Attenuation of platelet reactivity by enoxaparin compared with unfractionated heparin in patients undergoing haemodialysis

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Abstract

Background. Increased platelet reactivity presages adverse cardiac events. Because both haemodialysis and unfractionated heparin (UFH) can increase platelet reactivity, we compared platelet reactivity during haemodialysis when patients were anticoagulated with UFH or enoxaparin.

Methods. Patients \((n=20)\) underwent consecutive haemodialysis sessions with either UFH or enoxaparin in a random order. Blood was taken from the arterial end of the haemodialysis circuit at the initiation of haemodialysis before anticoagulation. Subsequently, blood was taken during dialysis from the venous end of the circuit 10 min after treatment with UFH or enoxaparin. Platelet reactivity was assessed with the use of flow cytometry by determining the capacity of platelets to bind fibrinogen and the surface expression of P-selectin in response to adenosine diphosphate (ADP, 0 and 0.2 \(\mu\)M). Results were compared with the use of two-way repeated measure ANOVA.

Results. Platelet reactivity in arterial blood obtained at the beginning of dialysis prior to patients being treated with either UFH \([0.2\mu\text{M ADP-induced capacity to bind fibrinogen} = 28 \pm 15\% \text{ (SD)}]\) or enoxaparin \((30 \pm 18\%)\) was similar \((\text{P} = 0.15)\). In contrast, platelet reactivity was less after treatment with enoxaparin compared with UFH \((P = 0.006)\). The 0.2 \(\mu\)M ADP-induced capacity to bind fibrinogen in venous blood obtained 10 min after anticoagulation was 34 \pm 11\% after treatment with UFH and 22 \pm 11\% after treatment with enoxaparin.

Conclusions. Anticoagulation with enoxaparin during haemodialysis is associated with less platelet reactivity compared with UFH. Accordingly, enoxaparin use may contribute to a lesser risk of cardiac events in patients with end-stage renal disease treated with haemodialysis.

Keywords: enoxaparin; kidney; platelets

Introduction

Cardiovascular disease is the primary cause of death among patients with end-stage renal disease (ESRD) who are treated with dialysis, and accounts for 44% of deaths [1]. The prevalence is in part related to older age and the high incidence of diabetes [2]. Rapid progression of atherosclerosis is combined with limited success of coronary revascularization procedures in this patient group [3].

Platelet reactivity can be determined by characterizing the activation of platelets in response to a low concentration of agonist and identifies those at high and low risk of subsequent cardiac events after percutaneous coronary intervention [4]. The flow cytometric analysis of platelet function that we have employed facilitates characterization of platelet function independent of complex interactions between platelets, the vessel wall, proteins in blood, and changes in the rheology and composition of the dialysis circuit [5–7]. We found that patients with ESRD have increased platelet reactivity [5]. We also found that platelet reactivity is increased further by exposure of platelets to the dialysis membrane [5].

We have determined that exposure of blood from healthy subjects and patients with coronary disease (without renal failure) to unfractionated heparin (UFH) increases platelet reactivity [8,9]. Because platelet reactivity is lower when blood is anticoagulated in vitro with enoxaparin compared with UFH [9], we hypothesized that platelet reactivity would be lower when haemodialysis was performed with enoxaparin rather than UFH as the anticoagulant. Accordingly, we compared platelet reactivity when haemodialysis was performed with either UFH or enoxaparin. Each patient was treated with both UFH and enoxaparin, in random order, in consecutive dialysis sessions.
Subjects and methods

Subjects

In a protocol approved by the University of Vermont Institutional Review Board, 20 subjects were enrolled after obtaining written informed consent. Eligible patients were those undergoing haemodialysis for ESRD, >18 years of age and treated with haemodialysis for at least 1 month. Patients were excluded if they were taking an anti-platelet drug other than aspirin or were treated with a glycoprotein IIb–IIIa inhibitor in the previous month. Conditions that led to exclusion included an acute coronary event in the past 3 months, an intercurrent illness such as pneumonia or congestive heart failure, any haematological disorder, malignancy, recent major haemorrhage from any source, a terminal illness with expected survival of <6 months, hypersensitivity to the medications being tested or the inability to provide informed consent.

Blood sample collection and anticoagulation

The first sample of blood was taken from the dialysis catheter immediately after its insertion into the arterial portion of the arteriovenous fistula (henceforth called arterial blood) and before anticoagulation. Patients were then treated with either UFH or enoxaparin in a random order (determined by the flip of a coin) during consecutive haemodialysis sessions. The second sample was drawn from a catheter placed in the venous portion of the arteriovenous fistula and comprised effluxing blood leaving the haemodialysis machine after patients had undergone dialysis for 10 min (henceforth called venous blood). For patients with a permanent in-dwelling dialysis catheter (n=8), blood samples were obtained similarly from the outlet and inlet ports of the catheter. Patients were treated with a dosage of UFH that previously had been determined to increase the partial thromboplastin time (PTT) determined 1 h before the end of haemodialysis to 50–70 s.

Each patient was treated with a single dose of 0.7 mg/kg enoxaparin before haemodialysis. This dosage has not been associated with an increased risk of haemorrhage or thrombotic complications of the dialysis circuit [10]. Enoxaparin in this dose is associated with an anti-factor-Xa concentration of 0.75±0.05 U/ml 1 h after administration, an anti-Xa concentration of 0.38±0.02 U/ml after 4 h, and no evidence of accumulation after repeated use for 4 weeks [11]. Because UFH and enoxaparin affect tests such as the aPTT and the anti-Xa concentration of 0.75±0.05 U/ml after 4 h, and no evidence of accumulation after repeated use for 4 weeks [11]. Because UFH and enoxaparin affect tests such as the aPTT and the anti-Xa concentration of 0.75±0.05 U/ml after 4 h, and no evidence of accumulation after repeated use for 4 weeks [11].

Because UFH and enoxaparin affect tests such as the aPTT differently, dosages of each agent were selected based on their clinical efficacy rather than equivalent anticoagulant effects. Dialysis was performed with a cellulose acetate membrane and the membranes were reused after sterilization.

All blood was drawn into syringes containing corn trypsin inhibitor [CTI; 32 μg/ml, 1:10 (v/v), Enzyme Research, South Bend, IN] with the use of the two-syringe technique. CTI is a specific inhibitor of coagulation factor XIIa and was used as the anticoagulant to avoid altered activation of platelets by conventional anticoagulants such as citrate [8].

Determination of platelet reactivity

Assays were performed by adding 5 μl of whole blood to microcentrifuge tubes containing 60 μl of HEPES-Tyrodes buffer (5 mmol/l HEPES, 137 mmol/l NaCl, 2.7 mmol/l NaHCO₃, 0.36 mmol/l NaH₂PO₄, 2 mmol/l CaCl₂, 4 mmol/l MgCl₂ and 5 mmol/l dextrose, pH 7.4), fluorochrome-labelled ligands and agonist [adenosine diphosphate (ADP) 0 and 0.2 μM]. A peridinin chlorophyll protein (per-CP)-conjugated antibody to glycoprotein IIIa (CD61; Becton Dickinson, San Jose, CA) was used as an activation-independent marker of platelets. This antibody does not inhibit binding of fibrinogen to the activated conformer of glycoprotein IIb-IIIa. Fluorescein isothiocyanate (FITC)-conjugated fibrinogen was used to assess activation of glycoprotein IIb-IIIa. A phycoerythrin (PE)-conjugated antibody to P-selectin (CD62, Becton Dickinson) was used to assess α-granule degranulation. Fibrinogen (Enzyme Research) was conjugated with FITC with the use of FITC-Celite (Calbiochem, La Jolla, CA) [12]. Labelling of fibrinogen with FITC-Celite does not alter binding of fibrinogen to the activated conformer of glycoprotein IIb-IIIa [13].

The reaction mixture was incubated at room temperature for 15 min. Subsequently, platelets were fixed and the red cells were lysed by the addition of 100 μl of Optilyse-C (1.5% formaldehyde, Immunotech, Wesbrook, ME). All assays were performed in duplicate. We have demonstrated previously that the intra-assay coefficient of variation is <10% [4,5]. To assess the extent of non-specific association of proteins with platelets, blood was added to control tubes with FITC-labelled albumin and PE-labelled non-immune IgG. Flow cytometric analysis was performed with a fluorescence-activated cell sorter (Epics Elite EPS, Coulter). The population of platelets was identified based on particle size (forward and 90° side scatter) and on association with CD61 antibody. The control ligands (albumin–FITC and IgG–PE) were used to determine a threshold above which activation-dependent binding was present.

Statistical analysis

Values are means ± SD. Platelet reactivity in the arterial blood when the blood was subsequently anticoagulated with UFH and enoxaparin was compared with the use of a paired Student’s t-test. Similarly, platelet reactivity in venous blood was compared with the use of a paired t-test. The significance of change in platelet reactivity when blood was anticoagulated with UFH and enoxaparin was determined with the use of a two-way repeated measure analysis of variance (ANOVA). The use of two-way ANOVA permits the comparison of change in platelet reactivity with one anti-coagulant in each individual with the change in platelet reactivity when blood is treated with the second anti-coagulant in the same individual. Significance was defined as P ≤ 0.05.

Results

The clinical characteristics of the patients studied are shown in Table 1. The mean age of the patients enrolled was 63 ± 14 years, and 50% of patients were male. Risk factors associated with coronary artery disease were prevalent and 70% of patients were taking aspirin. The dosage of UFH was 6030 ± 2946 U administered as a single bolus at the initiation of dialysis, and the dosage of enoxaparin was 62 ± 22 mg. Administration of
Enoxaparin was safe except for self-limited lower gastrointestinal bleeding in one patient that was associated with a drop in haemoglobin of <3 g/dl. No difference was observed in the time to achieve haemostasis after removal of the dialysis needle between the two anticoagulants (data not shown).

Platelet reactivity with UFH and enoxaparin

Platelet reactivity as assessed by the 0.2 μM ADP-induced capacity of platelets to bind fibrinogen in the arterial blood before initiation of dialysis was similar when patients were treated subsequently with UFH (28 ± 16) or enoxaparin (30 ± 18) (P = 0.15, Table 2). Anticoagulation with UFH during haemodialysis was associated with a 20% increase in the capacity to bind fibrinogen in response to 0.2 μM ADP (venous blood 34 ± 11; Figure 1). In contrast, anticoagulation with enoxaparin was associated with a 27% decrease in the 0.2 μM ADP-induced capacity of platelets to bind fibrinogen (venous blood 22 ± 11, P = 0.006 by repeated measure ANOVA; Figure 2).

Platelet reactivity with respect to ADP-induced P-selectin expression was similar in the arterial blood before initiation of dialysis when patients were treated subsequently with UFH or enoxaparin (P = 0.21, Table 2). Although the effect of UFH and enoxaparin on ADP-induced P-selectin expression paralleled that seen when the capacity to bind fibrinogen was assessed, the...
results obtained after 10 min in the venous sample were not significantly different (Table 2).

Discussion

Our results demonstrate that platelet reactivity as determined by the capacity of platelets to bind fibrinogen in response to a low concentration of ADP in patients undergoing haemodialysis is significantly greater when dialysis is performed with UFH compared with enoxaparin as the anticoagulant. Thus, anticoagulation with enoxaparin attenuates the heightened platelet reactivity seen in patients with ESRD that is increased further by haemodialysis. Because we have found that platelet reactivity identifies patients at high and low risk of complications after percutaneous coronary intervention [4], the results of this study have potentially important clinical implications. Lower platelet reactivity should decrease the likelihood of thrombosis and thereby the risk of cardiac events during haemodialysis when patients are treated with enoxaparin rather than UFH.

Increased reactivity of platelets seen after their exposure to the haemodialysis circuit may have diverse determinants [5]. Changes in platelet rheology, shear forces imposed by the dialysis circuit, changes in temperature, and altered chemistry of the dialysate which is alkaline and rich in calcium are all factors that may contribute [14,15]. This study identifies the anticoagulant used during haemodialysis as a determinant of platelet reactivity during haemodialysis.

Treatments with low molecular weight heparins such as enoxaparin have been shown to be superior to treatment with UFH in a variety of clinical circumstances. A reduced incidence of cardiac events has been seen when patients with acute coronary syndromes are treated with enoxaparin compared with UFH [16]. Several small studies have demonstrated the safety and efficacy of low molecular weight heparins during haemodialysis in patients with ESRD [10,11]. The time after catheter removal to obtain haemostasis was shorter when 21 patients were treated with low molecular weight heparin compared with UFH [17]. In another study, 33 patients with a history of increased bleeding during haemodialysis when treated with UFH had a lower incidence of both bleeding and clot formation in the dialyser when haemodialysis was performed with low molecular weight heparin for 6 months [18]. Analogous to our results, treatment with UFH compared with enoxaparin is associated with a pro-thrombotic state [19]. The concentration of prothrombin fragment 1 + 2, a marker of thrombin generation, is increased during haemodialysis with UFH and unchanged when haemodialysis is performed with enoxaparin. Accordingly, our results provide additional evidence that performance of haemodialysis with enoxaparin will be associated with a less pro-thrombotic state than when haemodialysis is performed with UFH.

We characterized different components of platelet activation. The binding of fibrinogen reflects activation of glycoprotein IIb–IIIa. P-selectin expression reflects the release of z-granules. We used a low concentration of ADP because we have found that lower concentrations of ADP discriminate inter-individual differences to a greater extent [4,5]. In addition, the capacity to bind fibrinogen was a better descriptor of cardiac risk than was P-selectin expression [5]. In this study, we demonstrated that the capacity to bind fibrinogen was significantly less when haemodialysis was performed with enoxaparin compared with UFH. Although changes in P-selectin expression were parallel, these changes did not achieve statistical significance. The relatively small number of patients recruited and the greater inter-individual variability of 0.2 μM ADP-induced P-selectin expression contribute to the inability to distinguish the effects of enoxaparin and UFH. Intra-individual changes in platelet reactivity over time may have contributed to variability in the response to UFH and enoxaparin. Factors contributing to such changes include platelet turnover and subclinical changes in infectious or inflammatory status. All patients in the study underwent haemodialysis with membranes made of cellulose acetate. Thus, differences in platelet reactivity observed between the two anticoagulants may not be significant in patients undergoing haemodialysis with different types of synthetic membranes.

Haemodialysis can precipitate myocardial ischaemia resulting in angina and myocardial infarction [20]. Multiple factors may contribute, including reduction in blood oxygen saturation, increase in haemoglobin–oxygen affinity, increased myocardial contractility, increased heart rate and increased frequency of arrhythmias [20]. Increased platelet reactivity that is accentuated further by exposure of blood to the dialysis circuit may contribute [5]. The proximate clinical benefit associated with the attenuation of platelet reactivity during dialysis is anticipated to be a reduction in the incidence of silent and manifest ischaemia during dialysis. Larger studies will be required to assess such clinical end points.

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References


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