J Thromb Thrombolysis (2011) 32:417–425
DOI 10.1007/s11239-011-0621-6

Comparison of unfractionated heparin, low-molecular-weight heparin, low-dose and high-dose rivaroxaban in preventing thrombus formation on mechanical heart valves: results of an in vitro study

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Published online: 20 July 2011

Abstract Thromboembolism and bleeding after mechanical heart valve replacement are still unsolved problems, particularly for patients requiring anticoagulative bridging therapy. The aim of this study was to investigate whether rivaroxaban, a new oral selective and direct coagulation factor Xa inhibitor, is as effective as enoxaparin and unfractionated heparin (UFH) in preventing thrombus formation on mechanical heart valves using an in vitro system. Blood from healthy male donors was anticoagulated with either UFH, enoxaparin, rivaroxaban at 300 ng/ml, ($n = 10$ each), or rivaroxaban at 30 ng/ml ($n = 3$). Mechanical aortic valve prostheses were placed into the in vitro testing system THIA II and exposed to the anticoagulant blood mixtures at a pulsatile flow for 60 min. Overall thrombus weight, coagulation parameters, and electron microscopic features of thrombus formation on the valve surface were quantified as endpoints. The mean thrombus weights were 163 ± 64 mg for group 1 (UFH), 341 ± 63 mg for the group 2 (enoxaparin), 238 ± 83 mg for group 3 (rivaroxaban 300 ng/ml) and 1.739 ± 16 mg for group 4 (rivaroxaban 30 ng/ml). Whereas high-dosed rivaroxaban showed no significant differences compared to UFH or enoxaparin, low-dosed rivaroxaban generated a massive thrombus generation, thus differing significantly from all other treatment groups regarding the thrombus weight. We hypothesize that high-dose rivaroxaban is a competitive oral available alternative to UFH and LMWH's, that might be a worthwhile alternative for patients in need of anticoagulative bridging therapy. Prospective studies have to evaluate if rivaroxaban might even overcome the limitations of OAC in patients after implantation of artificial heart valves.

Keywords Anticoagulation · Rivaroxaban · Mechanical heart valves · Thrombosis

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Introduction

Lifelong oral anticoagulation (OAC) is mandatory after mechanical heart valve replacement to prevent thromboembolic events. Even under adequate therapy with oral vitamin K antagonists (VKA), a considerable number of embolic events occur: approximately 1–4% patients per year are affected [1–3]. Furthermore, major and minor bleedings caused by anticoagulation develop in 2–9% of the patients per year [4, 5]. The established treatment with VKA is still associated with a number of drawbacks, such as unpredictable pharmacokinetics, including various drug and food interactions. A narrow therapeutic window also necessitates frequent coagulation monitoring [6].

Current bridging strategies [7, 8], which are applied when surgical procedures are required, recommend that OAC be temporarily discontinued after mechanical heart valve replacement. The established anticoagulant bridging strategy comprises parenteral administered, continuously infused unfractionated heparin (UFH).

One major complication of heparin is the rare, but potentially life-threatening development of an immune-mediated platelet activation that causes heparin-induced thrombocytopenia (HIT) and secondarily results in a HIT-associated thrombosis syndrome (HITTS) [9]. Although the effects of LMWH on anticoagulation are more predictable, anticoagulative monitoring is not required, less heparin-induced osteopenia—another complication of long term treatment with heparins—and lower HIT rates are observed [10], no randomized clinical trials have evaluated the efficacy of LMWH versus UFH for periprocedural bridging. Whereas several observational studies favor the use of LMWH for perioperative bridging [11, 12], one controversial study still revealed arterial thromboembolism and stroke in 0.6% of the patients when oral VKA were discontinued and replaced by subcutaneous LMWH—in contrast to 0% of the patients who switched from VKA to UFH [13]. These results favouring UFH are obscured by the widespread interindividual differences in its pharmacokinetics, causing an increased rate of complications (especially minor and major bleedings) compared to LMWH (enoxaparin) and the need for intensive and costly coagulation monitoring in the hospital [14]. Thus, the search for more effective and safer antithrombotic agents that preferably can be administered orally and do not require frequent monitoring is still of extraordinary interest.

Rivaroxaban is a novel, orally available, direct FXa-inhibitor that has passed advanced clinical development for the prevention and treatment of thromboembolic disorders. The aim of this study was to test the hypothesis that rivaroxaban is as effective as enoxaparin and UFH in preventing thrombus formation on mechanical heart valves using an in vitro system.

Materials and methods

In vitro testing device

In the current study, we used the previously described and established [15–17] THIA II (Thrombosis Tester Helmholtz Institute Aachen II) system, a unique testing system for mechanical heart valve associated thrombosis. The design of the THIA II is based on a pulsatile blood circulation via a mechanical heart valve prosthesis. The system, schematically shown in Fig. 1, is composed of two flow-optimized and hemocompatible blood chambers consisting of PMMA (polymethylmethacrylate), thereby having only negligible impact on thrombus formation. These chambers are connected via the heart valve plane, in the current study retaining a St. Jude Medical 27 mm Aortic Valve™ and via a flow-sensor integrating bypass. Mimicking the left ventricular outflow tract, the chamber downstream of the prosthesis possesses the geometry of the human aortic root structure, including the sinuses (corresponding to a physiological annulus diameter of 27 mm) and generating a maximum reduction of shear stress. Upstream of the prosthesis, the blood chamber, mimicking the left ventricle, is shaped conical to optimize the filling volume and the blood flow pattern of the ejected blood volume. Both blood chambers are bordered by polyurethane membranes: the membrane situated upstream is actively displaced by a pneumatic driving system and the membrane situated downstream is connected to an air compliance chamber. Overall the system affords a systolic bulging of the upstream membrane into the chamber to force blood through the valve, while in diastole blood flows back from the downstream to the upstream chamber via the bypass. An external electronic control unit steers the cycle rate (bpm).

Pressures in the upstream and downstream chamber were recorded at a cycle rate of 60 bpm and a mean flow of 3.5 l/min, corresponding to a physiological cardiac output. Figure 2 shows typical pressure curves measured using the data acquisition unit (DAQ) in the THIAII.

By mimicking physiological conditions, thereby reducing the influence of temperature on clotting processes, the whole blood-containing system was assembled in a climate box at a constant temperature of 37°C.

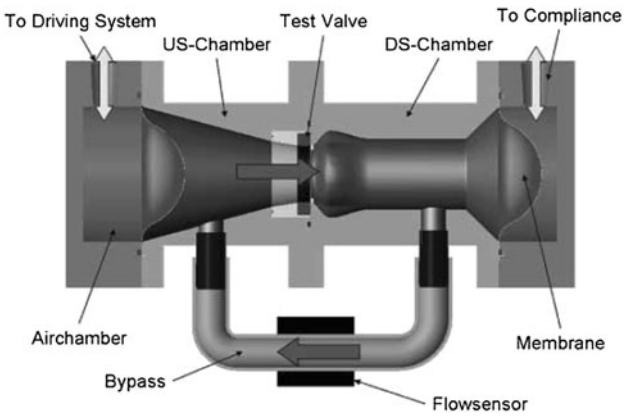

Fig. 1 Schematic experimental set up of the in vitro thrombosis testing device THIA II (ThrombosisTester Helmholtz Institute Aachen II). *US-Chamber* upstream chamber, *DS-Chamber* downstream chamber

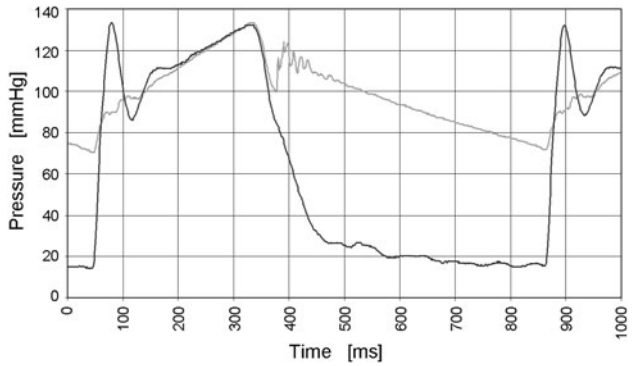

Fig. 2 Pressure curves measured using the data acquisition unit (DAQ) in the THIA II; upstream (*black*) and downstream (*grey*); 75 bpm, 3.5 l/min, 130/70 mmHg

Experimental setup

After initial screening for general cardiac, respiratory, gastrointestinal, renal, hepatic and neurologic disorders using an medical questionary supplemented by a physical examination and a blood sampling assaying the parameters of coagulation 33 young, healthy, male volunteers were enclosed in this study. Prior to each experiment the volunteers had to clarify and sign for the latitude of any intake of anticoagulant medication.

Blood (each 230 ml) was collected directly after antecubital venepuncture into prepared blood bags containing the specific anticoagulant being tested, namely: group 1 UFH 0.8 UI/ml ($n = 10$), group 2 enoxaparin 0.7 UI/ml ($n = 10$), group 3 rivaroxaban 300 ng/ml ($n = 10$) and group 4 rivaroxaban 30 ng/ml ($n = 3$) plasma. The high-dose rivaroxaban concentration was chosen in analogy to achieved peak ranges of 160–360 ng/ml plasma after oral bolus administration of 20 mg [18]. Furthermore stated UFH and LMWH concentrations were chosen with respect to the results of former in vitro investigations with thrombosis testing devices in our laboratories allocating the achievement of therapeutic ranges corresponding to the recommended loading doses of these drugs as used for perioperative bridging therapy [15–17]. The mechanical heart valves were exposed to a pulsatile blood flow at 60 bpm for a total duration of 60 min.

Electron microscopic analysis

To facilitate detailed analysis of the thrombotic material adhering to the prosthesis as regards the degree of erythrocytes, thrombocytes, and fibrin deposition, heart valves were removed from the THIA II after finishing the experiment. The prostheses were then prepared for electron microscopic analyses: this involved cleansing the valves of fluid blood components by placing them into Soerensen solution for 1 min twice, followed by fixation of the adherent thrombotic debris using glutardialdehyde solution (25% solution in H2O, M 100.1 mol/l of the anhydrous product). To prevent the fixed thrombus from oxidating, the heart valves were dehydrated in an ascending series of acetone solution (>99.5%, M 58.08 mol/l), treated with hexamethydisalazane (>99.9%, M 161.4 mol/l), and dried overnight under an exhaust system.

Scanning electron microscopic analysis was performed using a LEO 1530 scanning electron microscope (Gatech, Georgia Institutes of Technology, Atlanta, GA, USA). A defined surface region (100 × 150 µm) of the heart valve leaflets was analyzed in order to count erythrocytes and thrombocytes and to quantify the fibrin deposition according to an arbitrary scale from 0 to 4 (0 = no fibrin deposition, 1 = trace fibrin deposition, 2 = low fibrin deposition, 3 = moderate fibrin deposition, and 4 = heavy fibrin deposition). All microscopic analyses were conducted by two independent investigators blinded to the anticoagulant regimen used.

Laboratory parameters

The anticoagulative activity was assessed by performing global and specific coagulation tests using native blood drawn directly from the donors' veins as baseline values and blood drawn after anticoagulation at the beginning (0 min), during (at 30 min), and at the end (at 60 min) of the experiment.

In freshly citrated blood, aPTT (activated partial thromboplastin time) and TT (thrombin time) were measured using standard citrate tubes (Sarstedt©). Additionally in group 1 (UFH) the ACT (activated clotting time; using ACT plus, Medtronic©, Minneapolis, USA), in group 2 (enoxaparin) n-hep (anti-Xa-activity), and in group 3 and 4 (rivaroxaban groups) the anti-Xa-activity using an orgaran-calibrated assay was measured verifying an achievement of therapeutic levels of anticoagulation. The therapeutic range for the anti-Xa-activity-assay calibrated for LMWH's was 0.5–1.2 IU/ml and for the orgaran-calibrated anti-Xa-assay monitoring rivaroxaban 0.5–0.8 U/ml.

Hematologic monitoring was assessed determining the number of erythrocytes, platelets and hemoglobin levels in all treatment groups at timepoints 0 and 60.

All laboratory parameters were assessed by using routine methods employed at Martin-Luther University Halle-Wittenberg. Data are presented as mean ± SEM.

Statistical analysis

Statistical analysis comparing the differences between the treatment groups was performed using one-way ANOVA

with post-hoc analysis by SPSS software (V11.5). *P*-values < 0.05 were considered to be statistically significant.

Results

Thrombus weight

Mean thrombus weight, represented by the difference in weight of the heart valves gained by adherent thrombotic material on the surface of the heart valve pre- and postexperimentally, is an established and objective parameter indicating the overall remaining prothrombotic activity despite anticoagulant treatment as proven in our former studies [16–18]. Assessed mean thrombus weight in group 1 (UFH) was 163 ± 64 mg, in group 2 (enoxaparin) 341 ± 63 mg, in group 3 (rivaroxaban 300 ng/ml) 238 ± 83 mg, and in group 4 (rivaroxaban 30 ng/ml) mg 1739 ± 16 mg (Fig. 3). Whereas treatment with UFH and rivaroxaban at 300 ng/ml achieved comparably low thrombus weights, the enoxaparin group showed considerably higher valve weight differences, indicating a remarkably higher remaining procoagulant activity despite therapeutic levels of anticoagulation. However, no significant ($P < 0.05$) differences between high-dosed rivaroxaban, UFH and enoxaparin treatment were observed in post-hoc analysis, whereas low-dosed rivaroxaban at 30 ng/ml generated a massive increase in thrombus weight differing significantly from all other treatment groups.

Electron microscopic analyses

Regarding a more detailed analysis of the generated thrombotic debris adherent to the heart valves, electron micrographs (as shown exemplarily in Fig. 4) and their analysis showed in group 1 (UFH) of 66 ± 63, in group 2 (enoxaparin) of 7 ± 5, and in group 3 (rivaroxaban 300 ng/ml) 1 ± 1 mean adhering erythrocytes per standardized region on the surface of the mechanical heart valve (Fig. 5a). These data suggest that thrombotic erythrocyte deposition was considerably lower for high-dosed rivaroxaban. However, no statistical significant difference between the high-dose rivaroxaban, UFH and enoxaparin regarding erythrocyte deposition was observed.

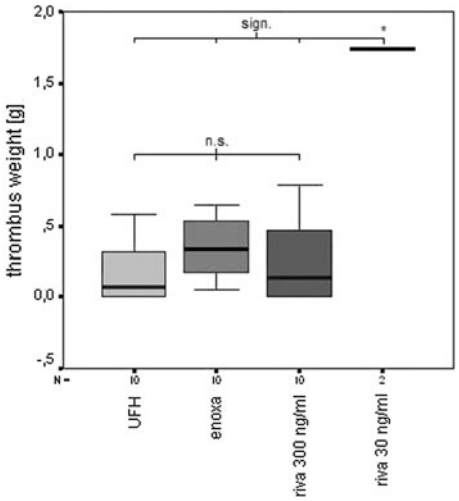

Fig. 3 *Box plots* depict the influence of UFH, enoxaparin, high-dose and low-dose rivaroxaban on thrombus weight. *UFH* unfractionated heparin, *enoxa* enoxaparin, *riva* rivaroxaban

Analysis of the degree of platelet deposition (Fig. 5b) showed in group 1 (UFH) a mean of 240 ± 67, in group 2 (enoxaparin) of 466 ± 134, and in group 3 (rivaroxaban 300 ng/ml) of 42 ± 10 adherent platelets per analyzed region. Thus, platelet deposition, as the second assessed electron microscopic parameter of thrombus formation on the mechanical heart valve, was lower for high-dosed rivaroxaban than for UFH and enoxaparin treatment. Statistical analysis showed a significant reduction of thrombocyte deposition in favor of the high-dose rivaroxaban treatment compared to enoxaparin ($P = 0.002$), whereas no significant difference compared to UFH was found.

The calculated fibrin deposition score, based on fibrin evaluation using an arbitrary scale, was 0.7 ± 0.37 in group 1 (UFH), 0.55 ± 0.12 in group 2 (enoxaparin), and 0.25 ± 0.13 in group 3 (rivaroxaban 300 ng/ml). Evaluating fibrin deposition (Fig. 5c) as the third parameter of thrombus formation assessed electron microscopically, high-dosed rivaroxaban treatment again generated less adherent fibrin masses than did the other treatment groups. Post-hoc analysis, however, did not demonstrate statistically significant differences between the groups concerning fibrin deposition.

Electron micrographs regarding the low-dose rivaroxaban group were prepared but not analysed continuative due to massive thrombotic debris adherent to the valves, precluding a conclusive analysis.

Laboratory analyses

As a specific parameter indicating the anticoagulant activity of UFH, ACT achieved partial supratherapeutic anticoagulant levels. Initial (0 min) mean ACT was 257 ± 10 s and reached a maximum mean ACT level at 405 ± 18 s after 30 min. This was followed by a stable ACT course with only little reduction in ACT for the remainder of the experiment, reaching 378 ± 5 s at the end of the experiment. The TT, as an unspecific and global coagulation parameter, attained therapeutic ranges starting from a mean baseline TZ of 12 ± 0 s, achieving 180 ± 0 s immediately after UFH application (0 min), followed by a slight decrease, reaching 144 ± 22 s after 30 min and 153 ± 16 s after 60 min. The aPTT, also an unspecific parameter for the anticoagulative activity of UFH, initially

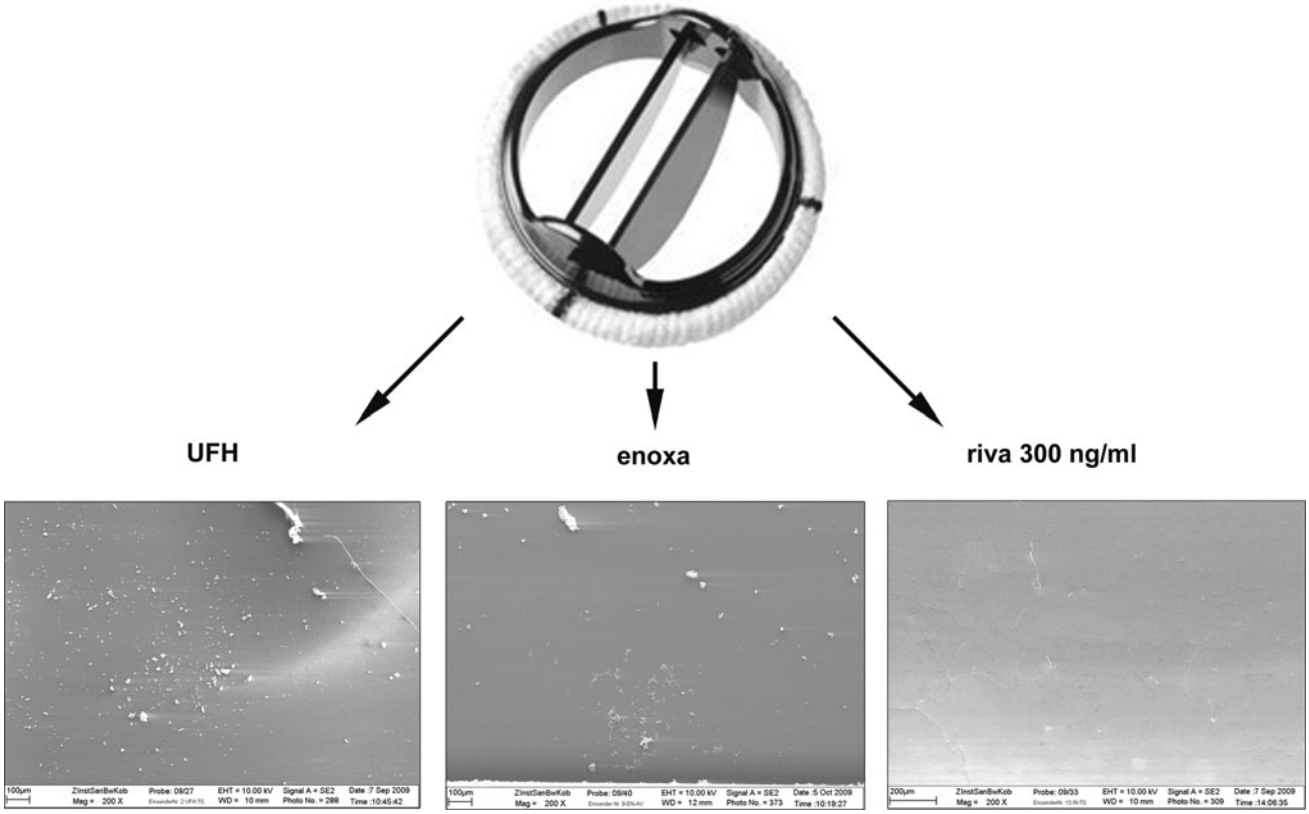

Fig. 4 Exemplary illustration of electron micrographs of thrombus formation on the heart valves in the treatment groups: unfractionated heparin (UFH), enoxaparin (enoxa), rivaroxaban 300 ng/ml (riva)

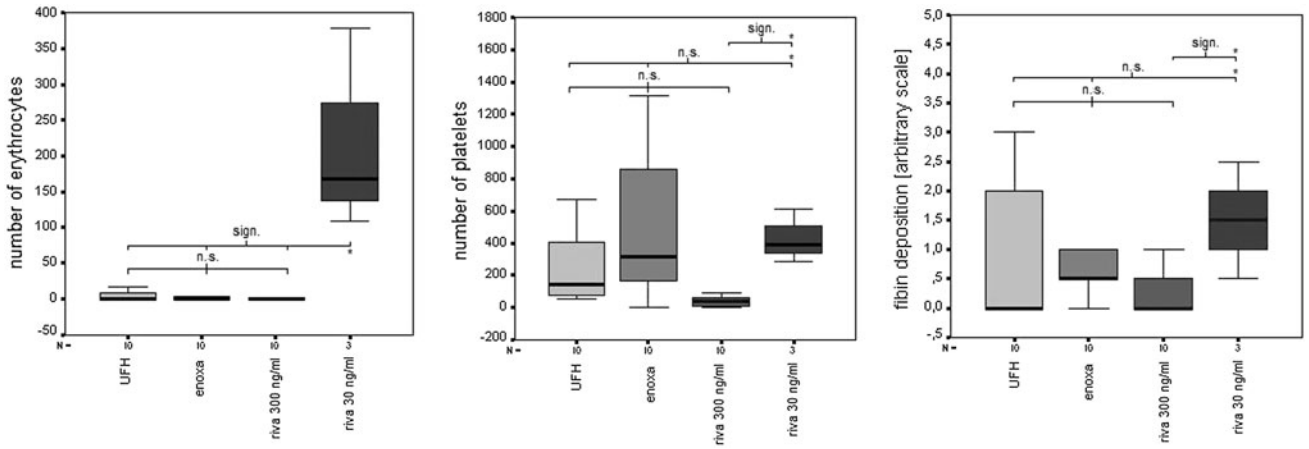

Fig. 5 **a–c** Results from scanning electron microscopy demonstrate the influence of the three anticoagulants on electron-microscopic features of thrombus formation. *Box plots* depict the number of erythrocytes (**a**), platelets (**b**), and the amount of fibrin (**c**) adherent to the heart valve surface. *UFH* unfractionated heparin; *riva* rivaroxaban

increased from the mean baseline of 44 ± 16 s to 156 ± 14 s, followed by a stable course without any decrease throughout the entire duration of the experiment.

Specifically monitoring the anticoagulative activity in the enoxaparin group, n-hep (anti-Xa-activity) measurements showed stable anti-Xa-levels throughout the entire experiment. The maximum anti-Xa-activity of 0.84 ± 0.34 UI/ml was achieved at the start of the experiment, showing a negligible decrease in the course and finally reaching 0.78 ± 0.41 UI/ml at the end of the experiment.

Anti-Xa-activity measurement in the rivaroxaban groups showed stable courses of anti-Xa-levels.

Mean baseline anti-Xa-activity was 0.056 ± 0.011 U/ml. Immediately after anticoagulation with rivaroxaban 300 ng/ml (0 min) anti-Xa-activity was 1.45 ± 0.013, after 30 min 1.44 ± 0.018, and after 60 min 1.44 ± 0.017 U/ml, whereas low-dosed rivaroxaban at 30 ng/ml achieved anti-Xa-activities at 0.70 ± 0.10 (0 min) and 0.66 ± 012 U/ml after 60 min.

Although the TT does not represent a recommended value for monitoring of the anticoagulant effect of rivaroxaban, the TT time course within our experiments was characterized by an initially delayed increase followed by a subsequent decrease for the remaining experimental period. The TT started from a mean baseline of 12 ± 0 s, reaching 14 ± 0 s immediately after administration of rivaroxaban at 300 ng/ml (0 min), slowly increasing to a maximum of 156 ± 8 s within 30 min, and finally decreasing to a mean TT of 136 ± 11 s.

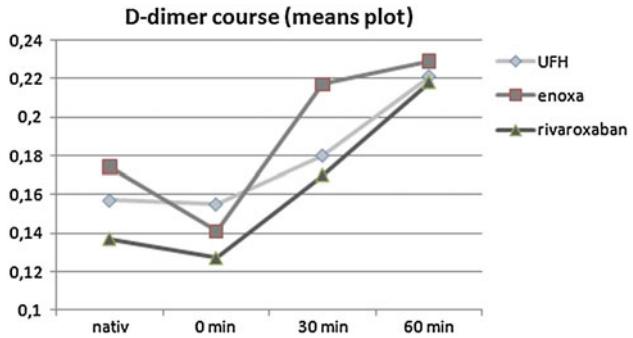

Fig. 6 Mean D-dimer before, at the beginning (0 min), after 30 min, and after 60 min of the experiment. D-dimer levels did not differ significantly between UFH, enoxaparin and high-dose rivaroxaban treatment for the entire duration of the experiment (P-value = 0.986 with ANOVA test for the mean D-dimers after 60 min)

Measuring a second unspecific coagulation parameter, the aPTT levels likewise showed delayed increases after rivaroxaban administration, starting from baseline levels at 32 ± 2 s up to 60 ± 2 s immediately after administration of rivaroxaban at 300 ng/ml (0 min). They reached the maximum level of 180 ± 0 s after 30 min, but remained stable for the rest of the experiment.

D-dimer levels did not differ significantly between UFH, enoxaparin and high-dosed rivaroxaban during the entire experiment (Fig. 6). After 60 min final D-dimer levels in group 1 (UFH) were 0.221 ± 0.041, in group 2 (enoxaparin) 0.289 ± 0.067, and in group 3 (rivaroxaban 300 ng/ml) 0.218 ± 0.023 mg/l. Thus, although not differing significantly from the D-dimer levels achieved by UFH and enoxaparin treatment, lower D-dimer levels were observed for high-dose rivaroxaban treatment than for the other anticoagulants tested.

Determination of erythrocytes, platelets, and hemoglobin levels showed mild decreases (Table 1) within all hematologic parameters corresponding to a slight consumptive coagulopathy and hemolysis as common and previously described by other groups in invitro investigations especially when mechanical heart valves are involved [19–21].

Discussion

Lifelong oral anticoagulation is considered to be essential to prevent thromboembolic events after implantation of biological or mechanical heart valves. However, several situations necessitate interruption of the OAC, for example, in preparation for surgical procedures or nonsurgical interventions such as cardiac catheterization. Interrupting

Table 1 Hematology

	0 min	60 min
Number of thrombocytes [gpt/l]		
UFH (mean ± SD)	221,43 ± 42,38	179,10 ± 29,97
Enoxaparin (mean ± SD)	205,40 ± 32,52	168,80 ± 29,86
Rivaroxaban 300 ng/ml (mean ± SD)	228,30 ± 30,64	172,50 ± 12,63
Rivaroxaban 30 ng/ml (mean ± SD)	213,64 ± 31,01	87,09 ± 57,18
Number of erythrocytes [gpt/l]		
UFH (mean ± SD)	5,19 ± 0,14	4,24 ± 0,28
Enoxaparin (mean ± SD)	5,09 ± 0,34	4,16 ± 0,16
Rivaroxaban 300 ng/ml (mean ± SD)	5,08 ± 0,41	4,14 ± 0,33
Rivaroxaban 30 ng/ml (mean ± SD)	5,08 ± 0,31	3,39 ± 0,67
Hemoglobin [g/dl]		
UFH (mean ± SD)	9,94 ± 0,21	9,01 ± 0,47
Enoxaparin (mean ± SD)	9,60 ± 0,54	8,71 ± 0,40
Rivaroxaban (mean ± SD)	9,73 ± 0,65	8,95 ± 0,55
Rivaroxaban 30 ng/ml (mean ± SD)	9,79 ± 0,61	7,94 ± 0,82

Mean platelet (gpt/l), erythrocyte (gpt/l) count and hemoglobin concentration (g/dl) at the beginning of the experiment (0 min) and at the end (60 min). *UFH* unfractionated heparin, *SD* standard deviation, *l* liter, *g* gram

anticoagulation treatment in patients with mechanical heart valves still poses unsolved problems such as bleeding or thromboembolic events. Not even one prospective, double-blind study has compared the 'gold standard' of intravenous UFH with alternative, subcutaneous LMWH therapy. New oral anticoagulants are promising alternatives for bridging therapy or even long term therapy in patients after mechanical heart valve replacement.

Rivaroxaban, a direct and selective coagulation Factor Xa inhibitor, is such a new drug. It affects clot-associated and free FXa activity as well as prothrombinase activity [22, 23]. Rivaroxaban is distinguished by a high oral bioavailability, a low propensity for food and drug interactions [24–27], a fast onset of action (maximum plasma concentrations are reached after 1.5–2 h), dose-dependent factor Xa-activities, and a balanced elimination, including hepatic metabolization as well as unchanged renal excretion [28–30]. Thus, pharmacokinetics are predictable, a fixed, once-daily dosing scheme is possible [23, 29] and problems such as avoiding accumulation of the drug, frequent coagulation monitoring, and dose adjustments are not clinically relevant [30, 31]. Furthermore, rivaroxaban is supposed to miss interactions with HIT-antibodies [32].

This new drug was primarily licensed in the European Union in 2008 after demonstration of its efficacy within the RECORD 1–4 [33–36] studies for the prevention of deep vein thrombosis and pulmonary embolism after total knee and hip replacement.

Recently a phase II study, the ATLAS ACS TIMI 46 study [37], generated the hypothesis of an existing trend towards a reduction in secondary cardiovascular events associated with rivaroxaban treatment as compared with placebo in patients after ACS under additional treatment with aspirin (ASS) with/without a thienopyridine. Although recently the phase III APPRAISE-2 study examining the use of apixaban, another selective factor Xa-inhibitor, in patients suffering from ACS, had to be halted prematurely due to increased bleeding complications, data from phase III studies using rivaroxaban in this setting are still outstanding. Currently the efficacy of rivaroxaban has been studied in comparison to warfarin in the ROCKET AF study as concerns prevention of stroke in patients with atrial fibrillation (AF) with promising results [38].

Although there is no need for monitoring under rivaroxaban treatment in clinical practice, in our in vitro study the orgaran-calibrated anti-Xa-activity-assay proved to be a good specific coagulation parameter for monitoring the anticoagulative activity of rivaroxaban: anti-Xa-activity levels were stable throughout the entire experiment after bolus administration. According to a recently published study [39], in our study the TT showed, in contrast to stable anti-Xa-levels using the orgaran-calibrated assay, a delayed increase and paradoxical decreases at the end of the experiment due to the development of thrombotic material on the valves thus reducing coagulation factors and fibrinogen and secondary affecting the TT. In conclusion the TT was shown to be not meaningful in displaying the anticoagulative activity of rivaroxaban.

The aPTT levels likewise showed delayed increases after rivaroxaban administration, finally reaching maximum levels of 180 ± 0 s after 30 min, but remaining stable for the rest of the experiment. According to recent findings [39], the aPTT, thus showing concentration-dependent increases after rivaroxaban administration, is not recommended to be an appropriate tool to measure rivaroxaban, due to the nonlinear relationship between the rivaroxaban concentration and the aPTT response as well as the great variability between different instruments and reagents used to measure the aPTT.

In conclusion, high-dosed rivaroxaban at 300 ng/ml plasma was shown to be as effective as UFH and enoxaparin in preventing mechanical heart valve associated thrombosis in the present study. These findings were confirmed by comparable generation of thrombus masses and missing significant differences in the electron microscopic analyses of the thrombotic debris (erythrocytes, thrombocytes, and fibrin deposition). Although there was a reduction in platelet deposition in favor of high-dose rivaroxaban as compared to enoxaparin ($P < 0.05$), a partial imbalance in the achieved level of anticoagulation comparing UFH, enoxaparin and high-dose rivaroxaban has to be accentuated discussing these results. Although ACT levels were initially targeted at 300–350 s as recommended for anticoagulant bridging therapy using UFH, in this study initial mean ACT was 257 s after bolus application of UFH followed by an increase up to 405 s after 30 min of experimental duration, thus achieving supratherapeutic ranges. Additionally high-dose rivaroxaban application also achieved supratherapeutic anti-Xa-activities using the orgaran-calibrated anti-Xa-activity assay. In conclusion, this study compared enoxaparin at therapeutic ranges with partial supratherapeutic UHF and high-dosed rivaroxaban treatment, thus resulting in a relative underdosing of enoxaparin.

However, low-dosed rivaroxaban, despite achievement of low therapeutic anti-Xa-activities, generated a massive increase in all parameters of thrombus formation including electron microscopic features, thus differing significantly from all other treatment groups regarding the thrombus weight.

Discussing the potential of rivaroxaban for perioperative bridging one has to consider the required off-set time for oral administration immediately prior to surgery. Although currently there are no official recommendations published, the mean terminal half life of rivaroxaban is 7–11 h suggesting that the drug should be stopped 24 h before

surgery. However, the time window depends on the relevant rivaroxaban dosage, which has to be identified in clinical studies as stated above.

According to our results we hypothesize that high-dosed rivaroxaban might be effective in preventing thromboembolic events after mechanical heart valve replacement and would therefore be an attractive alternative to UFH and LMWH in bridging therapy. Although not specifically tested in our study, this interesting new anticoagulant will perhaps even become a worthwhile alternative to VKA for OAC. Properly evaluating future indications of rivaroxaban prospective randomized trials are still outstanding and have to be carried out to bring more light into the possibilities of this promising substance.

Acknowledgments This study was funded by an unrestricted grant from BAYER-Schering® Pharma.

Conflicts of interest Mrs. Elisabeth Perzborn is an employee of Bayer HealthCare®.

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