The Influence of the Haemodialysis Procedure on Platelets, Coagulation and Fibrinolysis

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Abstract
In end-stage renal disease, in particular when treated with haemodialysis, the function of platelets, coagulation and fibrinolytic systems can be disturbed, thus contributing to either thrombotic or bleeding complications. It is important to know whether the currently used haemodialysis procedure itself (by biocompatible membranes and better anticoagulation with nadroparin) affects platelets, coagulation or fibrinolysis. In 15 patients who had been treated with chronic haemodialysis, we measured and compared platelet aggregation (induced by adenosine diphosphate, collagen and epinephrine), the markers of coagulation and fibrinolysis activation (thrombin-antithrombin complexes, thrombin fragments F1+2, D-dimer), and fibrinolytic parameters, i.e. fibrinogen, plasminogen, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 1 antigen and activity, before and immediately after the regular haemodialysis sessions. We did not find differences between pre- and post-haemodialysis platelet aggregation induced with all agents. Markers of coagulation and fibrinolysis activation remained unchanged during the process of haemodialysis. However, in post-haemodialysis samples, t-PA activity was significantly increased. Other fibrinolytic parameters remained unchanged. In conclusion, our results showed that the current technique of haemodialysis procedure does not affect platelet aggregation or activate coagulation, and therefore, does not contribute to a thrombotic tendency. However, it does directly affect fibrinolysis through activation of t-PA, which might be clinically relevant since this could increase the bleeding tendency in some haemodialysis patients.

Introduction

It is widely believed, mainly based on results from relatively old studies, that not only chronic renal disease but also the haemodialysis process by itself activates platelets, coagulation and fibrinolysis [1–11]. The haemodialysis procedure could influence haemostasis by two distinct pathways: first, by the effect of the dialysis membrane, the composition of the dialysis circuit, and changed rheology, and second, by the effect of added anticoagulants.
[12–19]. Noticeably, these factors have been significantly changed in the last decade. In the above-mentioned previous studies, it was mainly shown that haemodialysis activated platelets, coagulation and fibrinolysis [4–11]. Since activated platelets and coagulation could contribute to the occurrence of atherothrombotic events in haemodialysis patients, one could speculate that the haemodialysis procedure itself facilitates the development of atherothrombotic events. On the other hand, activation of fibrinolysis may lead to bleeding complications. Obviously, new data in regard to the effect of modern haemodialysis techniques and anticoagulants on platelets, coagulation and fibrinolysis are needed to accept or reject these assumptions. This unanswered issue was the rationale for performing the present study in which we measured platelet aggregation (which we believe most appropriately reflects the clinically significant activation of platelets), markers of coagulation and fibrinolysis activation, i.e. thrombin-antithrombin (TAT) complexes, thrombin fragments F1+2, and a specific fibrin degradation product (D-dimer), as well as fibrinolytic parameters, such as fibrinogen, plasmin, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1) antigen and activity, in patients before and immediately after regular haemodialysis sessions. Since we are aware that only ‘real’ concentrations and, consequently, ‘real’ conditions in the post-haemodialysis blood might be clinically relevant for possible thrombotic or haemorrhagic complications, we did not make any correction of post-haemodialysis values in regard to haemoconcentration (and the clearance of certain molecules), as described previously [20–22].

**Patients and Methods**

**Patients**

Fifteen patients (11 females, 4 males; mean age 64 years, range 45–76) treated by chronic haemodialysis for an average of 60 months (at least 12 and up to 300 months) were included in the study. Fifteen healthy, age-matched subjects (9 females, 6 males; mean age 65 years, range 48–80) served as controls. All patients had native vessels as a vascular access. All patients were treated by haemodialysis three times per week, using bicarbonate dialysate (polyamide (n = 11)

**Blood Sampling**

Whole blood was obtained from venous needles without venous stasis. The blood samples were obtained before (between 7 and 8 a.m.) and immediately after the haemodialysis session (between 11 and 12 a.m.). Blood taken for standard haematology parameters and platelet aggregation studies was immediately prepared for testing, and blood for measurements of coagulation and fibrinolytic parameters was centrifuged for 20 min at 3,000 g at 4°C. Plasma was then frozen and stored at –70°C until analysed.

**Standard Haematology Parameters**

Haemoglobin, haematocrit and platelet count were routinely determined on a Coulter haematology analyzer using EDTA blood samples.

**Platelet Aggregometry**

Platelet aggregometry was performed using an APACT 2 aggregometer (Labor, Germany). Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 2,000 g for 5 min. The remaining blood was recentrifuged at 2,000 g for 10 min, and platelet-poor plasma (PPP) was removed. All samples of PRP were adjusted to obtain a final platelet count of 250,000 ± 50,000 platelets/µl. The minimum and maximum transmittance was adjusted with PRP (0% transmission) and PPP (100% transmission), respectively. The light transmittance of PRP increased as the platelets aggregated. The ability of PRP to aggregate was tested with adenosine diphosphate (110 µmol/l; DiaAdin, DiaMed) in a final concentration of 4 and 8 µM, collagen 2 (110 µmol/l; DiaAdin, DiaMed) in a final concentration of 4 and 8 µM, collagen 2

**Markers of Coagulation and Fibrinolysis Activation, and Fibrinolytic Parameters**

TAT complexes and plasma prothrombin fragment (F1+2) were measured by ELISA using commercial assays (Behringwerke AG, Marburg, Germany). D-dimer was measured by ELISA (Tinteltest® t-PA, Biopool, Umeå, Sweden). Fibrinogen was determined by a clotting assay (Multifibren, Behring), plasminogen was determined by kinetic spectrophotometry (Berichrom Plasminogen, Behring). t-PA and PAI-1 antigens were determined by ELISA (Imulys® tPA and ImulyseTM PAI-1, Biopool). t-PA activity and PAI activity were determined by amidolytic assays (Spectrolyse®/fibrin, Biopool).

**Statistical Methods**

Measured variables were not distributed normally and were expressed as medians with ranges between the first and the third quartiles. The Mann Whitney U test assessed the differences between groups. A p value <0.05 was taken as statistically significant. Statistical analyses were performed by the Statistical for Windows computer program (StatSoft, Inc., Tulsa, Okla., USA).
**Results**

Compared with controls, patients had significantly lower values of haemoglobin (11.7 vs. 13.9 g/dl, ranges 10.4–12.1 vs. 12.6–14.5; p < 0.001), haematocrit (0.34 vs. 0.41 l/l, 0.32–0.38 vs. 0.37–0.43; p < 0.001) and platelet counts (169 vs. $1.245 \times 10^3 /\mu l$, 123–242 vs. 221–286; p < 0.01). Markers of both coagulation and fibrinolysis activation (pre-haemodialysis samples) were significantly increased in comparison with healthy controls: TAT 5.2 vs. 0 µg/l, ranges 4.2–17.7 vs. 0–4.2 (p = 0.02), F1+2 1.7 vs. 1.1 nmol/l, 1.4–1.9 vs. 1.0–1.2 (p < 0.001), and D-dimer 280 vs. 135 ng/ml, 170–460 vs. 120–150 (p < 0.01).

In patients, platelet aggregation (induced by adenosine diphosphate, collagen and epinephrine) measured in pre- and immediately post-haemodialysis samples did not differ significantly (table 1). Furthermore, the markers of coagulation and fibrinolysis activation (TAT, F1+2 and D-dimer) before and immediately after haemodialysis were not significantly different (table 2). In contrast, the comparison of levels of fibrinolytic parameters before and immediately after haemodialysis showed significantly increased t-PA activity (table 3). Other fibrinolytic parameters remained unchanged, although PAI activity decreased, and the change was not statistically significant (p = 0.08). Pre-haemodialysis levels of t-PA and PAI antigen and activity were comparable with those obtained in the controls, but no significant difference was found: t-PA antigen 6.8 vs. 8.6 g/ml, ranges 4.3–8.9 vs. 8.4–10.6, t-PA activity 0.4 vs. 0.5 IU/ml, 0.1–0.7 vs. 0.1–0.9, PAI-1 antigen 14.5 vs. 14.5 ng/ml, 9.5–21.4 vs. 11.1–16.7, and PAI activity 0.1 vs. 0.6 IU/ml, 0.0–2.4 vs. 0.4–0.9.

**Discussion**

During haemodialysis, platelets, coagulation and fibrinolytic systems could be importantly affected due to several known (velocity of procedure, type of membrane, artificial vascular access, circuit composition, and the type of anticoagulation) and unknown factors [12–19]. The aim of the present study was to explore the effect of the currently used, modern procedure of haemodialysis using synthetic membranes and low-molecular-weight heparin as anticoagulant on platelets, coagulation and fibrinolysis.

We first investigated the effect of haemodialysis on platelet aggregation. Several tests could be used to estimate platelet activation. We chose platelet aggregation (induced by several agents) as the most appropriate and suitable test for the clinically significant platelet activation in haemodialysis patients. The artificial circuit, changed rheology, and heparin might activate platelets [4, 5, 17, 19]. These effects could be counterbalanced by defective aggregation of platelets due to end-stage renal disease [15]. In the literature, contradictory results have

<table>
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<tr>
<th>Aggregation</th>
<th>Before HD</th>
<th>After HD</th>
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<tbody>
<tr>
<td>ADP 4 µM</td>
<td>71.6 (42.5–79.1)</td>
<td>77.3 (55.3–89.0)</td>
<td>NS</td>
</tr>
<tr>
<td>ADP 8 µM</td>
<td>74.1 (51.9–79.4)</td>
<td>75.6 (55.9–86.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen 2 µg/ml</td>
<td>62.7 (31.7–67.8)</td>
<td>67.9 (31.2–75.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Epinephrine 10 µg/ml</td>
<td>67.4 (44.5–70.3)</td>
<td>71.3 (53.4–80.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

All values are medians with ranges between the first and the third quartiles. Results are expressed as percentages of the maximal aggregation obtained after 5 min of stimulation. HD = Haemodialysis; ADP = adenosine diphosphate; NS = not significant.

<table>
<thead>
<tr>
<th>Before HD</th>
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<tbody>
<tr>
<td>TAT, µg/l</td>
<td>5.2 (4.2–17.7)</td>
<td>4.9 (3.2–11.2)</td>
</tr>
<tr>
<td>F1+2, nmol/l</td>
<td>1.7 (1.4–1.9)</td>
<td>1.4 (1.1–2.0)</td>
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<tr>
<td>D-dimer, ng/ml</td>
<td>280 (170–460)</td>
<td>260 (210–410)</td>
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</table>

All values are medians with ranges between the first and the third quartiles. HD = Haemodialysis; NS = not significant.

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<tr>
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<th>p</th>
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<tbody>
<tr>
<td>Fibrinogen, g/l</td>
<td>4.6 (3.9–5.5)</td>
<td>4.8 (4.1–6.0)</td>
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<tr>
<td>Plasminogen activity (rel.)</td>
<td>0.76 (0.64–0.86)</td>
<td>0.76 (0.67–0.92)</td>
</tr>
<tr>
<td>t-PA antigen, ng/ml</td>
<td>6.8 (4.3–8.9)</td>
<td>8.9 (5.0–10.7)</td>
</tr>
<tr>
<td>t-PA activity, IU/ml</td>
<td>0.4 (0.1–0.7)</td>
<td>1.0 (0.0–1.95)</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/ml</td>
<td>14.5 (9.5–21.4)</td>
<td>11.8 (4.5–20.2)</td>
</tr>
<tr>
<td>PAI activity, IU/ml</td>
<td>0.1 (0.0–2.4)</td>
<td>0.0 (0.0–0.0)</td>
</tr>
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</table>

All values are medians with ranges between the first and the third quartiles. HD = Haemodialysis; NS = not significant.
been reported in regard to the effect of haemodialysis on platelet aggregation, probably related to the complex interaction of the vessel wall, changes in rheology, and different compositions of the dialysis circuit and anticoagulants on platelet function [4, 5, 15, 17, 19, 22–24]. Overall, our results did not show differences in platelet aggregation before and immediately after haemodialysis procedure, suggesting that the haemodialysis procedure probably does not significantly activate platelets.

Further, we explored the markers of coagulation and fibrinolysis activation (TAT, F1+2, D-dimer) before and immediately after the process of haemodialysis. In comparison with healthy controls, haemodialysis patients had an evidently activated coagulation system and activated fibrinolysis. We did not find any differences in markers of coagulation activation between pre- and post-haemodialysis samples. This could be due to the lack of activation of coagulation or, less likely, to augmented clearance of increased markers of coagulation activation. Our results are not in agreement with the majority of (older) studies, which mostly showed activation of coagulation after haemodialysis procedure, but are in line with newer studies that showed lack or only low activation of coagulation during haemodialysis procedure [6, 7, 14, 18]. Very likely, this seems to be due to the differences between previously used and improved, currently used modern procedures of haemodialysis by biocompatible membranes and better anticoagulation with low-molecular-weight heparins. Our observation that there is no change in D-dimer levels during haemodialysis is in line with previous studies [7–14]. It seems that increased activation of fibrinolysis is the consequence of extra-corporeal circulation and that it is related to dialysis membrane biocompatibility [12, 13]. Synthetic membranes appear to be more bio-compatible than others [23, 25]. Even though synthetic membranes were used in our patients, the fibrinolytic system was activated during haemodialysis. It might be that factors other than membranes are also important for fibrinolysis activation. The finding of increased t-PA activity immediately after haemodialysis procedure may be clinically important. The increase in fibrinolytic activity after haemodialysis may contribute to an increased bleeding tendency present in some susceptible haemodialysis patients.

In conclusion, our results showed that in haemodialysis patients, during the modern procedure of haemodialysis, the significant activation of platelet aggregation and/or coagulation does not take place, whereas in contrast, fibrinolysis is directly activated through activation of t-PA. These findings may be clinically relevant since fibrinolysis activation during the haemodialysis procedure can contribute to increased fibrinolytic potential and bleeding tendency present in some haemodialysis patients.

References


