

# Comparison of Fondaparinux, Low Molecular-Weight Heparin and Unfractionated Heparin in Preventing Thrombus Formation on Mechanical Heart Valves: Results of an In-Vitro Study

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**Background and aim of the study:** The study aim was to investigate the efficacy of three different anticoagulants in preventing thrombus formation on mechanical heart valves, using an in-vitro system.

**Methods:** Blood samples (470 ml) were taken from young and healthy male volunteers and anticoagulated with unfractionated heparin (UFH; n = 18), low molecular-weight heparin (LMWH; n = 18) or fondaparinux (n = 16). Bileaflet mechanical heart valves were placed in a new device - the 'Thrombosis Tester' - and exposed in a continuous circulation at a rate of 80 beats per min to the anticoagulated blood samples for a total exposure time of 60 min. Results for thrombus weight were skewed distributed and presented as log-transformed values.

**Results:** The weight of each valve was measured

before and after 1 h of perfusion; subsequent mean ( $\pm$ SD) thrombus weights were  $0.739 \pm 0.573$  g for UFH,  $0.789 \pm 0.099$  g for LMWH, and  $0.934 \pm 0.145$  g for fondaparinux ( $p = 0.397$  for comparison of all groups by ANOVA). Electron microscopy showed concordant results with regard to thrombus formation on heart valve surfaces.

**Conclusion:** Fondaparinux and LMWH were as effective as UFH in preventing thrombus formation on mechanical heart valves in vitro. The 'Thrombosis Tester' proved to be a new, unique instrument for investigating the ability of anticoagulants to prevent valve thrombosis on mechanical heart valves under in-vitro conditions.

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Lifelong oral anticoagulation (OAC) is considered to be essential for the prevention of thromboembolic events following the implantation of a mechanical heart valve (1). OAC treatment may be interrupted, however, in preparation for surgical procedures or non-surgical interventions such as cardiac catheterization. The 'gold standard' for adapting oral to parenteral anticoagulation in patients at high risk for thromboembolic complications is intravenous administration of unfractionated heparin (UFH) (2). To monitor this drug regimen, coagulation parameters (activated partial thromboplastin time; aPTT) must be assessed up to twice daily. Moreover, UFH must be infused constantly to ensure an adequate effect. Anticoagulation by UFH is associated with bleeding

and other complications that are related to the compound's low bioavailability, short plasma half-life, and other effects such as platelet activation and heparin-induced thrombocytopenia. Because considerable inter-individual differences occur with regard to UFH treatment, it is difficult to find an optimal dosage (3-5).

In several studies conducted during the past few years, low molecular-weight heparin (LMWH), when administered subcutaneously, has been found to be at least as effective as intravenous UFH in patients with pulmonary embolism, deep-vein thrombosis or acute coronary syndromes (6-9). Comparative studies have shown that anticoagulation by subcutaneously administered LMWH seemed to be safe in patients with mechanical heart valves (10-12). To the best of the present authors' knowledge, the efficacy of subcutaneous LMWH in comparison to intravenous UFH in preventing heart valve thrombosis has never been investigated in larger, randomized, prospective studies. However, subcutaneous LMWH is now being widely used as an alternative approach to intravenous UFH in patients with mechanical heart valves (13-15), a fact reflected in an endorsement by the American College of Chest Physicians for subcutaneously administered

This study is dedicated to Professor Helmut Reul, who died in 2004 at the age of 61.

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LMWH to manage anticoagulation during invasive procedures (16).

Fondaparinux, a pentasaccharide, is a selective inhibitor of coagulation factor Xa, and interrupts the blood coagulation cascade by inhibiting thrombin generation and the development of thrombus, without inactivating thrombin (17). This drug has been shown to be superior to LMWH in the prevention of deep-vein thrombosis after hip or knee replacement (18,19), and to be at least as effective and as safe as LMWH and UFH in patients with pulmonary embolism and deep-vein thrombosis (20).

It was hypothesized that fondaparinux might also be effective in preventing heart valve thrombosis. In 2003, a study was conducted to compare the efficacy of fondaparinux, LMWH and UFH in preventing valve thrombosis in an animal model (21). No differences were found in thrombus weight, while scanning electron microscopy failed to demonstrate any inter-group differences in terms of blood cell and fibrin deposition on the heart valve leaflets. It was concluded that fondaparinux and LMWH were at least as effective as UFH in preventing heart valve thrombosis in this small animal model (21).

However, due to several limitations of this model, other experimental conditions were sought under which anticoagulants could be compared with regard to their ability to prevent heart valve thrombosis, the aim being to prove the hypothesis that LMWH and fondaparinux are effective in preventing this condition.

## Materials and methods

### The THIA thrombosis tester

In order to conduct detailed research on heart valve thrombosis, an in-vitro test rig is required which itself has a negligible impact on thrombus formation. The tested heart valves must be easily assembled and disassembled, while during the test runs it must be both feasible and straightforward to take blood samples and/or to include drug additions. One further requirement is the use of human blood, since a standard blood bottle is limited to a volume of ca. 500 ml.

The THIA (Thrombostester Helmholtz Institut Aachen) thrombosis tester (Fig. 1), which was developed at the Helmholtz-Institute for Biomedical Engineering, RWTH-Aachen, Germany, overcomes these limitations and accomplishes the above-mentioned requirements when low or no-flow areas in heart valves are investigated in vitro. The test system permits in-vitro thrombogenic testing at blood volumes ranging from 300 to 800 ml, while comparative analyses not only of the inserted heart valves but also of the blood itself may be carried out. The concept of the THIA thrombosis tester (Fig. 2) is based on fluid

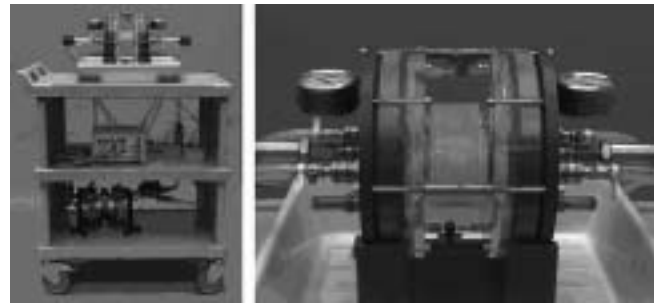


Figure 1: The THIA (Thrombostester Helmholtz Institut Aachen) thrombosis tester.

circulation in a blood chamber, separated by two mechanical heart valves assembled in oppositely opening directions.

The heart valves with silicone retainers are inserted into a flow-optimized and biocompatible polymethylmethacrylate (PMMA) compartment (constructed from acrylic glass) bordered by two elastic, biocompatible silicone membranes. The membranes bulge periodically due to compressed air pressure, and alternately force the fluid inside the blood chamber to circulate through the heart valves. The system air pressure and flow can be adjusted using regulators and throttles located at the inlet and outlet of the chambers, respectively.

During the experiments, the air chamber pressure is measured by pressure sensors in order to control the performance of the thrombosis tester. The differential pressure across the tested heart valves may also be monitored. An example of the differential pressure across a valve at 70 bpm is shown in Figure 3.

With an electronic control unit, the cycle rate of the tester, and therewith the beating rate of the heart valves, may be adjusted between 10 and 140 bpm. One tester cycle corresponds to one filling/bleeding phase of both air chambers, or one opening/closing phase of each heart valve, respectively.

An integrated data acquisition interface (DAQ-inter-

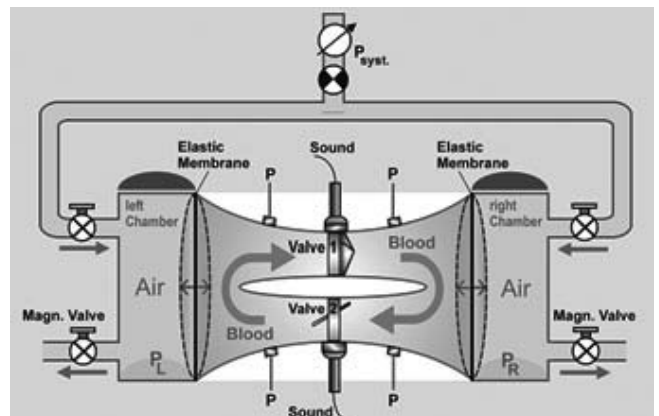


Figure 2: Schematic set-up of the THIA thrombosis tester.

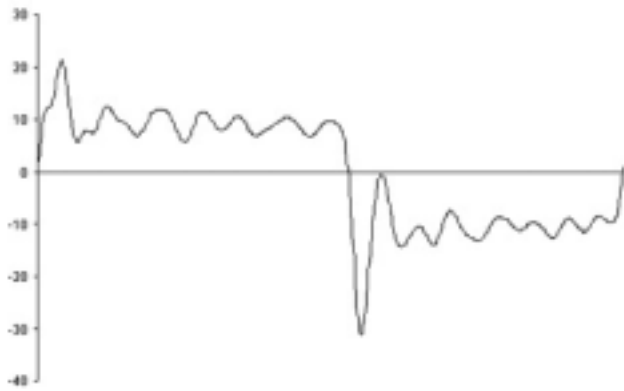


Figure 3: Differential pressure across a valve at 70 beats per min.

face) provides the opportunity of acquiring all relevant data (air chamber pressure, heart valve pressure, cycle rate). The monitoring and data acquisition software (LabView 7 Express; DAQ-Card 6024E, National Instruments, USA) enables pressure data acquisition at a default sample rate of 1,000 samples per second, and displays and/or saves data on user demand per tester cycle.

In order to use this set-up as an in-vitro thrombogenic tester, a negligible impact on thrombus formation (blood clotting) of the test system itself must be proven in advance. Hence, several investigations were conducted in the tester with a variety of mechanical heart valves and with non-anticoagulated blood. Numerous thrombus formations occurred for all valves used, but no thrombus formation was detected at the tester itself, with all surfaces (silicone and PMMA) of the tester being thrombus-free (Fig. 4). These data confirmed that the THIA tester would be well-suited for any investigations on thrombogenic effects.

#### Experimental setting

For initial experiments comparing different anticoagulants, venous blood samples (470 ml each) were taken from 26 healthy male blood donors. The blood was placed directly into a blood bag prepared with an anticoagulant: UFH (Liquemin N, Roche, Germany; n = 9); LMWH (Fragmin D, Pharmacia, Germany; n = 9); or fondaparinux (Arixtra, Sanofi-Synthelabo, France; n = 8). In order to measure aPTT levels, anti-Xa activity and fondaparinux concentrations, blood samples were collected into standard tubes containing sodium citrate (Braun, Melsungen, Germany)

Three different types of mechanical heart valve, namely Sulzer Carbomedics®, On-X® and ATS were



Figure 4: The inner surface of the thrombosis tester after an experiment with non-anticoagulated blood.

used in the investigations. The number of different heart valves was similar in each treatment group. Heart valves were exposed to blood for a total of 60 min, at a simulated heart rate of 80 bpm, in all experiments. All heart valves were removed from the thrombosis tester on completion of the exposure time. In order to quantify thrombi development on the valves, each valve was weighed before and after exposure; the difference in weights indicated the degree of thrombus formation and adherence.

Subsequently, scanning electron microscopic analyses (LEO 1530 instrument) were performed for further evaluation of the thrombi. The valves were placed in Sorensen solution (pH 7.4; 20 ml solution A: 0.1 M  $\text{KH}_2\text{PO}_4$  and 80 ml solution B: 0.1 M  $\text{NaHPO}_4$ ) for ca. 60 s to remove blood from the tissue surface. The valves were then placed in 25% glutaraldehyde (Merck, Germany) to fix the adhering thrombi. Following fixation, the valves were dehydrated in a graded series of acetone (Sigma Aldrich, Germany), and then further treated with hexamethyldisilazane (HMDS; Sigma Aldrich) to prevent oxidation. After washing, dehydration, and fixation, the heart valves were dried overnight in an aluminum plate under an exhaust system.

The APTT and anti-Xa activity were assessed using routine methods. Plasma fondaparinux concentrations were determined using a modified version of a commercially available anti-Xa activity test (Coamatic LMW Heparin Chromogenix, Milan, Italy) and a Hitachi 911 analyzer (Roche Diagnostics, Mannheim, Germany). The assay was calibrated for different concentrations of fondaparinux as standards.

The study was approved by the ethics committee of the Johannes Gutenberg-University Mainz, Germany. Participation of the blood donors was voluntary, and

each participant provided their written, informed consent.

### Statistical analysis

Differences between the three groups were assessed using ANOVA with post-hoc analysis. Results for thrombus weight were skewed distributed and presented as log-transformed values. All p-value computations were carried out using SPSS software (V11.5).

### Results

In order to compare treatment groups, the aim was to create the therapeutic range of aPTT, anti-Xa activity or fondaparinux concentration for the respective anticoagulant.

In experiments with UFH, the initial dose was 500 IU, over-anticoagulation was identified with aPTT >120 s, almost no thrombus adhering to the valve (as shown by scanning electron microscopy), and very low thrombus weight. In subsequent experiments, UFH was titrated down to a mean ( $\pm$ SD) of  $70 \pm 36$  IU, leading to an aPTT in the clinically relevant range of  $101 \pm 24.5$  s.

For LMWH, higher doses resulted in an anti-Xa activity of  $>1.5$  U/ml. Finally,  $75 \pm 12$  IU LMWH was used to produce a mean anti-Xa activity in the therapeutic range of  $0.69 \pm 0.42$  U/ml.

Initial studies with low-dose (0.5 mg) fondaparinux produced a plasma concentration of  $0.79 \pm 0.57$   $\mu$ g/ml (therapeutic range 0.5-1.0  $\mu$ g/ml). Experiments (n = 3) with saline as a control failed due to complete clotting of the heart valves after 15-20 min.

Tissue weight analyses before and after 1 h of perfusion indicated mean thrombus weights of  $0.739 \pm 0.573$  g for UFH (n = 18),  $0.789 \pm 0.099$  g for LMWH (n = 18), and  $0.934 \pm 0.145$  g for fondaparinux (n = 16) (p = 0.397 for comparison of all groups by ANOVA, log-transformed values were presented for skewed distribution).

Comparison of thrombus weight by post-hoc analyses showed no differences between the treatment groups; p-values were 0.929 (UFH versus LMWH), 0.383 (UFH versus fondaparinux), and 0.593 (LMWH versus fondaparinux).

Comparison by scanning electron microscopy showed similar results with regard to thrombus formation on the heart valve surfaces (Fig. 5).

### Discussion

In the present study, a new method was developed for the ex-vivo comparison of anticoagulant efficacy in preventing heart valve thrombosis. The study results showed fondaparinux and LMWH to be as effective as

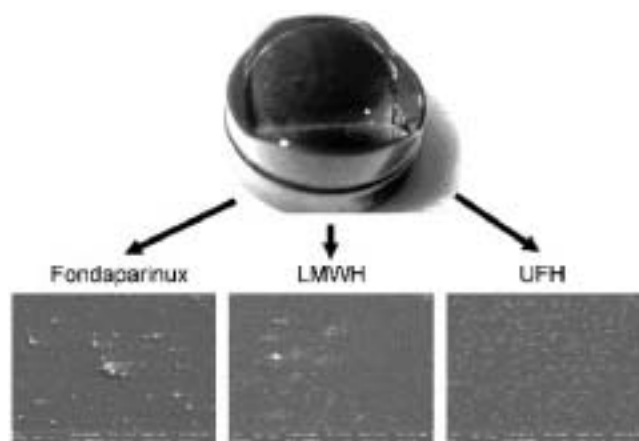


Figure 5: Electron micrographs of thrombus formation on heart valves in the three treatment groups: Fondaparinux, low molecular-weight heparin (LMWH), and unfractionated heparin (UFH).

UFH in preventing thrombus formation on mechanical heart valves in vitro, as shown by the degree of thrombus formation after 1 h of perfusion.

The interruption of anticoagulation treatment in patients with mechanical heart valves is an unsolved problem. Few large, prospective, double-blind studies have been reported comparing the 'gold standard' of intravenous UFH and the alternative therapy of subcutaneous LMWH. In order to obtain additional information regarding the ability of anticoagulants to prevent heart valve thrombosis, a new experimental method using a rabbit model was developed in 2003 (21), but had several limitations. Most importantly, a flow chamber was used with a fixed heart valve leaflet, and thus conditions in the chamber were static, with a more laminar flow (21,22). The newly developed THIA thrombosis tester is a unique in-vitro system which permits investigation of the ability of anticoagulants to prevent valve thrombosis on beating mechanical (or biological) heart valves, using human blood. In the present study, a heart rate of 80 bpm was used, though anticoagulants could be compared using different rates in this model.

Both the present and previous investigations showed thrombus formation on mechanical heart valves to be similar following anticoagulation with LMWH and UFH. This underlined the findings of another report in patients with mechanical heart valves whereby short-term LMWH therapy compared favorably with UFH (23). Likewise, based on the results of the present study, it is hypothesized that fondaparinux might be effective in preventing thromboembolic events after mechanical heart valve replacement, and would therefore be an alternative to UFH and LMWH. However, to the best of the present authors' knowledge, clinical data in patients receiving mechanical heart valves and

treatment with fondaparinux are lacking.

*In conclusion*, by using the THIA thrombosis tester, UFH, LMWH and fondaparinux were shown to have comparable effects on thrombus formation. However, the predictive accuracy of the system requires further investigation with regard to antithrombotic regimens following mechanical heart valve replacement.

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