ORIGINAL LABORATORY RESEARCH REPORT

Rivaroxaban Reduces the Dabigatran Dose Required for Anticoagulation During Simulated Cardiopulmonary Bypass

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BACKGROUND: Heparin is the standard anticoagulant for cardiopulmonary bypass (CPB); however, there are problems with its use that make the development of suitable alternatives desirable. Currently, no ideal alternative exists. We have previously reported that the direct thrombin inhibitor dabigatran can prevent coagulation in simulated CPB at high concentrations. These high concentrations may cause difficulties in achieving the reversal of dabigatran with idarucizumab, given the markedly different pharmacokinetics of the 2 drugs. Herein, we test the hypothesis that the addition of the anti-Xa drug rivaroxaban would provide suitable anticoagulation at a lower concentration of dabigatran given likely synergy between the 2 classes of drugs. The primary goal of the study was to investigate whether the addition of rivaroxaban reduces the concentration of dabigatran necessary to allow 2 hours of simulated CPB.

METHODS: The study was performed in sequential steps. Blood collected from consenting healthy donors was used throughout. First, we added graded concentrations of dabigatran and rivaroxaban alone and in combination and assessed inhibition of anticoagulation using thromboelastometry. Using results from this step, combinations of dabigatran and rivaroxaban were tested in both Chandler loop and simulated CPB circuits. Dabigatran and rivaroxaban were added before recalcification, and the circuits were run for 120 minutes. In both models of CPB, 120 minutes of circulation without visible thrombus was considered successful. In the Chandler loop system, idarucizumab was added to reverse anticoagulant effects. In the CPB circuits, the arterial line filters were examined using scanning electron microscope (SEM) to qualitatively assess for fibrin deposition.

RESULTS: In vitro analysis of blood samples treated with dabigatran and rivaroxaban showed that dabigatran and rivaroxaban individually prolonged clotting time (CT) in a dose-dependent manner. However, when combined, the drugs behaved synergistically. In the Chandler loop system, dabigatran 2400 and 4800 ng/mL plus rivaroxaban (150 ng/mL) effectively prevented clot formation and reduced the dynamics of clot propagation for 120 minutes. Idarucizumab (250–1000 µg/mL) effectively reversed anticoagulation. In the CPB circuits, dabigatran (2500 ng/mL) and rivaroxaban (200 ng/mL) were successful in allowing 120 minutes of simulated CPB and prevented fibrin deposition. Biomarkers of coagulation activation did not increase during simulated CPB. Heparin controls performed similarly to dabigatran and rivaroxaban.

CONCLUSIONS: The dual administration of oral anticoagulant drugs (dabigatran and Rivaroxaban) with different pharmacologic mechanisms of action produced synergistic inhibition of coagulation in vitro and successfully prevented clotting during simulated CPB. (Anesth Analg 2022;135:52–9)

KEY POINTS

- **Question:** Do lower concentrations of dabigatran administered with rivaroxaban prevent clotting during simulated cardiopulmonary bypass (CPB)?
- **Findings:** Dabigatran and rivaroxaban in combination produced synergistic inhibition of coagulation in vitro and effectively prevented clot formation during simulated CPB.
- **Meaning:** The combination of dabigatran and rivaroxaban, which inhibit successive steps in the coagulation cascade, may be considered as a potential anticoagulant strategy in a CPB setting.

GLOSSARY

ACT = Activated Clotting Time; **Angle** = speed of clot formation; **CPB** = cardiopulmonary bypass; **CT** = clotting time; **Dab** = dabigatran; **DMSO** = dimethyl sulfoxide; **ELISA** = enzyme-linked immunosorbent assay; **FDA** = Food and Drug Administration; **HIT** = heparin-induced thrombocytopenia; **Ida** = idarucizumab; **IgG** = immunoglobulin G; **LC-MS/MS** = liquid chromatography/mass spectrometry; **MA** = maximum strength of clot; **ND** = not detected; **PF4** = platelet factor 4; **PVC** = polyvinyl chloride; **R** = reaction time; **RapidTEG** = kaolin/tissue-factor-activated thromboelastography; **Riv** = rivaroxaban; **ROTEM** = rotational thromboelastometry; **RSRB** = Research Subjects Review Board; **SD** = standard deviation; **SEM** = scanning electron microscope; **TAT** = thrombin-antithrombin complex; **TEG** = thromboelastography

Since the initial development of cardiopulmonary bypass (CPB), heparin has been the standard anticoagulant. Heparin inhibits multiple steps of the coagulation cascade, which is beneficial due to the suppression of thrombin generation and consumption of coagulation factors. However, heparin is a less than ideal agent for anticoagulation for CPB for multiple reasons. It requires the intrinsic cofactor antithrombin to achieve anticoagulation, which, in some individuals, is not present in adequate quantity. As a family of large molecules derived from another species, it has significant immunogenicity that causes heparin-induced thrombocytopenia (HIT) with disturbing regularity.[1] Currently, no ideal alternative exists for heparin in this setting. The direct thrombin inhibitor bivalirudin is the most studied heparin alternative; however, it inhibits only 1 step in the coagulation cascade[2,3] and has been associated with both thrombotic and bleeding complications.[4] The development of more suitable heparin substitutes would improve the conduct of CPB, particularly in patients for whom heparin is contraindicated.

Dabigatran (Dab) is a direct-acting thrombin inhibitor currently US Food and Drug Administration (FDA)–approved as an oral formulation for thromboprophylaxis in atrial fibrillation and for patients with deep vein thrombosis. Dab is an attractive heparin alternative given the existence of idarucizumab, an effective and specific reversal agent. We have previously shown that Dab can be formulated in a solution suitable for parenteral use and, at high concentrations (≥7500 ng/ml), is an effective and reversible anticoagulant in simulated CPB with human blood.[5] However, at such high concentrations, the reversal of Dab may be problematic in vivo, given the markedly different volumes of distribution of the 2 drugs.[6]

It has been previously shown that direct thrombin inhibitors and anti-Xa agents are synergistic,[7] likely due to inhibition of sequential steps in the coagulation cascade. These synergistic effects between anti-Xa agents and direct thrombin inhibitors may be useful as an anticoagulant paradigm for CPB, providing acceptable anticoagulation at lower (clinically applicable) concentrations of individual drugs, thereby decreasing potential adverse effects. Rivaroxaban (Riv) is an inhibitor of activated factor X (Xa) and is currently FDA-approved as an oral formulation for thromboprophylaxis in atrial fibrillation and for patients with deep vein thrombosis or atherosclerosis. A reversal agent for Riv is also available as the FDA-approved agent andexanet alpha.

The primary goal of this proof-of-concept study was to test the hypothesis that the addition of a small concentration of Riv will significantly decrease the concentration of Dab needed to prevent clotting during simulated CPB using human blood.

METHODS

Subjects

The study was approved by the Institutional Review Board (University of Rochester Research Subjects Review Board [RSRB]), and written informed consent was obtained from all subjects. All aspects of the study were conducted according to ethical principles as set out in the Declaration of Helsinki. Six healthy nonpregnant donors 21 to 55 years old were recruited to provide blood. Subjects were excluded for recent infections, disorders of the immune system, hematological and clotting disorders, malignancies, and use of medications or herbal supplements that affect coagulation. Standard phlebotomy procedures performed by trained personnel were used to collect ≤150-mL whole blood per week. The collected blood was anticoagulated with 0.109-M Na-citrate and tested within 4 hours.

Establishing Synergy Between Rivaroxaban and Dabigatran

Dab (CLEARSYNTH) was initially dissolved in 0.1-M HCL to 16.6 mg/mL, followed by diluting to 120 µg/mL, using 50% dimethyl sulfoxide (DMSO). Six stock solutions were prepared: 120, 60, 30, 15, 7.5, and 3.75 µg/mL. Five-µL Dab from the corresponding stock solutions and 5 µL of solvent (50% DMSO) were added into 990 µL of citrated blood to create the final concentrations of Dab: 18.75, 37.5, 75, 150, 300, and 600 ng/mL. Riv (CLEARSYNTH) was dissolved in 100% DMSO and then diluted to 120 µg/mL using 50% DMSO in water. Six stock solutions of the drug were prepared: 120, 60, 30, 15, 7.5, and 3.75 µg/mL. Final Riv concentrations in blood were 18.75, 37.5, 75, 150, 300, and 600 ng/mL. For the initial step, several combinations of Dab and Riv were assessed using tissue factor-phospholipid stimulated thromboelastometry (EXTEM)[8] and kaolin/tissue-factor-activated thromboelastography (RapidTEG).[5] Dab (5 µL) and Riv (5 µL) from the corresponding stock solutions

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were added into 990-µL citrated blood to create the following concentrations: Dab 600 + Riv 18.75 (ng/mL); Dab 300 + Riv 37.5 (ng/mL); Dab 150 + Riv 75 (ng/mL); Dab 75 + Riv 150 (ng/mL); Dab 37.5 + Riv 300 (ng/mL); and Dab 18.75 + Riv 600 (ng/mL). For the control, 10 µL of solvent (50% DMSO) was added into 990 µL of the citrated blood. To test the interaction of Dab and Riv, 3 additional experimental combinations were added to the data matrix: Dab 600 + Riv 150 (ng/mL), Dab 300 + Riv 300 (ng/mL), and Dab 600 + Riv 600 (ng/mL).

A rotational thromboelastometry (ROTEM) delta (Instrumentation Laboratory) was maintained and calibrated as per manufacturer recommendation and was used for analysis. For the EXTEM test, 20-µL start-tem solution and 20-µL EXTEM solution were mixed with 300 µL of anticoagulant/blood. Reaction was monitored until the clotting time (CT) was established. All measurements were performed in duplicate.

RapidTEG measurements were performed as previously published.[5] In brief, 340 µL of blood was mixed with 10 µL of RapidTEG reagents and 20 µL of 0.2-M $CaCl_2$ and placed in a Thromboelastograph Analyzer 5000 (Haemoscope Corp). Reaction was monitored until reaction time (R), speed of clot formation (Angle), and maximum strength of clot (MA) were established.

Chandler Loop

The Chandler loop system is a revolving loop of polyvinyl chloride (PVC) tubing representing a simple bypass model. All experiments were run as previously described.[5] Briefly, Chandler loops were primed with a mixture of human whole blood plus PlasmaLyte A at 2:1 ratio. The Chandler loops were mounted in the apparatus (Ebo Kunze) and were circulated in a 37-°C water bath rotated at 6 revolutions per minute. Dab (2400 ng/mL) and Riv (150 ng/mL) were added 5 minutes before recalcification with $CaCl_2$ (5 mM). Chandler loops were run for 120 minutes or until thrombus was visible in the tubing. RapidTEG parameters (R, Angle, and MA) were recorded and analyzed at baseline (no drugs), 1 minute, 30 minutes, 60 minutes, and 120 minutes on drug administration. At the end of 120 minutes, idarucizumab (250 µM every 5 minutes) was added to reverse anticoagulative effects.

Dab and Riv concentrations were verified using liquid chromatography/mass spectrometry (LC-MS/MS) with a Dionex Ultimate 3000 UHPLC coupled to a Q Exactive Plus mass spectrometer (Thermo Scientific). Analytes were separated on a Waters Acuity UPLC BEH 2.1- × 50-mm Phenyl column, with 1.7 µm beads. The column was protected by a Waters Acquity UPLC BEH Phenyl Vanguard Pre-Column. LC-MS/MS quantitation was performed with the LC Quan node of the XCalibur software (Thermo Fisher), using fragment ion 289.10 m/z for Dab and 144.94 m/z for Riv. Peak areas were calculated by the software using a 10-ppm tolerance for the fragment ions. The mobile phases were A: 2-mM ammonium acetate in 0.1% formic acid, and B: 2-mM ammonium acetate in 95% methanol with 0.1% formic acid. The flow rate was set to 400 µL/min, and the column oven was set to 50 °C. Apixaban was used as an internal control.[9]

Cardiopulmonary Bypass

All CPB experiments were performed as previously described.[5] Briefly, a mixture of 115 mL of citrated human blood, 15 mL of 25% Human Albumin (Baxalta US Inc), and 20 mL of PlasmaLyte-A (Baxter Healthcare Corp) was circulated in a commercially available circuit consisting of 3/16″ (arterial) and 1/4″ (venous) PVC tubing, a Capiox FX 05 Baby-Fx oxygenator with biopassive coating (Xcoating, Terumo Cardiovascular systems) with integrated arterial filter, and a Hard shell reservoir. The circuit was set up in a CPB pump (COBE Cardiovascular, Inc). Six experiments were performed using concentrations of Dab (2500 ng/mL) and Riv (200 ng/mL) based on data from the Chandler loops, and 6 additional simulated CPB runs were also completed using heparin (3 U/mL) as an active control. Anticoagulants were administrated 5 minutes before recalcification with $CaCl_2$ (5 mM). Blood gases, electrolytes, ACT (Hemochron Signature Elite, Accriva Diagnostics, Inc) thromboelastography (TEG), and 4 plasma biomarkers of coagulation activation (Human Prothrombin Fragment 1 + 2 [F1 + 2], enzyme-linked immunosorbent assay [ELISA] HU8571, Biotang Inc), Fibrinogen (ELISA KA4794, Abnova), Human Fibrinogen Degradation Products (ELISA HU8475, Biotang Inc), and Human Thrombin-Antithrombin Complex (TAT, ELISA ab108907, Abcam) were measured in the blood samples obtained at 1, 30, 60, and 120 minutes of CPB. At the end of each CPB run, 2 2.5-cm diameter sections of the arterial filter were cut out and fixed in 2.5% glutaraldehyde, followed by dehydration in pure ethanol and incubation in 100% hexamethyldisilazane overnight. The filter sections were examined using a scanning electron microscope (SEM) at 500× power.

Statistical Analysis

The primary outcome of the study was to determine if the combination of Dab and Riv would maintain the fluidity of human blood for at least 120 minutes of simulated CPB.

For the in vitro experiments, CT values were measured. The repeated measures mixed model was used to study the synergistic effect of the combination of Dab and Riv on the prolongation of CT values in Table 1 and Figure 1. The repeated measure mixed

Table 1. Effects of Dab and Riv on the CT

Dab + Riv (ng/mL)	CT, seconds
Dab 600 + Riv 18.75	1095.5 ± 451.2
Dab 300 + Riv 37.5	835.0 ± 379.2
Dab 150 + Riv 75	744.5 ± 475.0
Dab 75 + Riv 150	738.3 ± 513.8
Dab 37.5 + Riv 300	558.7 ± 108.4
Dab 18.75 + Riv 600	681.1 ± 185.4

CT values are presented in Figure 1. Data are shown as mean ± SD; n = 6 individual subjects (blood samples obtained from 6 healthy donors) are used for each group. The repeated measures mixed model shows highly significant synergistic effect of the combination of Dab and Riv on the prolongation of CT values. See Supplemental Digital Content, Table S3 (http://links.lww.com/AA/D924) for more details of model fitting.

Abbreviations: CT, clotting time; Dab, dabigatran; Riv, rivaroxaban; SD, standard deviation.

model was also used to study the time effect from baseline "No Drug" to 120 minutes on each outcome in Table 2 and Supplemental Digital Content, Table S1 (http://links.lww.com/AA/D924). Wilcoxon signed-rank test was used to compare the clotting time values in Table 3. Statistical analyses were implemented with SAS 9.4 (SAS Institute Inc). The significance level was set at 0.05 for each analysis. Calculation of sample size was not performed due to the exploratory nature of this proof-of-concept study.

RESULTS

Synergistic Effect of Dabigatran and Rivaroxaban

Both Dab and Riv significantly increased CT in a dose-dependent fashion, demonstrating similar effects at doses ≥300 ng/mL (Figure 1, Table 1, and Supplemental Digital Content, Table S2, http://links.lww.com/AA/D924). At the maximal single dose of 600 ng/mL, Dab and Riv increased CT to 506 ± 197 and 431 ± 158 seconds, respectively (Figure 1). Then, we combined Dab and Riv, measured CT (Figure 1, Table 1, and Supplemental Digital Content, Table S2, http://links.lww.com/AA/D924), and applied the repeated measures mixed model to evaluate supraadditive (synergistic) effects. In this model, the outcome variable was the clotting time (CT); the independent variables included the concentrations of Dab, Riv, and their interaction. The predictive model of clotting time based on the model fitting is

$$CT = 513.8 + 1.067 \times 10^{-2} \times Dab + 1.552 \times 10^{-3} \times Riv + 6.004 \times 10^{-5} \times Dab \times Riv$$

The standard errors and *P* values of all estimated coefficients are incorporated in Supplemental Digital Content, Table S3 (http://links.lww.com/AA/D924). Since the coefficient of the interaction was positive and highly significant, the combination of Dab and Riv showed highly synergistic effect in prolonging clotting time. As anticipated, the clotting time increased with the concentrations of both Dab and Riv (Supplemental Digital Content, Table S2, http://links.lww.com/AA/D924).

Dab and Riv effects were also measured using RapidTEG platform (Supplemental Digital Content, Figure S1, http://links.lww.com/AA/D924). R measured by RapidTEG exhibited similar trends to ROTEM, although the magnitude of the effects was much lower compared to ROTEM. Due to its great sensitivity, ROTEM was not practical to measure the anticoagulant effects of Dab at high concentrations (>2400 ng/mL). Similarly, ACT values were out of range for the Dab doses higher than 600 ng/mL. Therefore, RapidTEG was an optimal and reliable

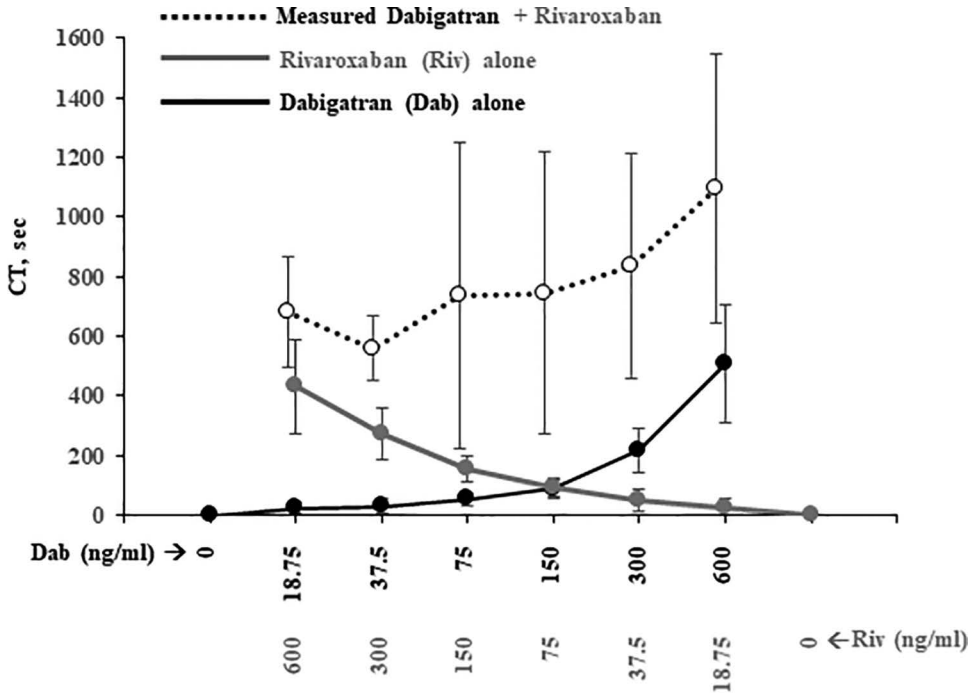

Figure 1. CT measured by ROTEM. Black circles with the solid line represent data where Dab alone was added into the whole blood. Gray circles with the solid line represent data, where Riv alone was added to whole blood. White circles with the dotted black line represent data where the combinations of Dab and Riv were added into the whole blood. Data are shown as mean ± SD, n = 6 individual subjects. Repeated measures mixed model was applied to evaluate supraadditive (synergistic) effects of the combinations of Dab and Riv (see model fitting results in Results and in Supplemental Digital Content, Table S3, http://links.lww.com/AA/D924). CT indicates clotting time; Dab, dabigatran; Riv, rivaroxaban; ROTEM, rotational thromboelastometry; SD, standard deviation.

Table 2. RapidTEG Parameters and Drugs Levels Measured During 120 Minutes in Chandler Loop System With Dab 2400 ng/mL and Riv 150 ng/mL

TEG variable/measured drug concentration	Dab 2400 ng/mL + Riv 150 ng/mL (with Ca 5 mmol/L added)				
	No drug	1 min	30 min	60 min	120 min
R, min	0.6 ± 0.05	72.9 ± 10.69	69.5 ± 11.66	65.2 ± 16.05	46.5 ± 8.81
Angle, °	67.4 ± 5.3	2.0 ± 0.62	3.2 ± 1.42	2.8 ± 1.2	5.2 ± 2.75
MA, mm	55.0 ± 7.86	21.3 ± 7.64	20.0 ± 7.71	27.9 ± 8.44	32.7 ± 12.56
Dab, ng/mL (MS)	ND	2302 ± 487	2305 ± 455	2287 ± 305	2230 ± 695
Riv, ng/mL (MS)	ND	136 ± 34	137 ± 30	136 ± 21	124 ± 8

R, Angle, and MA were measured by RapidTEG. Drugs concentrations in the blood samples were measured by LCMS. Statistical analysis was performed using the repeated measure mixed model. *P* values for the various comparisons are presented in Supplemental Digital Content, Table S4 (http://links.lww.com/AA/D924).

Abbreviations: Dab, dabigatran; ND, not detected; MA, maximum strength of clot; MS, mass spectrometry; R, reaction time; RapidTEG, kaolin/tissue-factor-activated thromboelastography; Riv, rivaroxaban.

Table 3. Effects of Dab vs Dab + Riv on Clot Formation in Chandler Loop System

	Time until visible clot formation (in min)					
Dab + Riv	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Riv 150	22	25	25	15	26	16
Dab 2400 + Riv	>120	>120	>120	>120	>120	110

All concentrations are listed in ng/mL. Wilcoxon signed-rank test was used to compare the median clotting time of Dab + Riv with that of Riv 150 ng/mL alone.

Abbreviations: Dab, dabigatran; Riv, rivaroxaban.

platform to measure the anticoagulant effect of high Dab doses used in the Chandler loop and simulated CPB models.

Chandler Loop

The addition of Riv (150 ng/mL) to Dab (2400 ng/mL) prevented clot formation in 5 out of 6 volunteers (Table 3), while Dab alone (at 2400 ng/mL) did not prevent clot formation for 120 minutes. The combination of Dab and Riv significantly reduced the clot strength (MA) and the dynamics of clot propagation (Angle; Table 2 and Figure 2A). Interestingly, the anticoagulant effects of the drugs gradually declined toward the end of 120 minutes, despite the fact that the drug concentrations remained stable throughout the whole run (Table 2).

Idarucizumab added after 120 minutes in up to four 250-µg/mL stepwise (250 µg/mL every 5 minutes) reversed anticoagulation, initiating clot formation in the loops (the earliest at 4 minutes, the latest at 20 minutes; Figure 2B). Importantly, Riv reversal was not required to block anticoagulation.

CPB Simulation

To achieve full anticoagulant efficacy (in 6 out of 6 volunteers) for the CPB settings, Dab and Riv concentrations were increased up to 2500 and 200 ng/mL accordingly. Although Dab alone at this concentration showed extensive fibrin deposition (Figure 3A), in combination with Riv, all simulated CPB runs were completed with no evidence of gross thrombus for 120 minutes. No fibrin deposition on the filters was seen on the electron micrographs (Figure 3B). The values of R, measured by TEG, were significantly elevated after the addition of the drugs, while Angle and MA were depressed, indicating reduced clot propagation and strength (Supplemental Digital Content, Table S1, http://links.lww.com/AA/D924). Blood gases and electrolytes were stable during 120 minutes of the CPB experiment (Supplemental Digital Content, Table S1, http://links.lww.com/AA/D924). Concentrations of plasma F1 + 2, fibrinogen, and thrombin/antithrombin during CPB (at 1, 30, 60, and 120 minutes) were not different compared to baseline (no drugs; Supplemental Digital Content, Figure S2, http://links.lww.com/AA/D924). Fibrinogen degradation products were undetectable.

Heparin (3 U/mL), used as a positive control, prevented clot formation and fibrin deposition in all 6 volunteers (Figure 3C). Plasma levels of F1 + 2 and fibrinogen were not different from baseline (Supplemental Digital Content, Figure S2, http://links.lww.com/AA/D924), exhibiting similar trends observed in the Dab/Riv-treated groups. However, in the Heparin-treated group, the levels of thrombin/antithrombin significantly declined at 1, 30, 60, and 120 minutes versus baseline.

DISCUSSION

This study demonstrates that Riv and Dab have supraadditive (synergistic) effects on coagulation as measured by thromboelastometry and that this synergy may be used to reduce the concentration of Dab that provides acceptable anticoagulation for 2 in vitro models of simulated CPB using human blood. This represents a step toward the development of an ideal anticoagulant alternative to heparin in patients for whom heparin is contraindicated.

The ideal anticoagulant agent for CPB should:

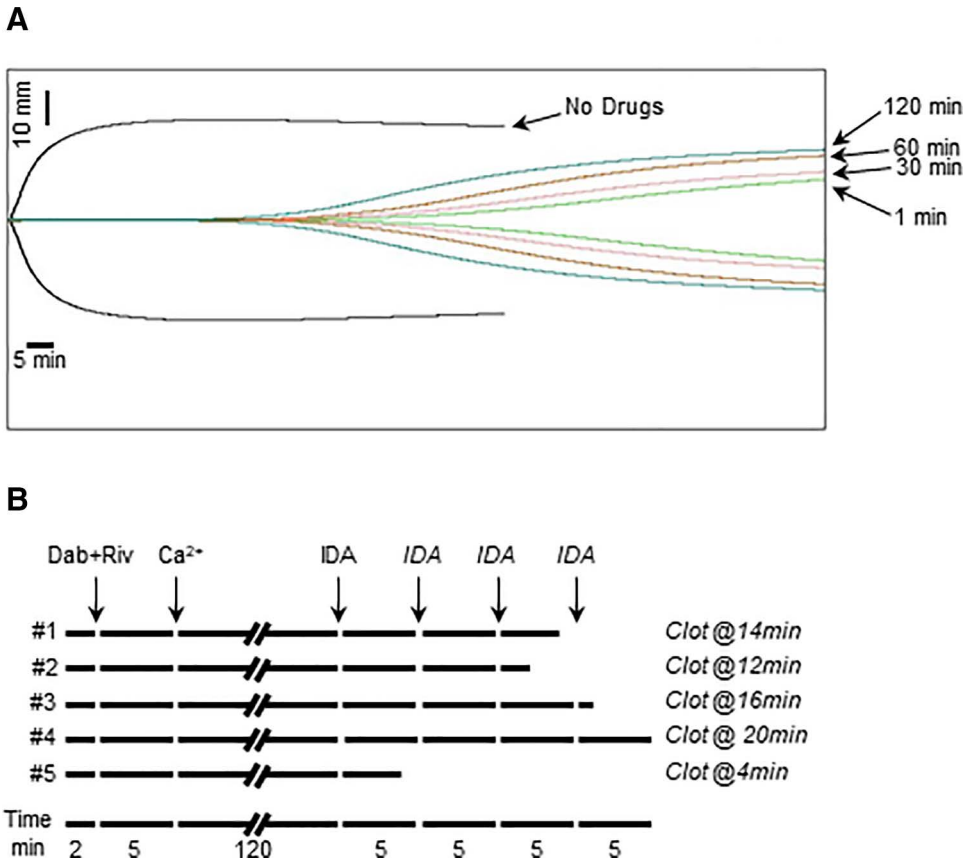

Figure 2. Representative RapidTEG traces and a scheme for Ida administrations. A, Representative traces recorded during the Chandler loop experiments with Dab (2400 ng/mL) + Riv (150 ng/mL). The following time points are shown: "No Drug," 1 minute, 30 minutes, 60 minutes, and 120 minutes. B, Ida (250 μM, Ida) was added at the end (120 minutes) of a Chandler loop run. If a clot was not formed during 5 minutes, the next bolus of Ida (250 μM) was administrated. Ida (250 μM) was administrated every 5 minutes until a clot was detected in the loop. Dab indicates dabigatran; Ida, idarucizumab; RapidTEG, kaolin/tissue-factor-activated thromboelastography; Riv, rivaroxaban.

- 1. completely inhibit coagulation during CPB
- 2. act directly, that is, without the need for intrinsic cofactors
- 3. lack serious adverse hemodynamic effects
- 4. lack immunogenic potential
- 5. have effects on coagulation that are easily measured using point-of-care testing
- 6. either have an ultrashort half-life, or be rapidly and completely reversible with a nontoxic agent.

Heparin meets some of these criteria and has been successfully used for CPB for decades. However, its effects are dependent on adequate circulating concentrations of antithrombin, which are not always present in patients requiring CPB, and more seriously, the antigenicity of heparin results in the development of HIT with a prevalence of 0.1% to 5%. HIT is a serious antibody-mediated reaction characterized by platelet-activating immunoglobulin G (IgG) that recognizes platelet factor 4 (PF4)/heparin complexes. HIT antibodies (antiheparin/PF4) develop in up to 60% of cardiac surgical patients.[10] While the incidence of frank HIT is significantly lower at 1% to 2%,[11] the presence of antibodies alone is associated with adverse outcomes,[12] and HIT may increase mortality by 50%.[13]

The direct thrombin inhibitor bivalirudin has been used successfully for CPB[4] and is currently recommended as an alternative to heparin for patients with HIT in available guidelines.[14,15] However, bivalirudin inhibits only one step in the coagulation cascade, has no reversal agent, has a prolonged duration of effect in patients with decreased kidney function, and necessitates significant changes in the conduct of CPB and cardiac surgery when it is used in this setting.

We have previously shown that Dab is an effective anticoagulant for simulated CPB using human blood.[5] As Dab may be reversed with the monoclonal antibody idarucizumab, it meets all of the above requirements for an ideal CPB anticoagulant, and at least in vitro performs as well as standard heparin. However, while Dab distributes a large volume approximating the extracellular compartment, idarucizumab distributes only to the intravascular space,[16] complicating reversal, especially of the high concentrations of Dab needed when used alone. The synergistic action of Riv with Dab allows CPB to proceed with a 4-fold reduction in Dab, potentially making reversal a more realistic possibility. Riv is itself reversible with andexanet alpha (recombinant coagulation factor Xa, inactivated), theoretically allowing restoration of normal hemostasis using both reversal agents. In addition, as Riv inhibits activated factor X, which precedes thrombin in the coagulation cascade, the combination may be superior to Dab alone in achieving the first

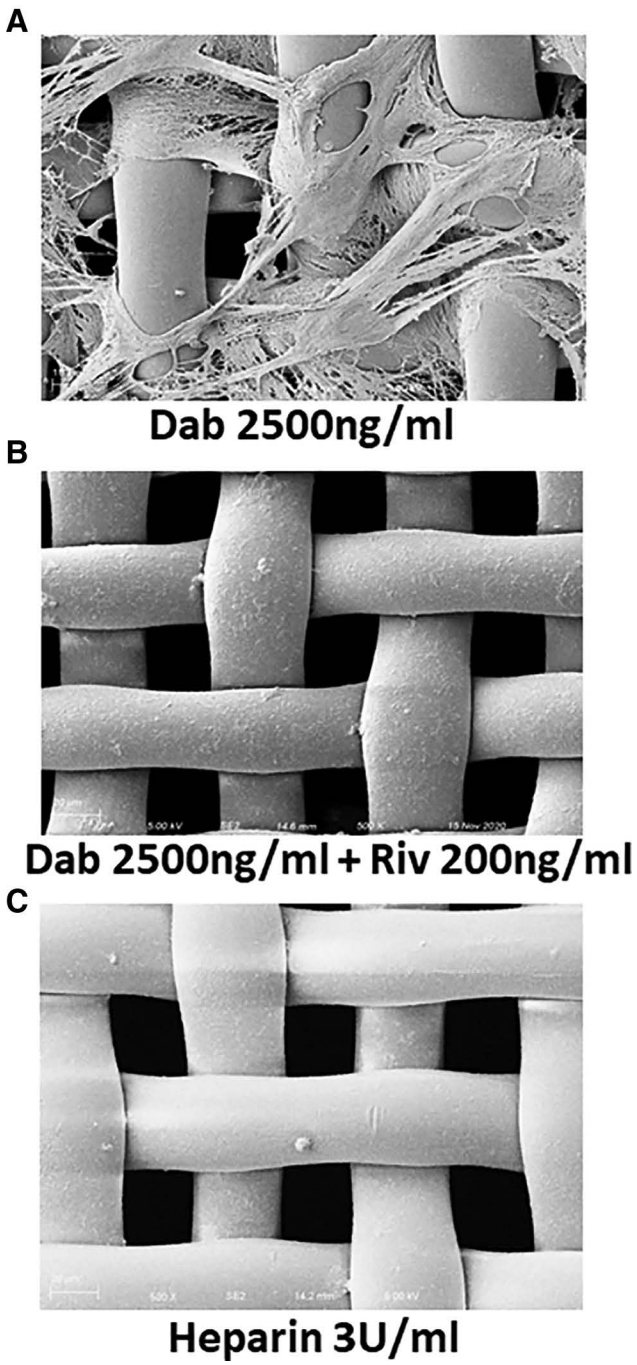

Figure 3. Representative electron microscopy pictures (×500) of the arterial filter meshes. Pictures were obtained after the following experimental conditions: (A) Dab (2500 ng/mL), (B) Dab (2500 ng/mL) + Riv (200 ng/mL), and (C) Heparin (3 U/mL). Dab indicates dabigatran; Riv, rivaroxaban.

requirement of the ideal anticoagulant. It should be noted that our study does not provide data to support any of these 3 conjectures, but only lays the groundwork for further study.

This study has many limitations. As an in vitro study, it cannot provide data on the effects of an intact organism on the pharmacokinetics or pharmacodynamics of the 2 anticoagulants used.

The drugs may have adverse effects in vivo as well, either alone or in combination. While the Dab and Riv we used are structurally identical to the active forms ultimately effective in plasma of the FDA-approved oral medications, giving these drugs intravenously is quite different from giving an oral preparation. We have established only efficacy of our dosing forms and route. As established, safety profiles of the approved products are not relevant to our proposed use, and further development toward clinical use must include toxicity assessment, particularly as there is some evidence that both Riv and Dab in high concentration may be associated with hepatic toxicity.[17,18]

While our drug combinations fulfill most of the requirements for an ideal anticoagulant for bypass, so far, we have not found a point-of-care test that would be realistically useful. While we are able to show measurable results with the RapidTEG R time, as opposed to the ACT, a test that takes up to an hour to result is not useful in clinical practice.

The anticoagulation effects of Dab and Riv in the blood samples obtained from the healthy individuals do not necessarily reflect the complex balance of pre- and anticoagulant factors of cardiovascular patients, and additional studies will be required to assess the effects of our drugs combinations in this population, particularly in patients with HIT.

As this is an exploratory proof-of-concept study, we did not perform the sample size calculation. One limitation of the data analysis is the normality assumption. Due the small sample size, it is infeasible to test the assumption based on the data. Hopefully, our result can be used as the basis for a larger study to find the deterministic synergistic effects of the combination of Dab and Riv.

Overall, acknowledging all limitations mentioned above, our study demonstrates that the combination of Dab and Riv provides acceptable anticoagulation in simulated CPB. The next steps will be to test this combination of anticoagulants and reversal agents in an intact animal model, assessing both toxicity and efficacy as an anticoagulant-reversal combination. ■

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DISCLOSURES

Name: Sergiy M. Nadtochiy, PhD.
Contribution: This author helped design the study, record and analyze the data, write significant portions of the manuscript, and review and approve the final manuscript.
Conflicts of Interest: None.

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Contribution: This author helped perform significant portions of lab procedures, write portions of the manuscript, and review and approve the final manuscript.
Conflicts of Interest: None.
Name: Ronald E. Angona, MS, CCP.
Contribution: This author helped design the CPB apparatus, operate CPB simulations, and review and approve the final manuscript.
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REFERENCES

1. Lee GM, Arepally GM. Heparin-induced thrombocytopenia. *Hematology Am Soc Hematol Educ Program.* 2013;2013:668–674.
2. Koster A, Dyke CM, Aldea G, et al. Bivalirudin during cardiopulmonary bypass in patients with previous or acute heparin-induced thrombocytopenia and heparin antibodies: results of the CHOOSE-ON trial. *Ann Thorac Surg.* 2007;83:572–577.
3. Veale JJ, McCarthy HM, Palmer G, Dyke CM. Use of bivalirudin as an anticoagulant during cardiopulmonary bypass. *J Extra Corpor Technol.* 2005;37:296–302.
4. Dyke CM, Smedira NG, Koster A, et al. A comparison of bivalirudin to heparin with protamine reversal in patients undergoing cardiac surgery with cardiopulmonary bypass: the EVOLUTION-ON study. *J Thorac Cardiovasc Surg.* 2006;131:533–539.
5. Nadtochiy SM, Baldzizhar A, Stefanos T, et al. High-dose dabigatran is an effective anticoagulant for simulated cardiopulmonary bypass using human blood. *Anesth Analg.* 2021;132:566–574.
6. Hegemann I, Ganter C, Widmer CC, Becker M, Müller D, Spahn DR. Ongoing redistribution of dabigatran necessitates repetitive application of idarucizumab. *Br J Anaesth.* 2018;121:505–508.
7. Giardino EC, Haertlein BJ, de Garavilla L, et al. Cooperative antithrombotic effect from the simultaneous inhibition of thrombin and factor Xa. *Blood Coagul Fibrinolysis.* 2010;21:128–134.
8. Korpallová B, Samoš M, Bolek T, et al. Role of thromboelastography and rotational thromboelastometry in the management of cardiovascular diseases. *Clin Appl Thromb Hemost.* 2018;24:1199–1207.
9. Schmitz E, Boonen K, Van Den Heuval D, et al. Determination of dabigatran, rivaroxaban and apixaban by ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulants. *J Thromb Hemost*. 2014;12:1636–1646.
10. Visentin GP, Malik M, Cyganiak KA, Aster RH. Patients treated with unfractionated heparin during open heart surgery are at high risk to form antibodies reactive with heparin:platelet factor 4 complexes. *J Lab Clin Med.* 1996;128:376–383.
11. Shore-Lesserson L, Baker RA, Ferraris VA, et al. The Society of Thoracic Surgeons, the Society of Cardiovascular Anesthesiologists, and the American Society of ExtraCorporeal Technology: clinical practice guidelines-anticoagulation during cardiopulmonary bypass. *Anesth Analg.* 2018;126:413–424.
12. Bennett-Guerrero E, Slaughter TF, White WD, et al. Preoperative anti-PF4/heparin antibody level predicts adverse outcome after cardiac surgery. *J Thorac Cardiovasc Surg.* 2005;130:1567–1572.
13. Seigerman M, Cavallaro P, Itagaki S, Chung I, Chikwe J. Incidence and outcomes of heparin-induced thrombocytopenia in patients undergoing cardiac surgery in North America: an analysis of the nationwide inpatient sample. *J Cardiothorac Vasc Anesth.* 2014;28:98–102.
14. Linkins LA, Dans AL, Moores LK, et al. Treatment and prevention of heparin-induced thrombocytopenia: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* 2012;141:e495S–e530S.
15. Shore-Lesserson L, Baker RA, Ferraris V, et al. STS/SCA/AmSECT clinical practice guidelines: anticoagulation during cardiopulmonary bypass. *J Extra Corpor Technol.* 2018;50:5–18.
16. Eikelboom JW, Quinlan DJ, van Ryn J, Weitz JI. Idarucizumab: the antidote for reversal of dabigatran. *Circulation.* 2015;132:2412–2422.
17. Guglielmo N, Orso D, Mestroni R, Montanari G. A case of liver failure due to dabigatran treated with venovenous hemodiafiltration and idarucizumab. *Curr Drug Saf.* 2020;15:227–230.
18. Licata A, Puccia F, Lombardo V, et al. Rivaroxaban-induced hepatotoxicity: review of the literature and report of new cases. *Eur J Gastroenterol*. 2018;30:226–223.