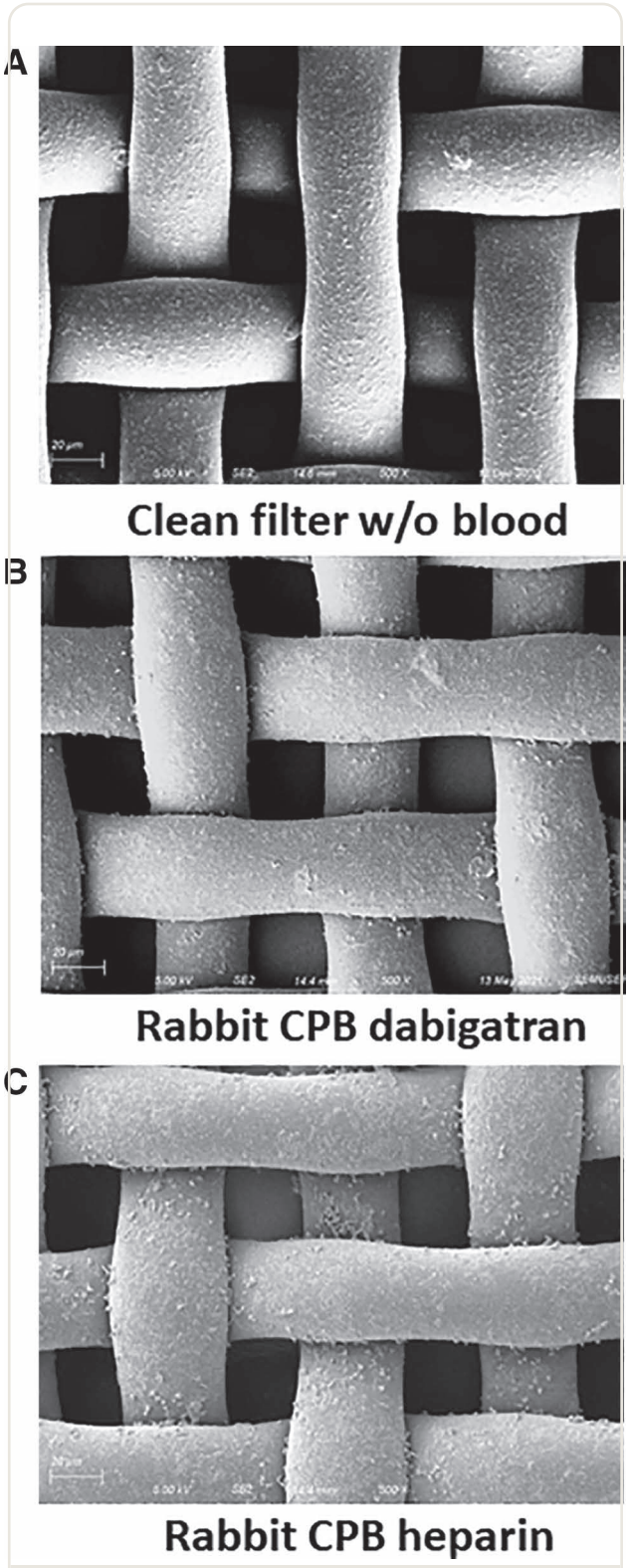

Fig. 3. Representative electron microscopy pictures (×500) of the arterial filter meshes. Pictures were obtained after the following conditions: (*A*) clean filter, (*B*) dabigatran group, and (*C*) heparin group.

Third, we did not use a survival model, so potential adverse effects of bypass using dabigatran could not be assessed. Future studies will have to address organ toxicity and the risk of excess bleeding.

Fourth, neither reaction time nor activated clotting time had concentration/response ranges adequate for use as a point-of-care test to measure the anticoagulant effects of dabigatran group during CPB. Any future clinical use likely mandates the development of an effect measurement method that returns results across the range of dabigatran concentrations from 0 to 100 μg/ml in a few minutes.

Fifth, although the loading dose of dabigatran was effective in achieving the target concentration in all but one rabbit, subsequent measurements demonstrated continual accumulation during CPB (fig. 2). It can be assumed that anesthesia, thoracotomy, and especially CPB had a significant effect on dabigatran clearance, likely through decreased renal clearance and/or decreased hepatic blood flow and drug metabolism. Thus, although all animals finished 120 min of bypass without evidence of thrombus formation, we cannot say that the targeted concentration of 90 μg/ml would have achieved the same result.

Last, although rabbits allowed us to use a commercially available CPB circuit, it was not an ideal model because rabbit thrombin was much more resistant to inhibition than human thrombin. Our Chandler loop experiments showed that rabbit blood required a dabigatran concentration eight times higher than we had found in previous work with human blood.[9] However, in previous studies establishing the rabbit as a useful model for CPB,[24–26] anticoagulation using heparin at doses typical for human CPB was reported to be effective. The impossibility of achieving target activated clotting times despite doses greater than 1,600 units/kg was an unanticipated finding of our study. We assume that this may have been due partially to interspecies differences in anticoagulant sensitivity (as with dabigatran), but also possibly to our use of the cartridge-based activated clotting time, rather than the older tube-based activated clotting time device used in published studies. Given the complete lack of fibrin deposition on the arterial line filters in the heparin group, we conclude that the low activated clotting times were more a measurement phenomenon and did not reflect significant thrombin activation during bypass.

There are also many other significant physiologic differences between healthy rabbits and human patients presenting for cardiac surgery. Cardiac patients often present with significant comorbidities, including renal dysfunction that have important effects on drug distribution and elimination. The pharmacokinetics of both dabigatran and idarucizumab will have to be worked out in this population before human use can be contemplated.

Despite these limitations, we have shown clearly that dabigatran can provide adequate anticoagulation for CPB

in an intact animal. The number of medications that have been found effective in this setting is quite small, and at this point dabigatran is the only drug other than heparin that can be reversed. Although there is much work to be done before we can declare this is a safe and effective drug for human cardiac surgery with CPB, we believe it is an important first step toward a truly workable alternative to heparin anticoagulation. Next steps must include a survival model, tests of organ function to assess toxicity, and the development of protocols to reverse the anticoagulant effects with idarucizumab.

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Competing Interests

Dr. Eaton is a holder of Provisional Patent Application No. 62/814,454. Anticoagulant Compositions and Uses Thereof. The other authors declare no other conflict of interest.

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Supplemental Digital Content

Supplemental Figures and Tables, https://links.lww.com/ALN/D96
Fig. S1. Dabigatran concentrations in rabbit plasma.
Fig. S2. The relationship between reaction time and activated clotted time and dabigatran.
Fig. S3. Hemoglobin concentrations.
Fig. S4. Quality of fit of pharmacokinetic data.
Fig. S5. Experimental scheme.
Fig. S6. Micrographs of rabbit brains and kidneys.
Table S1. Time to clot in Chandler loops.
Table S2. Intersubject pharmacokinetics variability.
Table S3. Individual dabigatran bayesian pharmacokinetics estimates.
Table S4. Targeted dosing regimens.

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