Impact of bivalirudin or unfractionated heparin on platelet aggregation in patients pretreated with 600 mg clopidogrel undergoing elective percutaneous coronary intervention

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Received 14 December 2007; revised 15 March 2008; accepted 18 April 2008; online publish-ahead-of-print 7 May 2008

Aims
The aim of this study was to assess the impact of bivalirudin or unfractionated heparin (UFH) on platelet aggregation in patients, pretreated with a 600 mg loading dose clopidogrel, undergoing elective percutaneous coronary intervention (PCI).

Methods and results
Patients (n = 100) were recruited consecutively in the setting of a double-blind, randomized trial. Bivalirudin or UFH was administered during PCI. Blood was drawn immediately before PCI, following administration of bivalirudin or UFH directly after PCI, and 24 h after PCI. Adenosine diphosphate (ADP)-induced platelet aggregation was assessed with light transmission aggregometry (LTA) and multiple electrode aggregometry (MEA). Before PCI, ADP-induced platelet aggregation was similar in UFH and bivalirudin patients (P = 0.99 for LTA; P = 0.28 for MEA). Administration of bivalirudin during PCI resulted in significant additional suppression of platelet aggregation (P = 0.012 for LTA; P = 0.008 for MEA). Administration of UFH did not have a significant influence on platelet aggregation (P = 0.42 for LTA; P = 0.78 for MEA). Platelet aggregation was again similar in the two groups 24 h after PCI (P > 0.05 for LTA and MEA).

Conclusion
Bivalirudin, given during PCI in patients pretreated with 600 mg of clopidogrel, is in contrast to UFH associated with further inhibition of platelet aggregation.

Keywords
Bivalirudin ● Clopidogrel ● Unfractionated heparin ● Platelet aggregation ● Whole blood aggregometry

Introduction
Platelets play a pivotal role in the pathogenesis of thrombotic complications after percutaneous coronary intervention (PCI).1,2 The use of optimal antiplatelet and antithrombotic regimens is critical in reducing adverse events among patients undergoing PCI. Dual-antiplatelet therapy consisting of P2Y12 receptor blockade by thienopyridines, such as clopidogrel in addition to cyclooxygenase inhibition by aspirin is an effective pharmacological tool to prevent thrombotic vascular events.3–6 Pretreatment with clopidogrel using loading doses ranging from 300 to 600 mg administered prior to PCI is routinely performed, whenever possible, to achieve a high degree of platelet inhibition by the time the coronary intervention is performed.6 Clopidogrel pretreatment has further reduced the incidence of thrombotic events after PCI.7,8

In addition to an adequate antiplatelet regimen, different agents are currently in use for adjunctive anticoagulant treatment during PCI. Traditionally, unfractionated heparin (UFH) has been the standard anticoagulant administered during coronary interventions.9 UFH offers several shortcomings, such as its potential to activate platelets in therapeutic concentrations,10 the need for repeated laboratory measurements to monitor its effect and the inability to inhibit thrombin bound to fibrin or factor Xa bound to platelets.11 However, P2Y12 receptor antagonists have the potential...
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Methods

Study population

Patients (n = 100) with coronary artery disease (CAD) undergoing elective PCI were recruited consecutively in the setting of the Intracoronary Stenting and Antithrombotic Regimen-Rapid Early Action for Coronary Treatment (ISAR-REACT)-3 trial (ClinicalTrials.gov Identifier: NCT00262054); a prospective, randomized, and double-blind trial. The purpose of this study is to determine whether bivalirudin given during elective PCI is associated with better outcomes compared with UFH. The primary endpoint of ISAR-REACT-3 is a composite rate of death, myocardial infarction, urgent target vessel revascularization within 30 days, or in-hospital major bleeding. Inclusion criteria were age >18 years and an elective PCI procedure. Exclusion criteria were ST-elevation myocardial infarction within the last 48 h, cardiogenic shock, ACSs with a positive troponin T, malignancies, active bleeding or bleeding diathesis, recent trauma or major surgery in the last month, history of intracranial bleeding or structural abnormalities, suspected aortic dissection, pericarditis, subacute bacterial endocarditis, oral anticoagulation therapy with coumarin derivatives within the last 7 days, treatment using UFH within 6 h or low-molecular weight heparin within 8 h before randomization, treatment with bivalirudin within 24 h before randomization, planned staged PCI procedure within 30 days from index procedure or prior PCI within the last 30 days, relevant haematological deviations (haemoglobin <100 g/L or platelet count <100 x 10^9/L) and renal insufficiency [current dialysis, glomerular filtration rate (GFR) <30 mL/min, or serum creatinine >30 mg/L]. All patients received a single high dose of 600 mg of clopidogrel at least 2 h before catheterization. Patients were randomized after diagnostic angiography and before PCI to receive either bivalirudin or UFH during PCI. Sealed envelopes were used for the randomization process. Double-blinding was achieved using similarly appearing vials in the two treatment groups. Thereby, operators were completely blinded to the treatment regimen. All patients received the drug to which they were randomized. Bivalirudin was given as an intravenous bolus of 0.75 mg/kg directly before PCI, followed by an intravenous infusion of 1.75 mg/kg/h for the duration of the procedure. UFH was administered as an intravenous bolus of 140 UI/kg followed by an infusion of placebo for the duration of the procedure. Blood for platelet function testing was drawn at three different time points. First, blood was taken from the arterial sheath after diagnostic angiography immediately before PCI after clopidogrel pretreatment and at a second time point directly after PCI following administration of either bivalirudin or UFH. A third blood sample was taken by venipuncture 24 h after the procedure. The first tube drawn at all time points was labelled as a discard and was not used for platelet function testing. ADP-induced platelet aggregation was assessed with light transmission aggregometry (LTA) in the entire study population (n = 100) and with multiple electrode platelet aggregometry (MEA) in a subset of the study population (n = 43). The study was approved by the institutional ethics committee and patients gave written informed consent for participation.

Light transmission aggregometry

LTA was used to assess ADP-induced platelet aggregation in citrated platelet-rich plasma (PRP) using the platelet aggregation profiler (PAP B) aggregometer (Moebel, Berlin, Germany) with a constant stirring rate of 1200 rpm at 37°C. The final platelet count was adjusted to 250 x 10^9/L with autologous platelet-poor plasma (PPP). PRP (0% light transmission) and PPP (100% light transmission) served as references. After baseline adjustment, ADP of final concentrations 5 and 20 μM was added and aggregation recorded for 6 min. The analysed parameter was maximal aggregation (% within the first 6 min after addition of ADP.

Multiple electrode platelet aggregometry

Aggregation in whole blood was assessed on a new generation impedance aggregometer with multiple electrode aggregometry (MEA). The device used is called Multitrack Analyzer (Dynabyte, München, Germany), indicating the multiplicity of channels and sensors per channel of the device. One Multitrack test cell incorporates two independent sensor units. One unit consists of two silver-coated highly conductive copper wires with a length of 3.2 mm. After dilution (1:2 with 0.9% NaCl solution) of hirudin-anticoagulated whole blood (25 μg/ml, Refudan, Hirudin blood collection tubes, Dynabyte, München, Germany) and stirring for 3 min in the test cuvettes at 37°C, ADP of final concentration 6.4 μM was added and aggregation was continuously recorded for 5 min. The increase of impedance because of the attachment of platelets to the electrodes is detected for each sensor unit separately and transformed to arbitrary aggregation units (AU) that are plotted against time. Aggregation measured with MEA is quantified as AU and area under the curve of arbitrary units (AU x min). MEA is capable of detecting the effect of clopidogrel treatment and the results of MEA, prior to, and after clopidogrel treatment, correlate well with LTA. No centrifugation steps are needed for MEA and assessment of platelet aggregation can be done in approximately 10 min.

Statistical analysis

The primary aim of this platelet substudy was to assess the platelet response to treatment with bivalirudin or UFH. In order to do so, platelet function was determined before and after administration of
the drug of interest. The number of patients included in the study was based on the assumption that administration of bivalirudin results in a 5% absolute decrease (from 30 ± 8 to 25 ± 8%) and no change for UFH of maximal ADP (5 μM)-induced platelet aggregation assessed with LTA. Choosing a power of 80% and a two-sided α value of 0.05 an overall sample size of at least 82 was required (nQuery advisor, version 5.0, Statistical Solutions, Cork, Ireland). Variables of baseline characteristics are presented as mean ± standard deviation (SD), counts or percentages, and median with inter-quartile range (IQR). Categorical variables were compared using χ² test. Kolmogorov–Smirnov test was used to test normal distribution of continuous data. Normally distributed continuous variables were compared with two-sided unpaired t-test. As most of the platelet function data were not normally distributed, these data were presented as median with IQR and were compared with two-sided Wilcoxon test and Wilcoxon matched-pairs signed-ranks test as appropriate. P-values < 0.05 were considered statistically significant.

**Results**

The study cohort comprised 100 patients who were treated with elective PCI, of whom 52 patients received intravenous bivalirudin treatment and 48 patients received intravenous UFH during the PCI procedure. Baseline characteristics of the patients included in this study are demonstrated in Table 1. Variables were well balanced between the two groups except for the proportion of patients with a history of bypass surgery, which was borderline significant.

**Light transmission aggregometry**

Before PCI, ADP (5 μM)-induced platelet aggregation [median (IQR)] was similar in patients treated with bivalirudin and UFH [30.0% (22.8–46.5) vs. 32.5% (20.8–48.0); P = 0.99]. Directly after PCI following administration of bivalirudin or UFH, ADP-induced platelet aggregation (5 μM) was 24.0% (17.8–39.0) in patients treated with bivalirudin and 29.5% (20.0–49.3) in patients treated with UFH. Figure 1 displays box-plot analyses of ADP-induced platelet aggregation (5 μM) before and after administration of either bivalirudin or UFH during PCI. As demonstrated in Figure 1, administration of bivalirudin resulted in significant additional suppression of ADP-induced platelet aggregation. Administration of UFH did not have a significant influence on ADP-induced platelet aggregation. Figure 2 demonstrates the absolute change (Δ) of ADP-induced platelet aggregation (5 μM) because of administration of either bivalirudin or UFH during PCI. This change differed significantly between the two groups (P = 0.018). The difference of the absolute change (Δ) of ADP-induced platelet aggregation (5 μM) between both groups was −6.5% (95% CI −12.8 to −0.12). ADP-induced platelet aggregation (5 μM) was again similar in the two treatment groups 24 h after PCI [39.0% (23.0–50.0) for bivalirudin patients vs. 34.0% (26.8–45.3) for UFH patients; P = 0.53]. Using 20 μM ADP, administration of bivalirudin also resulted in significant additional
suppression of ADP-induced platelet aggregation ($P = 0.006$). Administration of UFH did not have a significant influence on ADP-induced platelet aggregation (20 μM) ($P = 0.94$).

**Multiple electrode aggregometry**

Assessed using MEA before PCI, ADP-induced platelet aggregation (median (IQR)) was similar in bivalirudin patients ($n = 23$) and UFH patients ($n = 20$) [180.0 AU × min (78.0–244.0) vs. 233.5 AU × min (136.0–342.0); $P = 0.28$]. Directly after PCI following administration of bivalirudin or UFH, ADP-induced platelet aggregation was 109.0 AU × min (58.0–168.0) in bivalirudin patients and 236.5 AU × min (134.5–304) in patients treated with UFH. As demonstrated in Figure 3, administration of bivalirudin resulted in significant additional suppression of ADP-induced platelet aggregation assessed with MEA. Administration of UFH did not have a significant influence on ADP-induced platelet aggregation assessed with MEA. ADP-induced platelet aggregation was again similar in the two groups 24 h after the procedure [113.0 AU × min (74.0–171.0) for bivalirudin patients vs. 175.5 AU × min (134.3–280) for UFH patients; $P = 0.10$].

**Discussion**

To the best of our knowledge, this is the first study that compared parameters of platelet aggregation before and after administration of either bivalirudin or UFH during PCI in patients pretreated with a 600 mg loading dose of clopidogrel.

The first main result of the study is that bivalirudin results in a significant additional inhibition of ADP-induced platelet aggregation in patients pretreated with a high loading dose of clopidogrel. This antiplatelet effect of bivalirudin was consistently observed with two different methods of platelet function testing: LTA and MEA.13–26 The inhibition of platelet function achieved with bivalirudin was only transient. No differences were observed for parameters of platelet function between the two groups at 24 h after administration of the study drug. This is because of the short half-life of the drug, which was shown to be approximately 25 min.27 The second main result of the study is that in patients pretreated with 600 mg of clopidogrel before PCI, administration of UFH does not result in a significant increase of platelet aggregation.

Many variables affect platelet aggregation in concert and interindividual variability after loading with a 600 mg loading dose of clopidogrel is extensive. Moreover, the additional inhibition observed for bivalirudin is—albeit being statistically significant—not as extensive that an overlap of platelet aggregation values between both groups would not occur. The observed additive inhibition of platelet function because of bivalirudin treatment is of interest, especially in light of the reduced rate of bleeding complications reported for bivalirudin with provisional glycoprotein IIb/IIIa (GP IIb/IIIa) blockade when compared with UFH plus planned GP IIb/IIIa blockade in the REPLACE-2 trial.13,14 It seems that the drug, because of its pharmacological properties, may exert positive effects on both bleeding rates and platelet function inhibition.

Saucedo et al.28 studied platelet function, including ADP-induced platelet aggregation, with serial measurements in 60 patients undergoing PCI and randomized to one of four different groups: patients receiving UFH or bivalirudin only and patients receiving additional treatment with eptifibatide (plus UFH or bivalirudin).

Only a small number of patients ($n = 14$) was investigated in the UFH only and bivalirudin only group, and clopidogrel (300 mg) was administered 15–30 min before PCI. Subsequent measurements of platelet function (from baseline up to 24 h after PCI) demonstrated numerically more pronounced inhibition of
ADP-induced platelet aggregation in the bivalirudin group at 2 and 8 h after the start of the procedure. However, these differences did not reach the level of statistical significance.

Anand et al. compared platelet function using the Cone and plate(let) analyzer in patients undergoing PCI receiving UFH or bivalirudin. Only a small proportion (n = 12) of the study population (n = 50) received pretreatment with a 300 mg loading dose of clopidogrel. In this study, platelet function was only measured before and directly (5 min) after treatment with bivalirudin or UFH. In line with the results of our study, bivalirudin was able to achieve additional inhibition of platelet function, as administration of bivalirudin showed an additive effect in further decreasing platelet surface coverage.

Platelets are pivotal in the pathogenesis of ACSs and play a paramount role in the development of thrombotic events during and after PCI. Multiple signalling pathways mediate platelet activation and aggregation. It has been demonstrated, that activation of the P2Y12 signalling pathway enhances thrombin generation. The P2Y12 receptor was found to be involved in tissue factor-induced thrombin formation in PRP. Thrombin is considered to be the most potent stimulus activating platelets in subnanomolar concentrations by binding to the protease-activated receptors (PARs), PAR-1 and PAR-4. PAR activation results in the secretion of ADP-containing dense granules. ADP released from dense granules again acts in an autocrine fashion on the platelet ADP receptors. This endogenous ADP release may be reduced in patients treated with bivalirudin resulting in lower values of ADP-induced platelet aggregation. Moreover, a yet unexplored direct influence of bivalirudin on the P2Y12 signalling pathway cannot be excluded.

We did not find a significant influence of UFH on platelet aggregation in our study population. In previous studies, UFH was shown to activate platelets in therapeutic concentrations and to increase ADP-induced platelet aggregation. However, all patients included in this study were pretreated with a high loading dose of 600 mg clopidogrel administered at least 2 h before the PCI procedure. Adequate clopidogrel pretreatment resulting in partial P2Y12 receptor blockade may have counteracted the pro-aggregatory effects of UFH in this study. This finding is supported by the data from Storey and coworkers, who demonstrated that enhancement of platelet aggregation by UFH is attenuated in the presence of the P2Y12 receptor antagonist cangrelor.

This study has limitations that merit mention. We only investigated parameters of platelet function in vitro in patients with stable CAD undergoing elective PCI. Results of the present study cannot be extrapolated to patients with ACS who have increased platelet reactivity. Whether the influence of bivalirudin on platelet aggregation confers a better clinical outcome when compared with UFH treatment in patients pretreated with clopidogrel before elective PCI has to be clarified in sufficiently powered, large prospective studies. The ISAR-REACT-3 trial addresses this question.

In conclusion, bivalirudin, given during PCI in patients pretreated with 600 mg of clopidogrel, is in contrast to UFH associated with further inhibition of platelet aggregation. This additional antiplatelet effect of bivalirudin may be beneficial in patients undergoing elective PCI.

Conflict of interest: none declared.

Funding
This study was funded from Deutsches Herzzentrum, Munich, Germany (grant KKF 1.1-05, 984323). The ISAR-REACT-3 trial was supported in part by Nycomed Pharma GmbH, Unterschleißheim, Germany (distributor of Bivalirudin in Europe) and the grant KKF 1.1-05 (984323) from Deutsches Herzzentrum, Munich, Germany.

References
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