Dabigatran is Less Effective Than Warfarin at Attenuating Mechanical Heart Valve-Induced Thrombin Generation

Iqbal H. Jaffer, MBBS; Alan R. Stafford, MSc; James C. Fredenburgh, PhD; Richard P. Whitlock, MD, PhD; Noel C. Chan, MBBS; Jeffrey I. Weitz, MD, FAHA

Background—Patients with mechanical heart valves (MHV) require warfarin to prevent thromboembolism. Although dabigatran was as effective as warfarin for stroke prevention in atrial fibrillation when compared with warfarin in patients with MHV, the study was stopped early because of more strokes and bleeding with dabigatran. To determine why dabigatran was less effective than warfarin, we compared their effects on thrombin generation induced by MHV.

Methods and Results—Thrombin generation in the absence or presence of valve leaflets or sewing ring segments (SRS) was quantified. Studies were done in control plasma, plasma depleted of factors (F) XII, XI, or VII, plasma containing varying concentrations of dabigatran, or plasma from patients on dabigatran or warfarin with varying dabigatran concentrations or international normalized ratio (INR) values. Mean endogenous thrombin potential (ETP) increased 1.2-, 1.5-, and 1.8-fold in the presence of leaflets, Teflon SRS, and Dacron SRS, respectively. Whereas ETP in FVII-depleted and control plasma was similar, ETP was reduced to background levels in FXII-depleted plasma and abrogated in FXI-depleted plasma. Dabigatran had little effect on ETP at concentrations below 400 ng/mL, whereas in plasma from warfarin-treated patients, ETP was suppressed with INR values over 1.5.

Conclusions—MHV induce thrombin generation via the intrinsic pathway and generate sufficient thrombin to overwhelm clinically relevant dabigatran concentrations. In contrast, warfarin is more effective than dabigatran at suppressing MHV-induced thrombin generation. These data explain why dabigatran failed in MHV patients and suggest that strategies targeting FXII or FXI may suppress the root cause of thrombosis in such patients. (J Am Heart Assoc. 2015;4:e002322 doi: 10.1161/JAHA.115.002322)

Key Words: anticoagulants • coagulation • thrombin • valves

Patients with mechanical heart valves (MHV) require lifelong anticoagulation to prevent thromboembolic events.1,2 Traditionally, warfarin has been the anticoagulant of choice in such patients. By decreasing the functional levels of factors (F) II, VII, IX, and X, warfarin attenuates the extrinsic, intrinsic, and common pathways of blood coagulation.3 In patients with MHV, warfarin is dose-adjusted to maintain the international normalized ratio (INR) between 2 and 3.5, depending on the type of valve, its anatomical location, and the presence of patient factors associated with an increased risk of thromboembolism.2,4

Although effective, warfarin is burdensome for patients and physicians because it requires frequent coagulation monitoring and dose adjustments, reflecting, at least in part, numerous food- and drug-drug interactions.3 In addition, there is a risk of serious bleeding with warfarin, including intracranial bleeding, which can occur even when the INR is within the therapeutic range.5 The limitations of warfarin prompted the development of non-vitamin K antagonist oral anticoagulants (NOACs) that can be given in fixed doses without routine coagulation monitoring. One such agent is dabigatran etexilate, an oral thrombin inhibitor, which is licensed for stroke prevention in patients with atrial fibrillation,6 and for treatment of venous thromboembolism.7 At a dose of 150 mg twice daily, dabigatran was superior to warfarin for reduction in the risk of stroke or systemic embolism in patients with atrial fibrillation, and was associated with significantly less intracranial bleeding.6

From the Thrombosis and Atherosclerosis Research Institute (I.H.J., A.R.S., J.C.F., J.I.W.), Population Health Research Institute, (R.P.W., N.C.C.), and Departments of Surgery (I.H.J., R.P.W.), Medical Sciences (I.H.J., J.I.W.), Medicine (A.R.S., J.C.F., J.I.W.), Biochemistry and Biomedical Sciences (J.I.W.), McMaster University, Hamilton, Ontario, Canada.

Correspondence to: Jeffrey I. Weitz, MD, FAHA, Thrombosis and Atherosclerosis Research Institute, 237 Barton Street East, Hamilton, Ontario, Canada L8L 2X2. E-mail: weitzj@taari.ca

Received July 8, 2015; accepted August 3, 2015.

© 2015 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley Blackwell. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
Recently, dabigatran was compared with warfarin in 2 strata of MHV patients in the Randomized, Phase II Study to Evaluate the Safety and Pharmacokinetics of Oral Dabigatran Etxilate in Patients after Heart Valve Replacement (REALIGN); those with newly implanted valves and those with valves implanted more than 3 months prior to randomization. In both strata, the dose of dabigatran could be increased from 150 to 300 mg twice daily in order to maintain trough drug levels above 50 ng/mL. The study was stopped early because there were 9 strokes in the dabigatran group and none in the warfarin group. Furthermore, in patients with newly implanted valves, there was a trend for more pericardial bleeding with dabigatran than with warfarin; a finding that precluded dabigatran dose escalation. The results of this study prompted a black box warning advising against the use of dabigatran in patients with MHV.

The explanation for the failure of dabigatran in patients with MHV remains elusive. We hypothesized that like other blood-contacting medical devices, MHV initiate clotting by activating FXII and the intrinsic pathway, thereby triggering the local generation of thrombin in concentrations that exceed those of dabigatran. Contemporary bileaflet MHV consist of 2 semicircular leaflets that rotate about struts attached to a valve housing covered with Dacron (polyethylene terephthalate) or Teflon (polytetrafluoroethylene) to form a sewing ring that enables surgical implantation. Thrombosis often starts on the sewing ring, but can also involve the leaflets. To test the hypothesis that MHV are prothrombotic because they activate FXII, we first determined whether leaflets or sewing ring segments (SRS) from bileaflet MHV promote thrombin generation, and examined the extent to which corn trypsin inhibitor (CTI), a specific inhibitor of FXIIa, or depletion of FXII, FXI, or FVII attenuate this phenomenon. To understand why dabigatran failed in REALIGN, we then compared the capacities of dabigatran and warfarin to attenuate leaflet- or SRS-induced thrombin generation.

Materials and Methods

Materials

FVII-, FXI-, and FXII-depleted plasma and anti-FXII antibody were from Affinity Biologics (Ancaster, ON). CTI and human FXII and FXI were from Enzyme Research Laboratories Inc (South Bend, IN). The thrombin-directed fluorescent substrate, Z-Gly-Gly-Arg-AMC, was from Bachem (Bubendorf, Switzerland), and Technothrombin TGA CAL SET, the thrombin generation calibrator set, was from Technoclone (Vienna, Austria). Valve leaflets from 19 mm Carbomedics MHV and Dacron vascular conduits were generously provided by Mr. S. Murphy of Sorin Group (Markham, ON), whereas Teflon vascular conduits were kindly provided by Hamilton Health Sciences (Hamilton, ON). Dabigatran, the active form of the prodrug dabigatran etexilate, was a gift from Dr. J. van Ryn (Boehringer-Ingelheim, Biberach, Germany).

Plasma

Thrombin generation assays were performed in platelet-poor plasma. To create a pool of control plasma, 90 mL of blood was collected from the antecubital veins of each of 11 healthy volunteers into 10 mL of 3.8% sodium citrate. After twice subjecting the blood to centrifugation at 3000g for 20 minutes at 4°C, plasma was harvested, pooled, and stored in 3-mL aliquots at −80°C until used. The protocol was approved by the Research Ethics Committee at McMaster University, and informed written consent was obtained from all volunteers.

Citrated plasma samples from patients taking warfarin were obtained from the Coagulation Laboratory at Hamilton Health Sciences. Although the INR was provided, no patient identifiers or clinical information was included, thereby obviating the need to obtain consent. Individual patient samples were stored in 3-mL aliquots at −80°C until used.

Dabigatran

Dabigatran was dissolved in dimethyl sulfoxide (DMSO) and diluted to 60 μg/mL. After addition to control plasma to 1200 ng/mL, samples were further diluted with control plasma to obtain final dabigatran concentrations ranging from 50 to 800 ng/mL; concentrations that span the trough and peak levels measured in patients taking dabigatran etexilate in doses of 150 to 300 mg twice daily. Final DMSO concentrations were 1% or less; levels that in pilot experiments had no effect on thrombin generation.

Plasma from atrial fibrillation patients receiving dabigatran etexilate at a dose of 110 or 150 mg twice daily were systematically collected at peak and trough as previously described. Dabigatran concentrations were determined using the dilute thrombin time with dabigatran calibrators (Hemoclot Thrombin Inhibitor Assay; Hyphen BioMed, Neuville-sur-Oise, France); a test that yields values that are highly correlated with those measured by mass spectrometry.

Valve Leaflets and Sewing Ring Segments

Intact valve leaflets were used, whereas SRS were cut from Dacron or Teflon vascular conduits. Dacron conduits were cut lengthwise to form sheets and some were pressed flat with a

DOI: 10.1161/JAHA.115.002322

Journal of the American Heart Association
Dabigatran and Mechanical Heart Valves

Jaffer et al

described above. Thrombin generation pro

DOI: 10.1161/JAHA.115.002322

Thrombin Generation Assays

Assays with valve leaflets and similarly sized SRS were conducted in uncoated 24-well plates (Evergreen Scientific, Los Angeles, CA), the wells of which were of sufficient size to accommodate the devices. To enable exposure of both sides of the devices to plasma, well bottoms were rendered concave with paraffin wax. Briefly, plates were heated by floating them in a water bath heated to 70°C. To each well was added 200 μL of melted Flix-O paraffin wax (Montreal, PQ) and plates were then placed on an oscillating platform heated to 37°C to generate concave well bottoms. To wells without or with a valve leaflet or SRS was added 200 μL of pre-warmed plasma. After a 5 minute incubation at 37°C, a 200-μL aliquot of solution containing 1 mmol/L Z-Gly-Gly-Arg-AMC and 25 mmol/L CaCl₂ in 0.02 mol/L HEPES, pH 7.4, 0.15 mol/L NaCl (HBS) was added to each well, and substrate hydrolysis was monitored at 1-minute intervals for 90 minutes at excitation and emission wavelengths of 360 and 460 nm, respectively, with an emission cutoff filter of 455 nm using a FlexStation 3 fluorescence plate reader (Molecular Devices, Sunnyvale, CA). Studies with 5-mm Dacron or Teflon SRS were performed in uncoated 96-well flat bottom plates (Evergreen Scientific). To wells without or with single SRS cutouts was added 40 μL of plasma followed by 10 μL of 0.02 mol/L HEPES buffer, pH 7.4. After a 5 minute incubation at 37°C, a 50-μL aliquot of 1 mmol/L Z-Gly-Gly-Arg-AMC in HBS containing 25 mmol/L CaCl₂ was added and substrate hydrolysis was monitored as described above. Thrombin generation profiles were analyzed using Technoclone Technothrombin TGA evaluation software (Vienna, Austria), and assays were calibrated with the Technothrombin TGA CAL SET according to the manufacturer’s instructions.

Although SRS were used only once, valve leaflets were cleaned and reused. Thus, after removal from wells, leaflets were submerged in deionized water in 25-mL polystyrene tubes, and tubes were subjected to vortex mixing to dislodge surface clot. To remove residual protein, leaflets were placed in a beaker and washed sequentially with 40-mL aliquots of 10% acetic acid, 0.5% SDS, and 100% acetone. After each wash step, leaflets were rinsed with 40 mL deionized water and the beaker was immersed in an ultrasonic bath for 3 minutes. Individual leaflets were then dried with delicate task wipes (Kimwipes) and stored in airtight containers until used. Cleaning procedures were those recommended by the manufacturer, and control experiments confirmed that leaflet-induced thrombin generation was unaffected by repeated cleaning (data not shown).

In some experiments, CTI was added to plasma, or the studies were performed in FVII-, FXI-, or FXII-depleted plasma instead of control plasma. To compare the capacity of dabigatran and warfarin to attenuate valve leaflet- or SRS-induced thrombin generation, 200-μL aliquots of plasma containing dabigatran in concentrations ranging from 50 to 800 ng/mL, or 200-μL aliquots of plasma from dabigatran- or warfarin-treated patients were added to wells containing a valve leaflet or SRS and thrombin generation was monitored and quantified as described above.

Individual thrombin generation parameters were plotted separately as a function of dabigatran concentration or warfarin INR value. A 2-parameter exponential decay model (y = ae⁻ᵇᵗ) was fitted to the relationship between each thrombin generation parameter and anticoagulant level (SigmaPlot, Version 11; Systat Software Inc, San Jose, CA).

Statistical Analysis

Due to limited amounts of patient plasma, studies were done in triplicate; all other experiments were performed at least 6 times. Data are presented as mean±SEM. Means of data were compared using Student’s t test or using 1-way analysis of variance (ANOVA) followed by post hoc analysis with the Tukey HSD test for normally distributed data or the Mann–Whitney-U test followed by post-hoc analysis with Dunn’s test for non-parametric data. Analyses were performed using IBM SPSS (Version 22) and P values <0.05 were considered statistically significant.

To generate dose-equivalency plots, matched thrombin generation values for warfarin and dabigatran were interpolated at fixed intervals using regression analysis of the observed data. The interpolated values with the two drugs were then plotted against each other to generate a smooth dose-equivalency curve, and the corresponding 95% confidence interval was calculated.
Results

Valve Leaflets and SRS Enhance Thrombin Generation

Thrombin generation in the absence of leaflets or SRS (background) was compared with that in their presence (Figure 1). Compared with background, leaflets and similarly sized Teflon and Dacron SRS significantly shortened the lag time by 1.2-, 1.5-, and 2.7-fold, increased peak thrombin by 1.2-, 1.5-, and 1.6-fold, and increased endogenous thrombin potential (ETP) by 1.2-, 1.5-, and 1.8-fold, respectively (Table 1). In 96-well plates, Teflon and Dacron SRS significantly shortened the lag time by 1.7- and 1.7-fold, increased peak thrombin by 1.8- and 1.3-fold and increased ETP by 1.4- and 1.2-fold, respectively, compared with values measured in their absence (Table 2). Therefore, valve leaflets and SRS are prothrombotic, and SRS promote thrombin generation to a greater extent than valve leaflets.

Table 1. Thrombin Generation Parameters for Leaflet and SRS

| Condition       | Lag Time (min) | Peak Thrombin (nmol/L) | ETP (nmol/L·min) |
|-----------------|----------------|------------------------|------------------|
| Background      | 26.5±0.63      | 273.2±18.2             | 4400.9±192.1     |
| Leaflet         | 22.5±0.40      | 319.8±10.7             | 5170.9±90.3*     |
| Teflon SRS      | 17.3±1.18      | 415.7±33.2             | 6675.3±463*      |
| Dacron SRS      | 9.8±0.47       | 431.6±16.9             | 7950.9±203.5      |

Thrombin generation values were determined in plasma depleted of FVII, FXII, or FXI or supplemented with 200 μg/mL CTI were compared with those in control plasma. Assays were conducted in 96-well plates. Values represent mean±SEM of at least 6 experiments. ETP indicates endogenous thrombin potential; SRS, sewing ring segments.

Table 2. SRS Thrombin Generation Parameters in Control, FVII-, FXI-, or FXII-Depleted Plasma or in Plasma Containing CTI

| Condition       | Lag Time (min) | Peak Thrombin (nmol/L) | ETP (nmol/L·min) |
|-----------------|----------------|------------------------|------------------|
| Background      | Control plasma | 16.8±1.0              | 155.5±11.3       | 2153.5±49.4     |
| Teflon SRS      | Control plasma | 9.7±0.3               | 275.5±18.1       | 2955.5±54.9*    |
|                 | FVII-depleted  | 10.8±0.6              | 268.6±17         | 3211.7±71.8*    |
|                 | FXII-depleted  | 42.8±15*              | 22.5±8.2         | 564.1±215.3*    |
|                 | FXI-depleted   | 9.7±0.2               | 160.5±6.0        | 2716.7±214.5†   |
|                 | CTI            | 28.0±1.0†             | 57.4±3.4         | 1578.8±101.7†   |
|                 | Dacron SRS     | Control plasma        | 9.7±0.3          | 205.4±11.1†     | 2525.3±85.3†    |
|                 | FVII-depleted  | 11.5±0.9              | 214.2±15.4†      | 2585.4±69.4†    |
|                 | FXII-depleted  | 54.8±15*              | 27.6±12.8         | 762.3±347.7†    |
|                 | FXI-depleted   | 11.2±0.2              | 116.3±11.7‡      | 2327.4±28.9‡    |
|                 | CTI            | 28.3±4.4              | 44.6±10.5         | 1008.6±206.7†   |

Leaflets and SRS Promote Thrombin Generation via the Intrinsic Pathway

Thrombin generation in control plasma was compared with that in plasma depleted of FVII, FXII, or FXI. Leaflet- and SRS-induced thrombin generation in FVII-depleted plasma was similar to that in control plasma (Tables 2 & 3). In contrast,

Table 3. Leaflet Thrombin Generation Parameters in Control, FVII-, FXI-, or FXII-Depleted Plasma or in Plasma Containing CTI

| Plasma          | Lag Time (min) | Peak Thrombin (nmol/L) | ETP (nmol/L·min) |
|-----------------|----------------|------------------------|------------------|
| Control plasma  | 22.5±0.4       | 319.8±10.7             | 5170±90.3        |
| FVII-depleted   | 23.8±0.6       | 303.4±10.4             | 5795.7±181.9†    |
| FXI-depleted    | 60.4±4.3*      | 3.0±2*                 | 2.8±2.4*         |
| FXII-depleted   | 21.4±0.8       | 222.3±8.1             | 4784.7±146.3     |
| CTI (200 μg/mL) | 28±1.9         | 187.8±30.1*            | 4546.6±240.3     |

Thrombin generation parameters were determined in the presence of leaflets. Results in plasma depleted of FVII, FXII or FXI supplemented with 200 μg/mL CTI were compared with those in control plasma. Assays were conducted in 24-well plates. Values represent the mean±SEM of at least 6 experiments. CTI indicates corn trypsin inhibitor. *P<0.001 and †P<0.05 compared with control.
thrombin generation was attenuated in FXII-depleted plasma and abrogated in FXI-depleted plasma (Figure 2). Therefore, these findings suggest that both leaflets and SRS promote clotting via the intrinsic pathway.

To confirm the importance of the intrinsic pathway, we examined the effect of CTI, a potent inhibitor of FXIIa, on leaflet- and SRS-induced thrombin generation. CTI reduced leaflet-induced peak thrombin generation in a concentration-dependent manner to levels below background (Figure 3) and at 200 μg/mL, CTI reduced peak thrombin by 1.7-fold (Table 3). Likewise, CTI attenuated thrombin generation induced by Teflon and Dacron SRS (Table 2). Therefore, the effect of CTI together with the results in FXII- and FXI-depleted plasma suggests that the components of MHV trigger thrombin generation via FXIIa.

Comparison of the Effects of Dabigatran and Warfarin on Leaflet- and SRS-Induced Thrombin Generation

With valve leaflets, dabigatran prolonged the lag time and reduced peak thrombin and ETP in a concentration-dependent manner (Figure 4). Plots of thrombin generation parameters versus dabigatran concentration revealed a statistically significant (P<0.001), concentration-dependent reduction in peak thrombin and ETP (Figure 5). Concentrations of dabigatran ≥400 ng/mL were needed to significantly reduce leaflet-induced peak thrombin, whereas concentrations >200 ng/mL were needed to reduce ETP (Figure 5A and 5B). The effect of dabigatran was similar regardless of whether dabigatran was added to plasma or the plasma was collected from patients taking dabigatran (Figure 5B). With Teflon and Dacron SRS, dabigatran concentrations >200 ng/mL reduced both peak thrombin and ETP (Figure 5C and 5D). Therefore, dabigatran suppresses leaflet- and SRS-induced thrombin generation in a concentration-dependent manner, but only at concentrations considerably higher than the target trough concentration of 50 ng/mL used in the RE-ALIGN study.

In plasma from warfarin-treated patients, leaflet- and SRS-induced thrombin generation was markedly attenuated with
INR values above 1.5 (Figure 6A and 6B), and was almost abrogated with INR values in the 2 to 3.5 range.

To determine the concentrations of dabigatran required to suppress thrombin generation to a similar extent as warfarin at an INR of 2 to 3.5, dose equivalency plots were generated from regression analyses of the data in Figures 5 and 6. Dabigatran concentrations of 479 to 774 ng/mL and 254 to 488 ng/mL suppressed leaflet-induced peak thrombin and ETP, respectively, to similar extents as warfarin at an INR of 2 to 3.5 (Figure 7A and 7B). For Teflon and Dacron SRS, dabigatran concentrations of 205 to 370 ng/mL and 235 to 494 ng/mL, respectively, were needed to suppress peak thrombin to the same extent as warfarin at an INR of 2 to 3.5, whereas to reduce ETP induced by Teflon and Dacron SRS, dabigatran concentrations of 261 to 487 ng/mL and 267 to 776 ng/mL, respectively, were required (Figure 7C through 7F). These results suggest that dabigatran concentrations >200 ng/mL are required to suppress thrombin generation induced by MHV components to the same extent as warfarin at an INR of 2 to 3.5.

Discussion

The purpose of this study was to determine why dabigatran was less effective than warfarin at reducing thromboembolic complications in patients with MHV in the RE-ALIGN study.9 We show that valve leaflets and SRS, key components of contemporary bileaflet MHV, are prothrombotic and promote thrombin generation via the intrinsic pathway of coagulation. Furthermore, we demonstrate that warfarin suppresses leaflet- and SRS-induced thrombin generation at INR values of 1.5 or higher, whereas dabigatran concentrations >200 ng/mL are required to suppress thrombin generation to the same extent as warfarin at an INR of 2 to 3.5; dabigatran concentrations well above those achieved in the RE-ALIGN study, and prohibitive in patients because of the bleeding risk.14 Therefore, our work suggests that dabigatran failed to prevent thromboembolic complications in the RE-ALIGN study because the target trough dabigatran concentration was too low.

The observation that MHV components trigger thrombin generation via the intrinsic pathway is consistent with our
findings with catheters.\textsuperscript{18} Like the results with leaflets and SRS, catheter-induced thrombin generation was inhibited with CTI and attenuated in FXII- or FXI-depleted plasma.\textsuperscript{19} The role of the intrinsic pathway in catheter thrombosis in vivo is supported by the observations in rabbits that (1) catheters coated with CTI remained patent longer than uncoated catheters,\textsuperscript{19} and (2) knockdown of FXII or FXI with antisense oligonucleotides prolonged catheter patency, whereas knockdown of FVII had little effect.\textsuperscript{20} Similar results have been shown with other blood-contacting medical devices. Thus, an inhibitory antibody directed against FXIIa attenuated clotting to a similar extent as heparin in a rabbit model of extracorporeal membrane oxygenation, and FXI knockdown or an antibody against FXI reduced fibrin deposition on vascular grafts positioned within arterio-venous shunts in baboons.\textsuperscript{21–25} Therefore, like other blood-contacting medical devices, MHV are prothrombotic because they activate clotting via the intrinsic pathway.

Dabigatran inhibits thrombin in a 1:1 stoichiometric fashion. We hypothesize that MHV induce the generation of thrombin in concentrations that overwhelm those of dabigatran when it is administered at the usual therapeutic doses. This concept is supported by our studies with catheters in rabbits.\textsuperscript{26} Thus, at concentrations similar to those achieved at peak in humans taking an oral dose of 150 mg twice daily, dabigatran prolonged catheter patency, whereas at concentrations similar to trough levels, dabigatran had little effect.\textsuperscript{26} Peak and trough plasma dabigatran levels vary widely in patients\textsuperscript{14,27}, a finding considered in the RE-ALIGN protocol where dabigatran dose escalation to 300 mg twice daily was allowed in an attempt to maintain trough concentrations at 50 ng/mL or higher.\textsuperscript{8} Based on the results of our studies, a trough dabigatran concentration of 260 ng/mL would be needed to attenuate thrombin generation by components of MHV. Assuming renal function similar to that in the patients

Figure 6. Effect of warfarin on thrombin generation induced by leaflets or Dacron or Teflon SRS. Thrombin generation was determined in plasma from patients taking warfarin in the presence of leaflets (●) (A and B) or Teflon (▲) or Dacron (■) SRS (C and D). Values for peak thrombin (A and C) and ETP (B and D) are plotted against the INR values. Data points represent the mean of 3 determinations in each patient sample while the bars reflect SEM. Solid lines represent non-linear regression analysis and the red dashed lines in A and B represent the 95% confidence intervals. Blue dotted lines represent the background in the absence of leaflets or SRS. Leaflet and SRS thrombin generation assays were conducted in 24-and 96-well plates, respectively. ETP indicates endogenous thrombin potential; INR, international normalized ratio; SRS, sewing ring segments.
enrolled in the RE-ALIGN study, pharmacokinetic modeling predicts that dabigatran would need to be given at a dose of 620 mg twice daily to achieve this target trough concentration; a dose more than double the maximum dose administered in the RE-ALIGN study. Such doses are unlikely to be feasible in this patient population because there was already more bleeding with dabigatran than warfarin with the doses used in RE-ALIGN.9

In contrast to dabigatran, warfarin attenuates thrombin generation induced by leaflets or SRS with an INR of 1.5 or higher. Warfarin works because by reducing the functional levels of FIX, FX and prothrombin, it attenuates thrombin generation via the intrinsic and common pathways of coagulation. Therefore, these findings provide a plausible explanation as to why dabigatran was less effective than warfarin at preventing thromboembolic events in the RE-ALIGN study.

**Figure 7.** Dose equivalency plots for the effects of dabigatran and warfarin on thrombin generation induced by leaflets or Dacron or Teflon SRS. From regression analyses of the effect of dabigatran and warfarin on thrombin generation induced by leaflets (A and B) or Teflon (C and D) or Dacron SRS (E and F) illustrated in Figures 5 and 6, respectively, peak thrombin (A, C, E) or ETP (B, D, F) values at fixed intervals (2.5 nmol/L and 5 nmol/L-min, respectively) were interpolated. The interpolated values are plotted against each other to show the dose equivalency of dabigatran and warfarin for each parameter (solid line) and the associated 95% confidence interval (dotted lines). Shaded areas represent the doses of dabigatran that have effects equivalent to warfarin at an INR of 2 to 3.5; the therapeutic range for patients with MHV. ETP indicates endogenous thrombin potential; INR, international normalized ratio; MHV, mechanical heart valves; SRS, sewing ring segments.
Our findings have important clinical implications. The observation that SRS are more thrombogenic than leaflets is consistent with the observation that thrombosis on MHV often starts on the sewing ring.28 Bioprosthetic valves also have Dacron sewing rings and our finding that Dacron SRS are more thrombogenic than Teflon SRS raises the possibility that patients with newly implanted bioprosthetic valves would benefit from a 3-month course of warfarin to reduce the risk of thrombosis while the sewing ring undergoes endothelialization; a concept consistent with the results of large observational studies.29,30 The finding that dabigatran is less effective than warfarin at suppressing Dacron SRS-induced thrombin generation supports the recommendation that dabigatran be avoided in patients with newly implanted bioprosthetic valves as well as in those with MHV.10 Finally, our observation that warfarin produces maximum suppression of leaflet- or SRS-induced thrombin generation at an INR over 1.5 raises the question as to whether the target INR needs to be higher than 2 to 3 for patients with MHV in the mitral position or for those with risk factors for thromboembolism. A target INR of 2 to 3 is used for atrial fibrillation patients regardless of their risk of stroke,31 and our findings raise the possibility that the same approach could be used for patients with MHV. Clinical trials are needed to explore this possibility.

Our study has some limitations. We used leaflets and SRS as surrogates for intact MHV. Although intact MHV may be more thrombogenic than the individual components, they are unlikely to be less so. This concept is supported by the observation that dabigatran concentrations >600 ng/mL were required to prevent clotting when intact MHV were studied in a perfusion chamber.32 Another potential limitation is that we studied thrombin generation in a static system, which may magnify the prothrombotic stimulus, and in the absence of platelets. These are unlikely to be major confounders because in studies with catheters, our in vitro findings in plate-based assays were reproduced in rabbit models.18,26 Furthermore, when MHV were implanted in a conduit that bypassed the ligated descending aorta of pigs, dabigatran concentrations >250 ng/mL were needed to attenuate thrombus deposition.33

In conclusion, we show that the components of MHV induce thrombin generation via the intrinsic pathway, and that warfarin is better than dabigatran at inhibiting this process. Although higher doses of dabigatran suppress thrombin generation induced by MHV components, the dabigatran concentrations needed to have the same inhibitory effect as warfarin at an INR of 2 to 3.5 exceed those achieved in the RE-ALIGN study. Based on these findings, we hypothesize that the trend for more strokes with dabigatran than with warfarin in the RE-ALIGN study reflects the fact that MHV induced the local generation of thrombin in concentrations that overwhelmed those of dabigatran. Although it is uncertain whether oral FXa inhibitors would be superior to dabigatran in MHV patients, our plate-based assays provide a platform to explore this possibility.

Acknowledgments
We gratefully acknowledge Dr. Shannon Bates and Ms. Angela Russell for assistance in obtaining plasma samples, Professor Robin Roberts for helpful statistical advice, and Drs. Paul Reilly and Hugo Maas of Boehringer Ingelheim for their assistance with pharmacokinetic modeling. Dr. Weitz holds the Canada Research Chair (Tier I) in Thrombosis and the Heart and Stroke Foundation J.F. Mustard Chair in Cardiovascular Research.

Sources of Funding
This work was supported, in part, by the new investigator fund of Hamilton Health Sciences, a Canadian Cardiovascular Society-Bayer vascular resident award, grants-in-aid from the Heart and Stroke Foundation, and the Canadian Institutes of Health Research and a research grant from Boehringer Ingelheim.

Disclosures
Dr. Jaffer discloses receiving research support from Boehringer Ingelheim. Mr. Stafford, and Drs. Fredenburgh, Whitlock, and Chan have no relevant financial disclosures. Dr. Weitz discloses receiving consulting fees from Boehringer Ingelheim, Bayer, Bristol-Myers Squibb, Pfizer, Daiichi Sankyo, Janssen, ISIS Pharmaceuticals and Portola. No other potential conflicts of interest relevant to this article are reported.

References
1. Cannegieter S, Rosendaal F, Briet E. Thromboembolic and bleeding complications in patients with mechanical heart valve prostheses. Circulation. 1994;89:635–641.
2. Cannegieter S, Rosendaal F, Wintzen A, van der Meer F, Vandenbroucke J, Briet E. Optimal oral anticoagulant therapy in patients with mechanical heart valves. N Engl J Med. 1999;333:11–17.
3. Hirsh J. Oral anticoagulant drugs. N Engl J Med. 1991;324:1865–1875.
4. Whitlock RP, Sun JC, Frenses SE, Rubens FD, Teoh KH. Antithrombotic and thrombolytic therapy for valvular disease. Chest. 2012;141:e576S–e600S.
5. Landefeld CS, Rosenblatt MW, Goldman L. Bleeding in outpatients treated with warfarin: relation to the prothrombin time and important remediable lesions. Am J Med. 1989;87:153–159.
6. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S, Alings M, Xavier D, Zhu J, Diaz R, Lewis BS, Darius H, Dieper H-C, Joyner CD, Wallentin L. Dabigatran versus warfarin in patients with atrial fibrillation. N Engl J Med. 2009;361:1139–1151.
7. (Canada) B-I. BI Press Release: Pradaxa. Available at: http://www.boehringer-ingelheim.ca/en/news_press_releases/2014/4September201411.html. Accessed March 25, 2015.
8. Van de Werf F, Brueckmann M, Connolly SJ, Friedman J, Granger CB, Hartter S, Harper R, Kappetein AP, Lehr T, Mack MJ, Noack H, Eikelboom JW. A comparison of dabigatran etexilate with warfarin in patients with mechanical heart valves: the randomized, phase II study to evaluate the safety and pharmacokinetics of oral dabigatran etexilate in patients after heart valve replacement (RE-ALIGN). Am Heart J. 2012;163:931–937.e1.
Dabigatran and Mechanical Heart Valves

Jaffer et al.

DOI: 10.1161/JAHA.115.002322

Journal of the American Heart Association

11. Scharfschwerdt M, Thomschke M, Sievers H-H. In-vitro localization of initial
flow-induced thrombus formation in bileaflet mechanical heart valves. ASAIO J. 2009;55:19–23.

12. Hojima Y, Pierce J, Pisano J. Hageman-factor fragment inhibitor in corn seeds
fl

13. Mahoney W, Hermodson M, Jones B, Powers D, Corfman R, Reeck G. Amino-
acid-sequence and secondary structural-analysis of the corn inhibitor of
trypsin and activated Hageman-factor. J Biol Chem. 1984;259:8412–8416.

14. Reilly PA, Lehr T, Haertter S, Connolly SJ, Yusuf S, Eikelboom JW, Ezekowitz
2007;64:292–303.

15. Stangier J, Rathgen K, Stahl H, Gansser D, Roth W. The pharmacokinetics,
thrombin inhibitor, in healthy male subjects. Br J Clin Pharmacol. 2007;64:292–303.

16. Chan NC, Coppens M, Hirsh J, Ginsberg JS, Weitz JI, Vanassche T, Douketis JD,
Schulman S, Eikelboom JW. Real-world variability in dabigatran levels in
patients with atrial fibrillation: the RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy). J Am Coll Cardiol. 2014;63:321–328.

17. van Ryn J, Stangier J, Haertter S, Liesenfeld KH, Wienen W, Feuring M, Clemens A. Dabigatran etexilate—a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. Thromb Haemost. 2010;103:1116–1127.

18. Yau JW, Stafford AR, Liao P, Fenudrenburg JC, Roberts R, Weitz JI. Mechanism of
catheter thrombosis: comparison of the antithrombotic activities of fonda-
parinux, enoxaparin, and heparin in vitro and in vivo. Blood. 2011;118:6667–6674.

19. Yau JW, Stafford AR, Liao P, Fenudrenburg JC, Roberts R, Brash JL, Weitz JI. Corn
trypsin inhibitor coating attenuates the prothrombotic properties of catheters
in vitro and in vivo. Acta Biomater. 2012;8:4092–4100.

20. Yau JW, Liao P, Fenudrenburg JC, Stafford AR, Revenko AS, Monia BP, Weitz JI. Selective depletion of factor XI or factor XII with antisense oligonucleotides attenuates catheter thrombosis in rabbits. Blood. 2014;123:2102–2107.

21. Larsson M, Rayzman V, Nolte MW, Nickel KF, Bjorkqvist J, Jamsa A, Hardy MP,
Fries M, Schmidbauer S, Hedenqvist P, Broome M, Pragst I, Dickneite G, Wilson MJ, Nash AD, Panousis C, Renne T. A factor Xlla inhibitory antibody provides thromboprotection in extracorporeal circulation without increasing bleeding risk. Sci Transl Med. 2014;6:222ra17.

22. Cheng Q, Tucker EI, Pine MS, Sisler I, Matafonov A, Sun MF, White-Adams TC,
Smith SA, Hanson SR, McCarty OJ, Renne T, Gruber A, Gailani D. A role for
factor Xlla-mediated factor XI activation in thrombus formation in vivo. Blood. 2010;116:3981–3989.

23. Crosby JR, Marzec U, Revenko AS, Zhao C, Gao D, Matafonov A, Gailani D, MacLeod AR, Tucker EI, Gruber A, Hanson SR, Monia BP. Antithrombotic effect of antisense factor XI oligonucleotide treatment in primates. Arterioscler Thromb Vasc Biol. 2013;33:1670–1678.

24. Gruber A, Hanson SR. Factor XI-dependence of surface- and tissue factor-
initiated thrombus propagation in primates. Blood. 2003;102:953–955.

25. Matafonov A, Leung PF, Gailani AE, Grach SL, Puy C, Cheng Q, Sun MF, McCarty OJ, Tucker EI, Kataoka H, Renne T, Morrissey JH, Gruber A, Gailani D. Factor XII inhibition reduces thrombus formation in a primate thrombosis model. Blood. 2014;123:1739–1746.

26. Yau JW, Liao P, Fenudrenburg JC, Roberts RS, Weitz JI. Only high levels of
dabigatran attenuate catheter thrombosis in vitro and in rabbits. Thromb Haemost. 2014;112:79–86.

27. Stangier J, Clinical pharmacokinetics and pharmacodynamics of the oral direct
thrombin inhibitor dabigatran etexilate. Clin Pharmacokinet. 2008;47:285–295.

28. Dewanjee MK, Gross DR, Zhai P, Lanzo S, Shim H, Park K, Schaeffer DJ, Twardock AR. Thrombogenicity of polyethylene oxide-bonded Dacron sewing
ring in a mechanical heart valve. J Heart Valve Dis. 1999;8:324–330.

29. Merie C, Kober L, Skov Olsen P, Andersson C, Gislason G, Skov Jensen J, Torp-
Pedersen C. Association of warfarin therapy duration after bioprosthetic aortic
valve replacement with risk of mortality, thromboembolic complications, and
bleeding. JAMA. 2012;308:2118–2125.

30. Mehta SR, Weitz JI. Warfarin after bioprosthetic aortic valve implantation. JAMA. 2012;308:2147–2148.

31. Verma A, Cairns JA, Mitchell LB, Macle L, Stiell IG, Gladstone D, McMurtry MS, Connolly S, Cox JL, Dorian P, Ivers N, Leblanc K, Nattel S, Healey JS. 2014 focused update of the Canadian Cardiovascular Society guidelines for the management of atrial fibrillation. Can J Cardiol. 2014;30:1114–1130.

32. Maegdefessel L, Linde T, Krapiec F, Hamilton K, Steinsiefer U, van Ryn J, Raaz U, Buerke M, Werdan K, Schiltt A. In vitro comparison of dabigatran, unfractionated heparin, and low-molecular-weight heparin in preventing thrombus formation on mechanical heart valves. Thromb Res. 2010;126:e196–e200.

33. McKellar SH, Abel S, Camp CL, Suri RM, Ereh MH, Schaff HV. Effectiveness of
dabigatran etexilate for thromboprophylaxis of mechanical heart valves. J Thorac Cardiovasc Surg. 2011;141:1410–1416.