Comparison of bivalirudin, enoxaparin, and unfractionated heparin in preventing cardiac catheter thrombosis

Results of an in-vitro study

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Summary

Bivalirudin, a direct thrombin inhibitor binds specifically and reversibly to both fibrin-bound and unbound thrombin. Bivalirudin is approved for use as an anticoagulant in patients undergoing percutaneous coronary intervention. The OASIS-5 trial presented a significant increase in cardiac catheter thrombosis for the pentasaccharid fondaparinux compared to enoxaparin. Catheter thrombosis has never been reported in any trial using bivalirudin. Our study compared the development of catheter thrombosis for bivalirudin, enoxaparin, and unfractionated heparin in a controlled in-vitro environment. Ten healthy male volunteers were pretreated with aspirin 500 mg 2 hours before venesection of 50 ml of blood. The seven groups of anticoagulant combinations tested were: UFH, UFH + eptifibatide, enoxaparin, enoxaparin + eptifibatide, bivalirudin bolus, bivalirudin + eptifibatide, bivalirudin bolus + continuous infusion. The blood/anticoagulant mix continuously circulated through a cardiac guiding catheter for 60 minutes or until the catheter became blocked with thrombus. Thrombus development was assessed by weighing each catheter before and after the procedure. Electron microscopy was used to quantify the degree of erythrocyte, platelet and fibrin deposition. Following anticoagulation with bolus dose bivalirudin, the catheter was invariably occluded with thrombus after 33 minutes of circulation. However, a continuous infusion of Bivalirudin prevented the development of occlusive catheter thrombosis. In the bolus bivalirudin group the mean thrombus weight was significantly greater than in all other groups (p-value < 0.01 in all analyses). Bivalirudin given as a bolus was not sufficient to prevent cardiac catheter thrombosis in our in-vitro study. However, a continuous infusion of bivalirudin had similar anti-thrombotic efficacy compared to other treatment strategies.

Keywords

Cardiology, heparins, coagulation inhibitors, drug design, thrombosis

Introduction

Antithrombotic therapy is a crucial component of interventional cardiology. The benefits of this treatment in preventing stent thrombosis, equipment thrombosis (including catheter thrombus formation) and ischaemic complications in patients must be weighed against the risk of inducing bleeding complications. The increase in bleeding risk is associated with a higher risk of haemorrhagic events and death (1). The shortcomings of currently available anticoagulant drugs have promoted the ongoing development of new, powerful anticoagulant agents that demonstrate both antithrombotic efficacy and a reduced risk of bleeding.

The OASIS-5 trial showed that the selective coagulation cascade inhibitor fondaparinux, a factor Xa inhibitor, was superior to enoxaparin regarding bleeding and ischaemic complications. However, treatment with fondaparinux was associated with a significant increase in the development of a rare, unexpected adverse event: guide catheter thrombosis (2).

For fondaparinux we were able to show in our first in-vitro study that regardless of the dosage, cardiac catheter thrombosis always developed in experiments using fondaparinux, also at subtherapeutic dosages (3).

Bivalirudin is one of the more recently developed antithrombotic agents that reversibly targets the thrombin molecule, a key

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Received April 8, 2008
Accepted after major revision July 23, 2008
Prepublished online September 5, 2008
doi:10.1160/TH08-04-0220

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New Technologies, Diagnostic Tools and Drugs
factor in the coagulation cascade (4). It is a synthetic 20-amino acid peptide analogue of hirudin, a direct thrombin inhibitor (DTI) that binds specifically and reversibly to both fibrin-bound and unbound thrombin (5). DTIs have antiplatelet and anticoagulant effects but do not bind plasma proteins, thereby providing a more consistent dose-response effect than unfractionated heparin (UFH) (6). Intravenous bivalirudin has been approved for use in patients undergoing percutaneous coronary interventions (PCI) (1). Recently, the ACUTY trial found that clinical outcomes are comparable in patients with acute coronary syndrome (ACS) treated with glycoprotein IIb/IIIa inhibitors and in whom PCI is performed if UFH or enoxaparin is substituted by bivalirudin (7). Moreover, bivalirudin alone suppressed adverse ischaemic events to an extent similar to that of UFH plus glycoprotein IIb/IIIa inhibition, while significantly lowering the risk of major haemorrhagic complications (8, 9). Cardiac catheter thrombosis has not been reported in the ACUTY or any other trial using bivalirudin so far.

The incidence of catheter thrombosis during bivalirudin anticoagulation has only been investigated in patients undergoing brachytherapy (the Brachytherapy and bivalirudin evaluation = BRAVES study). This study showed an increased incidence of acute procedural intracoronary thrombosis in patients undergoing brachytherapy with β- and γ-radiation despite bivalirudin bolus and infusion anticoagulation (10, 11).

Administering bivalirudin as a single bolus during PCI without subsequent infusion must be considered clinical malpractice, since only the bolus plus infusion treatment is recommended (12, 13). Thus, given the mounting data supporting the use of bivalirudin during PCI and an unknown potential for catheter thrombosis, together with clinical practice that utilizes a range of bolus and infusion dosing regimens, we employed an in-vitro model to study the development of cardiac catheter thrombosis with bivalirudin (as bolus alone, or bolus plus continuous infusion), eptifibatide, UFH and enoxaparin as single agents or in combination.

Materials and methods

Ten healthy male volunteers were pretreated with 500 mg aspirin orally 2 hours before venesection and withdrawal of 50 ml of blood. The blood was collected into sample tubes containing anticoagulant. Seven groups of anticoagulant combinations were tested, and volunteers donated blood seven times, thus acting as their own controls. The groups were: group 1: UFH 0.8U/ml, group 2: UFH plus eptifibatide 1.7 µg/ml, group 3: enoxaparin 0.6U/ml, group 4: enoxaparin plus eptifibatide, group 5: bivalirudin bolus 0.3 µg/ml, group 6: bivalirudin bolus plus eptifibatide, and group 7: bivalirudin bolus plus continuous infusion (20 ± 5 µg/ml) of bivalirudin. The continuous infusion of bivalirudin was administered via a perfusion pump into the blood collecting system. The blood/anticoagulant mix was kept at 37°C (using a water bath) and continuously circulated (at a ‘slow’ flow rate of 3 ml/min by roller pump) through a 6F Multipurpose guiding catheter. All catheters were removed from the circulation, either when the exposure time of 60 minutes was completed or the catheter had been occluded by thrombosis and thus stopped the experiment. If thrombotic catheter occlusion occurred before 60 minutes (min) of circulation, this point was recorded. The mass of thrombus within each catheter was assessed by weighing each catheter before and after the procedure.

The study was approved by the ethics committee of the Martin Luther-University Halle-Wittenberg, Germany. Participation of the blood donors was voluntary, and participants provided their written informed consent.

Electron microscopic analysis

Electron microscopic analysis of the catheter lining was used to quantify the degree of erythrocyte and fibrin deposition. For this purpose, Sorensen solution (pH=7.4; 20 ml of solution A: 0.1 M KH₂PO₄ and 80 ml of solution B: 0.1 M NaH₂PO₄) was introduced into the circulation for approximately 60 seconds (s) to clean the catheter of blood. Thrombolytic agent that had formed in the catheter were fixed by introducing 25% glutaraldehyde into the circulation for a further 10 min. Thereafter, a 1 cm of the catheter tip was cut into half longitudinally. The catheter tips were dehydrated in a graded series of acetone solution. To prevent oxidation, they were further treated with hexamethyldisilazane. Tips were then dried overnight in an aluminium plate under an exhaust system (14).

For scanning electron microscopic analysis a LEO 1530 scanning electron microscope (Gatech®, Atlanta, GA, USA) was used to count the number of erythrocytes and to quantify the fibrin deposition on the inner surface of the catheter tips. The assessment was made in a 100x150 µm region central to the catheter tips (Fig. 1), the area where catheter-associated thrombi typically develop. A central field was selected to avoid edge artefacts which were observed in preliminary studies. The region of analysis central to the catheter tip was photographed, and the number of erythrocytes and platelets in this area counted. Fibrin deposition was quantified according to a scale from 0–4 (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 undertaken by two independent investigators blinded to the anticoagulant used.

Laboratory methods

For further comparison of the treatment groups, coagulation parameters (aPTT, ACT and anti-Xa activity) were compared (at baseline and end of each experiment) by using routine methods of the Johannes Gutenberg-University at Mainz.

Statistical analysis

Differences between the three groups were assessed using ANOVA with post-hoc analysis. All p-value computations were carried out using SPSS software (V11.5).

Results

Experiments were stopped after a maximum circulation time of 60 min. All experiments in groups 1 (UFH), 2 (UFH plus eptifibatide), 4 (enoxaparin plus eptifibatide), and 7 (bivalirudin bolus plus infusion) reached the maximum 60-min circulation time without thrombotic occlusion developing in the catheter. The mean circulation time in group 3 (enoxaparin) was 59.2 ± 2.5 min. Only one of the 10 experiments did not run for the total time of 60 min. In group 5 (bivalirudin bolus) the mean circulation
time was 33.1 ± 8.7 min and in group 6 (bivalirudin plus epifibatide) 41.2 ± 2.9 min. None of the 10 experiments in either group reached the 60-min mark (Table 1). The mean circulation time before thrombotic catheter occlusion in groups 5 and 6 was significantly lower than in all other treatment groups (p-value < 0.01).

**Thrombus weight**

The mean thrombus weight in group 1 (UFH) was 237 ± 60 mg, in group 2 (UFH plus epifibatide) 186 ± 52 mg, in group 3 (enoxaparin) 220 ± 47 mg, in group 4 (enoxaparin plus epifibatide) 196 ± 47 mg, in group 5 (bivalirudin bolus) 308 ± 79 mg, in group 6 (bivalirudin plus epifibatide) 255 ± 47 mg, and in group 7 (bivalirudin bolus + infusion) 225 ± 132 mg.

The thrombus weight was statistically similar in all treatment groups except for group 5 (bolus bivalirudin), in which the thrombus weight was significantly greater than in all other groups (p-value < 0.01 in all analyses).

**Anticoagulation**

The anticoagulant effect of each treatment group (which can be related to similar assays in the clinical setting) was assessed by measuring the activated clotting time (ACT), activated partial thrombin time (aPTT) and anti-Xa activity before and after each experiment.

The mean ACT in the UFH group was 253 ± 25 s, in the UFH plus epifibatide group 228 ± 25 s, in the bivalirudin bolus group 319 ± 32 s, in the bivalirudin plus epifibatide group 298 ± 29 s, and in the bivalirudin administered as a bolus plus infused continuously group 398 ± 52 s. In group 7 (bivalirudin bolus plus continuous infusion) ACT was also measured after 15, 30, and 45 min. After 15 min the mean ACT was 407 ± 41 s, after 30 min 387 ± 63 s, and after 60 min 262 ± 52 s.

For the enoxaparin and enoxaparin plus epifibatide groups, anti-Xa activity was monitored. In the enoxaparin group the mean activity was 0.84 ± 0.06 IU and in the enoxaparin plus epifibatide group 0.81 ± 0.08 IU.

The aPTTs in groups 1, 2, 5, 6 and 7 were >120 s in all experiments.

**Electron microscopic analysis**

We analysed a standardized region (100 x 150 μm) in the middle of the two halves of the catheter tips: representative examples for all seven groups are shown in Figure 1.

We calculated a fibrin deposition score of 0.93 ± 0.61 in group 1, 1.2 ± 0.22 in group 2, 1.83 ± 1.14 in group 3, 1.82 ± 0.84 in group 4, 1.9 ± 1.02 in group 5, 2.0 ± 0.37 in group 6, and 1.3 ± 0.75 in group 7. Fibrin deposition was (significantly, p < 0.05) higher in groups 5 and 6 than in all other treatment groups (Table 2).

There was no significant difference in the number of catheter adhering erythrocytes. However, erythrocyte deposition tended to be higher in the bivalirudin bolus group: 6.2 ± 7.1 in group 1, 1.7 ± 3.0 in group 2, 28.9 ± 45.7 in group 3, 9.2 ± 7.9 in group 4, 19.7 ± 52.9 in group 5, 6.5 ± 7.4 in group 6, and 1.5 ± 1.5 in group 7 (p-value for comparison between all groups: 0.107). In group 3 (enoxaparin), the number of adhering erythrocytes –171– was highly increased in one of the 10 experiments (Table 2).

In contrast to previous studies using the same method (15, 16), the blinded investigators were unable to reliably and reproducibly quantify the number of platelets, particularly on images with strong thrombus deposition in groups 5 and 6 (Fig. 1).

**Discussion**

Thrombin (coagulation factor IIa) is the centrepiece of the extrinsic and intrinsic coagulation pathways and represents the keystone of the twin processes of thrombosis and haemostasis. Through procoagulant, anticoagulant, and antifibrinolytic mechanisms, thrombin helps maintain vascular integrity in the face of haemorrhage. The same mechanisms, however, allow for the pathologic formation of thrombi in response to endothelial damage, which accompanies the erosion or rupture of endothelial plaques. Alone or incorporated into plaques, thrombi can cause vessel occlusion resulting in ACS (17). Inhibition of factor IIa results in effective inhibition of the coagulation cascade and inhibits thrombus generation (18).

The 20-amino acid peptide and hirudin analogue bivalirudin is a direct thrombin inhibitor whose pharmacological properties differ from those of the heparins. It binds directly with both fluid- and clot-bound thrombin (19).

Recently, the efficacy of newer agents such as bivalirudin and fondaparinux has been shown to be equivalent to that of the heparins in various antithrombotic indications. Bivalirudin and fondaparinux seem to be clinically very attractive due to the lower occurrence of bleeding complications and ischaemic events associated with these drugs (20). Indeed, UFH is likely to be replaced by safer and more effective antithrombins, such as bivalirudin (21).

**Table 1: Circulation time.** Mean circulation time of the seven different anticoagulation groups, standard deviation (SD), and minimum/maximum circulation time in minutes. P-value < 0.01 for comparison between the bivalirudin bolus and bivalirudin bolus + epifibatide groups to all other groups by ANOVA-test. UFH, unfractionated heparin.

<table>
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<th>UFH (n=10)</th>
<th>UFH + Epifibatide (n=10)</th>
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The Fifth and Sixth Organization to Assess Strategies in Acute Ischemic Syndromes (OASIS-5 and OASIS-6) trials compared the efficacy and safety of fondaparinux and enoxaparin in high-risk patients with unstable angina, non-ST elevation myocardial infarction (NSTEMI), and ST elevation myocardial infarction (STEMI). In both studies the risk of catheter-related thrombi was significantly increased in the fondaparinux-treated patients (2, 22).

In the case of bivalirudin, acute intracoronary thrombosis has been reported in patients undergoing vascular brachytherapy (10, 11). There are several similarities between bivalirudin and fondaparinux. Both inhibit only one coagulation cascade factor, bivalirudin factor II (thrombin) and fondaparinux factor Xa. Both have been associated with fewer bleeding complications compared to other antithrombotic agents, inhibiting more than one coagulation factor such as unfractionated heparin or enoxaparin (cascade factors Xa and II) in different trials (OASIS-5 and OASIS-6, ACUITY).

Our study used an in-vitro model to compare different antithrombotic strategies and their ability to prevent thrombus formation in cardiac catheters. Bivalirudin given as a bolus did not prevent cardiac catheter thrombosis in our in-vitro study. However, a continuous infusion of bivalirudin demonstrated an antithrombotic efficacy similar to that of other treatment strategies. Continuous infusion following the bolus of bivalirudin was necessary to get results comparable to those of other treatment regimens, whether by length of experimental period, number of catheter-adherent erythrocytes, or thrombus weights after 1 h of perfusion.

The underlying mechanisms for the higher occurrence of cardiac catheter thrombus formation for fondaparinux in our previous (3) and bivalirudin in our current in-vitro study cannot completely be explained at this point. An important role for a selective factor Xa-inhibitor such as fondaparinux may be promoted by kallikrein. The serine protease kallikrein derives from plasmatic prekallikrein (Fletcher factor) by enzymatic and catalytic activation of factor XIIa. It is a shortcut within the intrinsic coagulation cascade system. Kallikrein is involved in the intrinsic coagulation cascade, the kallikrein-kinin system, fibrinolysis, and inflammatory processes. In-vitro studies have shown that kallikrein, activated by factor XIIa, is able to transform prothrombin to thrombin after contact to artificial surfaces or layers such as cardiac catheters (23). This would explain why inhibiting only one coagulation factor (factor Xa or IIa) is not as effective as with other anticoagulants (e.g. UFH or low-molecular-weight heparins) in preventing thrombus formation on artificial surfaces since UFH and enoxaparin inhibit at least two coagulation factors: Xa (via AT-III) and thrombin.

In this regard, factor XII might represent a new target for preventing thrombosis and pathological coagulation, especially in combination with surface activation, artificial layers, and aggregation. However, series of investigations have convincingly shown that factor XII does not have a role in normal haemostasis (24, 25). Recently, experimentally induced thrombosis in factor XII-knockout mice provided evidence that factor XII-deficient mice are protected against ischaemic brain injury after obstructive clot formation (26). Based on these experiments, blocking factor XII could be a unique target for preventing obstructive clot formation in arterial thrombosis, while lowering the possible side effect of increased bleeding complications (27).

In our study, elective and inhibition of thrombin alone only seems sufficient when factor II is totally and permanently blocked by continuous infusion (as in group 7 of our study) of a direct thrombin inhibitor. The minimum molar concentration of bivalirudin that maintains blood in a fluid state must be higher than the molar concentration of prothrombin (28). In contrast to bival-

Figure 1: Electron microscopy. Scanning electron microscopic analysis (performed with a LEO 1530 scanning electron microscope, Gatech®, Georgia, USA) of the seven different anticoagulation groups.
Bivalirudin and cardiac catheter thrombosis

Maegdefessel et al. Bivalirudin and cardiac catheter thrombosis

Intravenous bivalirudin is approved for use as an anti-coagulant in patients undergoing percutaneous coronary intervention (PCI). However, although development of cardiac catheter thrombosis was found in large trials using unfractionated heparin, low-molecular-weight heparin, and fondaparinux in the PCI-setting, this adverse event was not investigated in trials using bivalirudin.

What does the paper add?
- Bivalirudin given as a bolus was not sufficient to prevent cardiac catheter thrombosis in our in-vitro study.
- However, a continuous infusion of bivalirudin had similar anti-thrombotic efficacy compared to unfractionated heparin and low-molecular-weight heparin.
- The development of catheter thrombosis with bolus bivalirudin in this study underlines the importance of using recommended dosing regimes for bivalirudin with a bolus followed by infusion in patients undergoing PCI.

Limitations
This is an in-vitro study which examined thrombus formation in cardiac guiding catheters under continuous flow conditions for a total time period of 60 min. In the setting of PCI, repeated flushes are performed to administer contrast media and other fluids (such as nitroglycerin or sodium chloride) and thrombus development may additionally be prevented by these flushes. Furthermore, our volunteers took only aspirin rather than aspirin and clopidogrel before blood was drawn. Most elective PCI is undertaken in patients who are receiving an established dual anti-platelet therapy and the use of aspirin alone in this model is at variance with this routine clinical practice. Our model may be more analogous to PCI in the ACS setting when patients often receive

What is known about the topic?
- Intravenous bivalirudin is approved for use as an anti-coagulant in patients undergoing percutaneous coronary intervention (PCI).
- However, although development of cardiac catheter thrombosis was found in large trials using unfractionated heparin, low-molecular-weight heparin, and fondaparinux in the PCI-setting, this adverse event was not investigated in trials using bivalirudin.

Table 2: Fibrin deposition and catheter adhering erythrocytes.
Results of the scanning electron microscopic analysis: mean fibrin deposition (quantified score according to a scale from 0 – 4; 0 = no fibrin deposition, 1 = trace fibrin deposition, 2 = low fibrin deposition, 3 = moderate fibrin deposition, and 4 = heavy fibrin deposition), standard deviation, minimum/maximum fibrin deposition. P-value for fibrin deposition <0.05 by ANOVA-test compared between the bivalirudin bolus and the bivalirudin bolus + eptifibatide groups to all other treatment groups. Mean number of catheter adhering erythrocytes, standard deviation (SD), minimum/maximum number of catheter adhering erythrocytes. P-value = 0.107 for catheter adhering erythrocytes for comparison between all groups by ANOVA-test. UFH, unfractionated heparin.

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irudin, UFH and enoxaparin catalyse the inhibition of thrombin and factor Xa by antithrombin. UFH and subsequently low-molecular-weight heparins do not inactivate factor Xa within the prothrombinase complex. Prothrombinase-bound factor Xa is the enzyme-activating prothrombin. Therefore, it is reasonable that UFH and enoxaparin keep blood in a fluid state in vitro by ensuring that any thrombin formed is rapidly neutralized (29).

Bivalirudin inhibits both fibrinogen binding and proteolytic functions of thrombin. It has a unique pharmacological profile because, unlike other DTIs such as argatroban and lepirudin, it is predominantly eliminated by proteolysis and not via an organ system and has a shorter half-life than argatroban and lepirudin. Its affinity to thrombin is intermediate compared that of argatroban, which is lower, and the higher affinity of lepirudin, which is higher (30). However, thrombin slowly cleaves bivalirudin that is bound to the catalytic site. After cleavage, the affinity of bivalirudin to thrombin decreases, thereby restoring the proteolytic function of thrombin. This means that although the initial binding of bivalirudin to thrombin is complete, later it becomes partial, thus enabling thrombin to participate in haemostatic reactions. Bivalirudin binds stoichiometrically to thrombin at a 1:1 ratio. Each molecule of bivalirudin can inhibit the action of a single molecule of thrombin, unlike heparin, which can free itself and become available for potentiating additional molecules of antithrombin (30). This may be an advantage in most PCIs to reduce the bleeding complications once the procedure is completed, but it probably becomes a disadvantage when only a bolus of bivalirudin is administrated, which may result in increased incidence of cardiac guiding catheter thrombosis as shown in our in-vitro study. Although interesting from a scientific point of view for an in-vitro study, using bivalirudin as a single bolus during PCI without continuing infusion must be considered clinical malpractice (21). Our in-vitro study would emphasize that a continuous infusion of bivalirudin and careful monitoring of ACT must be ensured in the setting of PCI.

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clotidogrel directly before (or during) coronary interventions, a point at which active metabolites of clotidogrel may not be present. In contrast to the healthy blood donors in our in-vitro experiments, the coagulation system is activated in patients with ACS (31). Thus, it has also been described that the coagulation cascade under in-vitro conditions is activated as well (32). Whether activation of the coagulation cascade under ACS or in-vitro conditions is comparable or different cannot be answered at this point.

Other important natural antithrombotic properties of healthy vessel walls in vivo, namely synthesis of prostacyclin, tissue factor pathway inhibitor, and thrombomodulin, which contribute to the fluidity of blood in vivo, are generally missing in in-vitro models. These agents can inhibit platelet-vessel wall interactions and platelet responses as well as decrease the thrombin production and prothrombotic functions of thrombin (thrombomodulin) (33). These important regulatory molecules, as well as the microcirculation in vivo, do not exist in our in-vitro model.

Moreover, another limitation of our study is that electron microscope analyses are limited due to massive thrombus formations in groups 5 (bivalirudin bolus) and 6 (bivalirudin bolus plus eptifibatide). Our blinded investigators were unable to reliably and reproducibly quantify the number of platelets, particularly on images of these two groups (Fig. 1).

References