Effectiveness of rivaroxaban for thromboprophylaxis of prosthetic heart valves in a porcine heterotopic valve model

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Abstract

OBJECTIVES: Warfarin is used to reduce the risk of stroke and thromboembolic complications in patients with mechanical heart valves. Yet, despite frequent blood testing, its poor pharmacokinetic and pharmacodynamic profiles often result in variable therapeutic levels. Rivaroxaban is a direct competitive factor Xa inhibitor that is taken orally. It inhibits the active site of factor Xa without the need for the cofactor antithrombin, and thus, its mechanism of action is differentiated from that of the fractionated heparins and indirect factor Xa inhibitors. No in vivo data exist regarding the effectiveness of rivaroxaban in preventing thromboembolic complications of mechanical heart valves. We tested the hypothesis that rivaroxaban is as effective as enoxaparin for thromboprophylaxis of mechanical valve prostheses using a previously described heterotopic aortic valve porcine model.

METHODS: A modified bileaflet mechanical valved conduit that bypassed the native, ligated descending thoracic aorta was implanted into 30 swine. Postoperatively, the animals were randomly assigned to groups receiving no anticoagulation (n = 10), enoxaparin at 2 mg/kg subcutaneously twice daily (n = 10) or rivaroxaban at 2 mg/kg orally twice daily (n = 10). The amount of valve thrombus was measured on post-implantation day 30 as the primary end point. Quantitative evaluation of radiolabelled platelet deposition on the valve prostheses was done and embolic and haemorrhagic events were measured as secondary end points.

RESULTS: Animals with no anticoagulation had a thrombus mean of 759.9 mg compared with 716.8 mg with enoxaparin treatment and 209.6 mg with rivaroxaban treatment (P = 0.05 for enoxaparin vs rivaroxaban). Similarly, the mean number of platelets deposited on the valve prosthesis was lower in the rivaroxaban group (6.13 × 10⁹) than in the enoxaparin group (3.03 × 10¹⁰) (P = 0.03).

CONCLUSIONS: In this study, rivaroxaban was more effective than enoxaparin for short-term thromboprophylaxis of mechanical valve prosthetics in the heterotopic aortic position. It reduced valve thrombus and platelet deposition on day 30 following implantation without increased adverse events. Future studies would provide additional support for clinical trials evaluating rivaroxaban as an alternative to warfarin for appropriately selected patients with prosthetic aortic valves.

Keywords: Factor Xa inhibitors • Heart valve • Thromboembolism • Valvular prosthesis

INTRODUCTION

Several new oral anticoagulation strategies are available for the prevention of thromboembolic complications [1, 2] in patients with atrial fibrillation (AF) and those at risk of deep venous thrombosis [3, 4]. These new medications—apixaban, dabigatran and rivaroxaban—have been developed as alternatives to warfarin for long-term anticoagulation and have been studied clinically. Interest is increasing in using these new oral anticoagulants for prophylaxis in patients with mechanical valves [5, 6]. Compared with warfarin, each of these medications has the advantages of rapid onset of action and predictable pharmacokinetic and pharmacodynamic profiles [7–9]. Unlike warfarin, these drugs are administered in standard fixed dosages and do not require frequent monitoring to assess therapeutic efficacy.

Rivaroxaban is an orally administered inhibitor of both free and prothrombinase complex-bound factor Xa [10, 11] that acts at the active site of factor Xa without the need for the cofactor antithrombin [12]. Rivaroxaban has proved to be safe and effective in preventing arterial and venous thrombosis after orthopaedic surgery [13, 14], preventing stroke associated with AF [15, 16] and treating pulmonary embolism [17]. However, the effectiveness of rivaroxaban in preventing thromboembolic complications has not been tested in the clinical setting of mechanical heart valve prostheses.

We hypothesized that rivaroxaban would provide thromboprophylaxis of mechanical bileaflet heart valves equivalent to common anticoagulation strategies. We tested this hypothesis using heterotopically positioned mechanical aortic valves in a porcine model [18, 19].
MATERIALS AND METHODS

Animal model

Approval for this study was obtained from the Mayo Clinic Institutional Animal Care and Use Committee. The heterotopic swine model for mechanical aortic valve placement has been reported previously by our laboratory [18]. In this preparation, a 19-mm modified bileaflet aortic valve conduit (St. Jude Masters Series; St. Jude Medical, Inc., St. Paul, MN, USA) is implanted in the descending aorta, which bypasses the native, ligated vessel; thus, all blood flow distal to the left subclavian artery flows across the mechanical valve in the conduit [18, 19]. All swine were castrated males weighing 35–40 kg at the time of operation.

Study design

Dosing study. The half-life of rivaroxaban is ~4 h in pigs, which is shorter than in humans, so in this study the drug was given twice daily. To identify a dose regimen similar to once-daily administration of 20 mg of rivaroxaban in humans (peak serum concentration $C_{\text{max}}$ [range], 222.6 [159.6–359.8] µg/l and trough serum concentration $C_{\text{min}}$ [range], 22.3 [4.3–95.7] µg/l), we first performed a dosing study (Phase 1) in which rivaroxaban (2 mg/kg twice daily) was administered to swine and haematological samples were drawn at time points of 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48 and 72 h. Our goal was to find the dose of rivaroxaban that corresponded to therapeutic levels in this animal model, and we aimed to have peak and trough values of 200 and 45 µg/l, respectively. In addition, we obtained drug levels in all 10 swine in the rivaroxaban treatment group (Phase 2).

In vivo thromboprophylaxis of mechanical valves. Specific details regarding this model of heterotopic aortic valve prostheses have been reported elsewhere [19, 20]. Thirty swine were randomly assigned to three postoperative anticoagulation arms: no anticoagulation ($n = 10$), twice-daily subcutaneous enoxaparin (dose, 2 mg/kg) ($n = 10$) and twice-daily oral rivaroxaban (dose, 2 mg/kg) ($n = 10$). The clinical formulation of rivaroxaban tablets was used in this study (Bayer AG, Leverkusen, Germany) and was given in feed to the animals. Because of the difficulty in maintaining a therapeutic window in swine with warfarin administration [18, 21], enoxaparin—a low-molecular-weight heparin—was used as the standard for anticoagulation. The rivaroxaban dose was determined from the results of our dosing study and the enoxaparin dose from previous reports [18, 20]. Swine randomization was masked to the investigator (Lawrence E. Greiten), to whom the drug treatment arm was masked.

End points

Valve thrombus. The primary end point of this study was the amount of thrombus on the prosthetic valve at 30 days postoperatively. At that time, the animals received heparin and were humanely killed under general anaesthesia. Valve thrombus was then measured and reported as mean (range) mg. Only thrombus present on the mechanical valve leaflets was measured by the investigator (Lawrence E. Greiten), to whom the drug treatment arm was masked.

Platelet deposition. As a second quantitative method, the mechanical valve platelet deposition was measured to assess both effect and thrombus burden for each of the experimental anticoagulation regimens. As previously described [18, 20], the amount of acute platelet deposition on each mechanical prosthesis was measured with autologous infusion of indium $^{111}$In autumomab pentetate-labelled platelets. At the time of humane killing, two 10-ml blood samples were obtained and the radioactivity (20 µCi) from the blood samples was measured by placing them into a scintillation radiocounter (Capintec, Inc., Ramsey, NJ, USA). Before quantification of thrombus burden, the explanted valve was placed into a scintillation radiocounter for radioactivity measurement. The average amount of radioactivity per platelet was calculated by comparing the animal’s blood platelet count and the radioactivity of each sample. Thus, the number of platelets on the mechanical valve could be calculated with the following equation: number of platelets on valve prosthesis = valve radioactivity/platelet radioactivity.

Haemorrhagic and thromboembolic complications. Secondary end points included occult haemorrhagic and thromboembolic complications. Research staff, to whom the treatment arm was masked, observed the swine daily for acute gastrointestinal haemorrhage and thromboembolic complications (i.e. neurological deficits, loss of lower extremity function or bowel ischaemia). Serial serum blood draws and faecal HemoQuant from each animal were used to assess possible occult haemorrhagic events, defined as a drop in serum haemoglobin of at least 2 mg/dl or an abnormally high level of faecal haemoglobin (>2 mg/g of stool). At the time of humane killing, a section of randomly selected outer renal cortex was collected from each swine and was sectioned and examined for gross and microembolic events.

Coagulation profile. We used haematological and multiple anticoagulation assays to assess the effect of rivaroxaban on the coagulation system. We obtained complete blood cell counts and measured prothrombin time, activated partial thromboplastin time, fibrinogen, anti-factor Xa levels, D-dimer and factor X chromogenic activity immediately before valve implantation (baseline), at trough ($C_{\text{min}}$) time periods on post-implantation day 10 and 20 and at the time of humane death (30 ± 2 days).

Statistical analysis

Valve thrombus (mg) and number of radiolabelled platelets deposited on valve prosthesis are reported as mean (range) and were treated as continuous variables, and compared through the Mann–Whitney U-test. Comparisons between treatment groups were made with the JMP 10 software (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Dosing study

In Phase 1 studies, animals treated with twice-daily oral rivaroxaban at 2 mg/kg consistently achieved a $C_{\text{max}}$ mean (range) of 124 (65–231) µg/l and a $C_{\text{min}}$ mean (range) of 24.6 (3.89–95.5) µg/l, which were in the therapeutic range and were comparable with drug levels in human dosing studies. Although $C_{\text{max}}$ levels were somewhat lower in comparison...
Figure 1: Plasma rivaroxaban peak and trough values. (A) Phase 1 dosing study (n = 5). (B) Phase 2 study (n = 10). Vertical bars indicate range.

Figure 2: Coagulation study results. There were no differences in (A) activated partial thromboplastin time (aPTT) or (B) prothrombin time (PT) among the groups. (C) Rivaroxaban (Rivox) and enoxaparin (Enox) increased anti-FXa activity compared with control. (D) Factor X chromogenic activity assay, percent activity. (E) Fibrinogen split products. Asterisk indicates P < .05; control: no anticoagulation; FEU: fractional equivalency unit; anti-FXa: anti-factor Xa. Error bars indicate standard error of the mean.
with human data, $C_{\text{min}}$ levels were similar in both swine and human studies. Phase 2 $C_{\text{max}}$ mean (range) and $C_{\text{min}}$ mean (range) values were 136.5 (50.6–534.6) and 51 (11.3–182.3) µg/l, respectively (Fig. 1).

Measurement of anticoagulation

No important differences were observed between groups in activated partial thromboplastin values (Fig. 2A), because many of the

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**Figure 3:** Mean valve thrombus. (A) Valve thrombus for each group on post-implantation day 30. (B) Valve thrombus on valves for the two treatment arms. Asterisk indicates $P < 0.05$; control: no anticoagulation; Enox: enoxaparin; Rivox: rivaroxaban. Error bars indicate standard error of the mean.
values were <20 s (laboratory detectable limit) or in the prothrombin time (Fig. 2B) (measured at trough levels). As expected, we observed increased anti-factor Xa levels relative to the control group (values lower than the detection limit [<0.05 IU/ml]) at all time points for animals receiving enoxaparin and rivaroxaban (Fig. 2C). Factor X activity and fibrinogen equivalent units were similar between groups (Fig. 2D and E).

**Valve thrombus**

One premature death occurred on postoperative day 30 in the rivaroxaban group secondary to a pulmonary abscess that eroded into the native aorta-conduit anastomosis. This animal was replaced subsequently with another experimental swine for 30 days of study. In animals with no anticoagulation, we observed a mean (range) valve thrombus weight of 760 (0–2298) mg compared with 717 (0–1490) mg in the animals receiving enoxaparin and 210 (0–1337) mg in those treated with rivaroxaban (Fig. 3A). Of note, when no thrombus was present on the explanted valve, the value of 0 was recorded.

Visible thrombosis was seen in 7 of the 10 swine with no anticoagulation, in 8 with enoxaparin treatment and in 4 with rivaroxaban treatment. When comparing the two treatment groups, we found significantly less thrombus burden on valves among the animals that received rivaroxaban ($P = 0.05$) (Fig. 3B). Likewise, the mean (range) number of platelets deposited on the mechanical valve prosthesis was significantly less in the rivaroxaban group ($6.13 \times 10^9$ [3.8 × 10^7–1.74 × 10^10]) than in the enoxaparin group (3.03 × 10^10 [2.5 × 10^8–8.18 × 10^10]; $P = 0.03$) (Fig. 4). Figure 5 shows representative post-mortem photographs of explanted valves from swine in the groups receiving no anticoagulation, enoxaparin and rivaroxaban.

**Thrombotic and haemorrhagic complications**

No overt haemorrhagic or thromboembolic complications were observed in animals in any of the three groups. Similarly, no evidence of occult or gross haemorrhage was found in any of the groups, according to serum haemoglobin and faecal haematocrit. Finally, on post-mortem kidney examination, no microemboli were seen.

**DISCUSSION**

The principal finding of this study is that the competitive and reversible direct factor Xa inhibitor rivaroxaban is effective for thromboprophylaxis of bileaflet mechanical heart valves implanted in heterotopic position in swine. This study is the first short-term animal study of rivaroxaban for anticoagulation of mechanical heart valves, to our knowledge. Our results show rivaroxaban to be superior to anticoagulation with low-molecular-weight heparin (enoxaparin), and its use resulted in the lowest amounts of valve thrombus and platelet deposition on postoperative day 30.

The concept of rivaroxaban as an alternative for oral long-term anticoagulation in patients with mechanical heart valves is promising because of the proven efficacy of this drug in treating patients with AF [15] and those at high risk of venous thromboembolism [4]. In a review of Phase 3 clinical trial data, Kwong [14] reported superior efficacy of rivaroxaban in reducing total venous thromboembolism in comparison with both the North American and European regimens of enoxaparin. In the double-blinded Rivaroxaban Once-Daily Oral Direct Factor Xa Inhibition Compared With Vitamin K Antagonism for Prevention of Stroke and Embolism Trial [22] of patients with AF, rivaroxaban was non-inferior to warfarin for the prevention of stroke or systemic embolism, and there was no significant difference in the risks of major bleeding between the two treatment arms.

If the safety and efficacy of rivaroxaban can be extended to patients with mechanical heart valve prosthesis, use of the drug would represent a major step forward in clinical care, because it is well tolerated in once-daily dosing and does not require
therapeutic efficacy monitoring. There are other potential applications in patients with prosthetic valves, including those with bioprostheses. Although conflicting data exist on the application of oral anticoagulation in patients after undergoing bioprosthetic aortic valve replacement [23, 24], the use of rivaroxaban would be an attractive alternative to warfarin if safety and efficacy are proved in appropriate randomized trials.

Unlike warfarin, rivaroxaban has rapid in vivo activity, achieving peak levels 2–4 h after ingestion and having a half-life of ~9 h in humans. Importantly, there are no known food interactions and limited drug interactions, and the pharmacological profile has not been shown to be effected by ethnicity [25]. Depending on clinical application, humans require once-daily administration of 10 or 20 mg, which provides sufficient factor Xa inhibition for anticoagulation. To achieve the target trough level of 50 μg/l in swine, doses of 2 mg/kg twice daily were necessary because of the different pharmacokinetic profile and a shorter half-life in swine than in humans.

Steady-state anticoagulation with warfarin in swine has been prohibitively difficult to achieve [21], and we have used twice-daily subcutaneous enoxaparin as a ‘standard’ anticoagulation regimen [19, 20]. An unexpected finding in this study was the reduced anticoagulation effect of enoxaparin, reflected by the lower anti-factor Xa levels compared with swine in previous studies [19]. There is no immediate explanation for this finding because the dosing calculation and administration of enoxaparin were identical to our previous studies, as was the formulary compound. It is possible that genetic changes in the swine provided by our breeder or a change in the dietary compound fed to the swine or both affected drug metabolism or absorption. In addition, although the assay conditions measuring anti-factor Xa did not change, the machine or the calibration reagent may have changed.

CONCLUSIONS

In this porcine model powered to test non-inferiority, we found rivaroxaban, an orally administered selective inhibitor of factor Xa, to be effective for thromboprophylaxis of mechanical heart valves implanted in a heterotopic position. However, because 4 of the 10 swine receiving rivaroxaban had detectable valve thrombosis, further investigations with a higher dose seem to be necessary to safely evaluate the effects that rivaroxaban may have in preventing valve thrombosis in this animal model. Future studies would provide additional support for clinical trials evaluating rivaroxaban as an alternative to warfarin for appropriately selected patients with prosthetic aortic valves.

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REFERENCES