Effect of anticoagulation on blood membrane interactions during hemodialysis

ROLAND HOFBAUER, DORIS MOSER, MICHAEL FRASS, RAFAEL OBERBAUER, ALAN D. KAYE, O. WAGNER, STYLIANOS KAPIOTIS, and WILFRED DRUML

Department of Medical and Chemical Laboratory Diagnostics, Department of Oral and Maxillofacial Surgery, Department of Internal Medicine I (MICU), and Department of Internal Medicine III, Nephrology and Dialysis, University of Vienna, Austria, and Department of Anesthesiology and Intensive Care Medicine, Texas Tech University, Health Science Center, Lubbock, Texas, USA

Effect of anticoagulation on blood membrane interactions during hemodialysis

Background. Adequate anticoagulation is a precondition to prevent extracorporeal blood clotting and to improve biocompatibility during hemodialysis. In this study, we performed a morphologic analysis by using scanning electron microscopy to compare three modes of anticoagulation—conventional unfractionated heparin (UFH), low molecular weight heparin (LMWH; dalteparin sodium), or sodium citrate during hemodialysis—on membrane-associated coagulation activation.

Methods. Fifteen patients on regular hemodialysis therapy were investigated. Five patients received UFH, five patients LMWH, and five patients sodium citrate as an anticoagulant during a standardized hemodialysis protocol using a single-use polysulfone capillary dialyzer. Membrane-associated clotting was evaluated using a scanning electron microscope. A dialyzer clotting score was used for quantitative description of coagulation activation on membrane segments.

Results. Using UFH as an anticoagulant revealed the most pronounced cell adhesion and thrombus formation and the highest dialyzer clotting score (11.5 ± 1.3 of a maximal 20 points). LMWH had a lower dialyzer clotting score than UFH (10.4 ± 1.2 of 20 points). During the use of sodium citrate, a negligible thrombus formation and the lowest dialyzer clotting score (1.6 ± 0.6 of 20 points, \( P < 0.05 \)) were observed.

Conclusion. The results of this investigation indicate that using sodium citrate as an anticoagulant during hemodialysis induces a lower activation of coagulation than both conventional and fractionated heparin, which might contribute to an improvement of biocompatibility of hemodialysis extracorporeal circulation.

Adequate anticoagulation is a precondition for any effective hemodialysis therapy both to prevent clotting in the extracorporeal circuit and to improve biocompatibility of artificial membranes. While unfractionated heparin (UFH) has been widely used, it is associated with several disadvantages, such as an increase in bleeding risk, consumption of antithrombin III, but also an activation of platelets and neutrophils, which may negatively affect the biocompatibility of the extracorporeal circuit. Moreover, because of its short half-life, a priming dose alone is often not enough to ensure the anticoagulation throughout the hemodialysis session. Alternatively, low molecular weight heparins (LMWHs) have a longer half-life and have been reported to induce less bleeding, less consumption of antithrombin III, and a lower proaggregatory effect on platelets [1, 2].

In recent years, sodium citrate has gained more popularity as an anticoagulant during hemodialysis, especially in patients with increased bleeding risks, because of the advantages of an efficient anticoagulation that is exclusively confined to the extracorporeal circulation and also an improvement of the biocompatibility by inhibition of activation of blood cells [3].

In this study, we used a scanning electron microscopic (SEM) analysis to compare three different anticoagulation regimes—conventional UFH, LMWH, and sodium citrate—to investigate the morphology of thrombus formation and cellular aggregation on the interior surfaces of hemodialysis membranes.

METHODS

Patients and hemodialysis therapy

Fifteen patients (median age 39 ± 11 years) on regular hemodialysis therapy were included in this investigation. Patient characteristics are shown in Table 1. The study was approved by the local institutional review board, and patients gave informed consent.

A standardized hemodialysis protocol was followed...
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UFH</th>
<th>LMWH</th>
<th>SCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28 ± 9</td>
<td>33 ± 11</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>Sex distribution</td>
<td>3:2</td>
<td>2:3</td>
<td>1:4</td>
</tr>
<tr>
<td>Body weight kg</td>
<td>58 ± 16</td>
<td>61 ± 13</td>
<td>57 ± 14</td>
</tr>
<tr>
<td>Primary disease</td>
<td>4 GN</td>
<td>5 GN</td>
<td>4 GN</td>
</tr>
<tr>
<td>Dialyzer clotting score</td>
<td>1 ATN</td>
<td>1 ATN</td>
<td>1 ATN</td>
</tr>
</tbody>
</table>

Patients were treated by three types of anticoagulation during hemodialysis therapy: unfractionated heparin (UFH), low molecular weight heparin (LMWH), and sodium citrate anticoagulation (SCA). Abbreviations are: GN, glomerulonephritis; ATN, acute tubulointerstitial nephritis.

during all treatments: A single-use hollow fiber hemodialysis membrane [polysulfone high performance steam, HemoFlow F8 HPS™; Fresenius, Bad Homburg, Germany (N = 15)] was used. Blood flow was adjusted to 250 ml/min, and the dialysis duration was four hours. Patients were allocated to three treatment groups of anticoagulation. In the first group, five patients received conventional UFH from porcine mucosa (Heparin Immuno™; Baxter-Immuno, Vienna, Austria). A bolus injection of 1000 U was followed by a constant infusion of 1000 U/hr. In the second group, five patients received LMWH dalteparin sodium (Fragmin™; Pharmacia, Uppsala, Sweden), a 85 U/kg body wt as bolus before the start of hemodialysis therapy. In the last group, five patients received sodium citrate (0.5 mol/liter; Mayerhofer Pharmaceuticals Inc., Linz, Austria), a rate of 50 mmol/hr into the arterial blood line and calcium chloride (0.5 mol/liter; Mayerhofer Pharmaceuticals Inc.) at a rate of approximately 10 mmol/hr into the venous line to maintain an ionized calcium concentration of approximately 1.1 mmol/liter. During citrate anticoagulation, the sodium and bicarbonate concentration in the dialysate was reduced. Patients did not receive other drugs affecting coagulation and/or platelets.

Hemodialysis membranes were removed at the end of the dialysis session and were gently rinsed with normal saline to clean the inside of the membrane capillaries. Within 10 minutes after removal, the hemodialysis membranes were prepared for SEM analysis.

Scanning electron microscopy

For SEM analysis, the hemodialysis membranes were opened, and the fibers were removed and were carefully cut longitudinally using a binocular microscope (SMZU™; Nikon, Tokyo, Japan) at a magnification of 40 times. Then the fiber fragments were twice gently flushed with phosphate-buffered saline (PBS). Flushing was standardized as follows: A 50 ml syringe was connected to the tube of the dialysis membrane. Three flushes were done on each membrane, with each flushing period lasting for 60 seconds. The flusher was not aware of the patients and their regimens because the membranes were blinded. Then the fiber fragments were fixed in 2.5% glutaraldehyde in a 0.04 m sodium cacodylate buffer (pH 7.4), rinsed three times with PBS, and dehydrated in a graded ethanol series. The samples were critical point dried and gold sputtered. Then the specimens were examined with a JOEL 6310 scanning electron microscope (JOEL, Tokyo, Japan) at an accelerated voltage of 5 to 15 kV [4].

Dialyzer clotting score

A scoring system for semiquantitative description of the clotting activation on the internal surfaces of the dialyzer membrane was employed. Five items were analyzed on a scale of 0 (minimum) to 4 points (maximum): fiber surface area involved by thrombus formation, fibrin net formation, erythrocyte aggregate formation, involvement of platelets in aggregate formation, and obstruction of the lumen. The reference area was assessed from the geometric presets of the dializer fibers. The scores in each item were added up to a “total dialyzer clotting score” (DCS).

Statistical analysis

The statistical evaluation was done using the SAS software (SAS Institute Inc., Cary, NC, USA). Data were analyzed nonparametrically by means of the Tukey range comparison. Results are expressed as mean ± sd.

RESULTS

Coagulation activations on the fibers of the dialyzers are illustrated by Figures 1 to 3. These scanning electron micrographs show the inner surfaces of the sample fibers of the dialyzers investigated. Coagulation activation on the fibers of the dialyzers is illustrated in Figure 1. A fibrin network with large amounts of aggregated erythrocytes, a red thrombus formation covering a high portion of the fiber surface and protruding into the fiber lumen, is evident in these micrographs. Similar observations were made in the dialyzers obtained from the other four patients.

Figure 2 shows the inner surface of the capillary fiber when LMWH has been used as anticoagulant during hemodialysis. A stronger fibrin network with erythrocytes is evident in these images, but a much lower number of erythrocytes are involved in the thrombus formation. A lower surface area is covered, and there is now major obstruction of the fiber lumen. Several platelets can be identified in this figure. Again, similar findings were demonstrated in dialyzers obtained from the other four patients with LMWH anticoagulation.

Figure 3 shows the findings on the inner surface of a capillary dialyzer when the patient received sodium citrate as the anticoagulant. Only a few single cells adhering to the inner surface were observed. No thrombus
formation was seen, and no fibrin network could be found (Fig. 3).

The semiquantitative evaluation using the DCS is given in Table 2. With UFH as anticoagulant during hemodialysis therapy, a total score of 11.5 ± 1.3 out of a maximum of 20 points was obtained. The total DCS using LMWH was 10.4 ± 1.2 points. In dialyzers with sodium citrate anticoagulation, the total DCS was only 1.6 ± 0.6 points ($P < 0.05$ vs. UFH and LMWH).

**DISCUSSION**

In a morphological analysis using a SEM evaluation of the inner surfaces of polysulfone hollow fiber dialyzer membranes, we compared three types of anticoagulation during hemodialysis therapy. The degree of coagulation activation was highest during the use of conventional UFH, lesser during LMWH, and virtually absent during the use of sodium citrate.

The clotting mechanism is a cascade of proenzyme-enzyme transformations, each activating the next until the final substrate, fibrinogen, is reached. Each factor in the coagulation cascade is short lived, only a matter of a few seconds, terminating with the rapid conversion of fibrinogen into fibrin, the main component of thrombus. The generation of clot-promoting activity by artificial surfaces depends on the activation of the Hageman or surface factor XII, which initiates and triggers a series of enzymatic reactions culminating in the generation of thrombin and the formation of fibrin. All of the elements of the blood, including platelets, fibrin, erythrocytes, and leukocytes, may enter into the formation of a thrombus. The three most important factors in the generation of a thrombus include slowing the blood flow in the blood circulation, changes in the blood vessel wall, changes in the blood, and the biocompatibility of the artificial membranes, respectively. Heparin, endogenously synthesized from the liver and mast cells, helps to maintain the fluidity of the blood in the systemic circulation. Heparin, newer small molecular weight heparins, and other glycosaminoglycans prevent the aggregations of platelets to form platelet thrombi [5–10]. Heparin, an acid with a pH of 6.93, enhances or potentiates antithrombin III (ATIII) and neutralizes the action of thrombin, thus preventing the formation of a fibrin clot. ATIII also binds to factors IXa, Xa, XIa, and XIIa. Under normal circumstances, the binding of ATIII to thrombin and the other activated factors of the intrinsic pathway occurs slowly. In the presence of heparin, the rate of ATIII binding is accelerated. Without ATIII, heparin has almost no anticoagulant action. The endothelial location of heparin sulfate permits binding and activation of ATIII at the blood-surface interface, where activated factors of the coagulation cascade are being generated.

A number of studies have demonstrated the impor-
Fig. 2. Scanning electron micrographs of the inner surface of a polysulfone hollow fiber dialyzer membrane using low molecular weight heparin as anticoagulant during hemodialysis therapy. (A–C) A stronger fibrin network is shown, but much less erythrocytes. (D) A platelet in a fibrin network using a high magnification. Similar observations were made in the dialyzers obtained from the other four patients.

tance of UFH and LMWH in modulating thrombus generation. LMWHs may be preferable to UFHs in the prevention of thromboembolism in general surgery [11]. LMWHs have been shown to be as effective and safe as intravenous UFH in patients with acute pulmonary embolism [12]. Heparinization of materials and medical devices can alter thrombogenicity. An in vitro study by Engbers et al reported that with polyurethane catheters, the adhesion of platelets onto the catheter surface is mainly determined by the rate of reaction between platelets and the material surface. Heparin-coated catheters showed a fourfold to fivefold reduction in platelet adhesion as compared with noncoated catheters. However, in a Scandinavian study, a comparison of thrombin generation during cardiopulmonary bypass with heparin-coated versus conventional cardiopulmonary bypass cir-
cuit demonstrated that the use of a heparin-coated circuit did not reduce thrombin generation [13–14].

Coagulation activation is obviously one of the most important barriers to adequacy of dialysis delivery. Recently, Shegal et al analyzed patient-related and technical factors determining dialysis delivery in 1836 treatments provided to 721 randomly selected patients on regular hemodialysis therapy [16]. Their study concluded that besides dialysis prescription, problems with vascular access and clotting are the most important limits to dialysis adequacy.

Coagulation activation presents one of the main determinants of biocompatibility. Clotting not only reduces the efficiency of dialysis therapy by mechanical obstruction of the dialysis membrane, but causes activation of other cascade systems such as the complement system,
Table 2. Dialyzer clotting score (DCS) of a polysulfone hollow fiber dialysis membrane during three types of anticoagulation during hemodialysis therapy: unfractionated heparin (UFH), low molecular weight heparin (LMWH), and sodium citrate anticoagulation (SCA)

<table>
<thead>
<tr>
<th></th>
<th>UFH</th>
<th>LMWH</th>
<th>SCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area involved</td>
<td>3.2 ± 0.4</td>
<td>2.4 ± 0.2</td>
<td>0.8 ± 0.2*</td>
</tr>
<tr>
<td>Fibrin net formation</td>
<td>1 ± 0.3</td>
<td>2.4 ± 0.2</td>
<td>0 ± 0*</td>
</tr>
<tr>
<td>Involvement of erythrocytes</td>
<td>3.6 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>0.8 ± 0.4*</td>
</tr>
<tr>
<td>Involvement of platelets</td>
<td>0.8 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>0 ± 0*</td>
</tr>
<tr>
<td>Obstruction of fiber lumen</td>
<td>3.8 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>0 ± 0*</td>
</tr>
<tr>
<td>Total “DCS”</td>
<td>11.5 ± 1.3</td>
<td>10.4 ± 1.2</td>
<td>1.6 ± 0.6*</td>
</tr>
</tbody>
</table>

* P < 0.05

such as an improvement in lipid metabolism, less activation of platelets, and less consumption of antithrombin III. Whether or not LMWHs—as frequently claimed—in fact reduce bleeding complications was not uniformly confirmed.

The lowest degree of clotting formation on the dialyzer membrane was observed when using sodium citrate as the anticoagulant. Citrate anticoagulation in hemodialysis therapy has become popular, especially for patients with a high risk for bleeding, because the anticoagulatory effect is exclusively confined to the extracorporeal blood volume with no coagulation alterations in the systemic circulation. Moreover, it is inexpensive and easy to deliver. The most important advantage besides the superb anticoagulatory effect is the improvement in the overall biocompatibility of the extracorporeal circuit during citrate anticoagulation. Calcium ions are not only required for blood coagulation, but are also a prerequisite for any cellular activation, such as of thrombocytes and neutrophils. Boehler et al have convincingly demonstrated that during citrate anticoagulation the release of proteolytic enzymes from neutrophils is, in fact, abolished [3].

Unfortunately, few studies are available comparing citrate with conventional means of anticoagulation. Janssen et al have compared 21 dialysis patients receiving the LMWH nadroparin calcium or sodium citrate. Analyzing the activated clotting time, activated partial thromboplastin time, factor anti-Xa, and prothrombin, these authors found that in contrast to citrate, LMWH induced systemic anticoagulation during hemodialysis [18–21].

In summary, the results of this morphological study using a SEM analysis, in which various anticoagulation regimes during hemodialysis therapy were compared with respect to coagulation activation on a polysulfone membrane hollow fiber, showed that clotting activation was most pronounced during conventional UFH heparin, less prominent during LMWH, and virtually absent during sodium citrate anticoagulation. Besides this optimal anticoagulatory effect, sodium citrate has additional advantages such as the strictly regional nature of anticoagulation and a superior effect on biocompatibility. Sodium
citrate anticoagulation should be evaluated in the future for broader use in regular hemodialysis patients.

ACKNOWLEDGMENTS

The authors thank Mrs. A. Worl, BNS, Assistant Managing Nurse, and Mr. A. Ecker, BNS, Managing Nurse, and the nursing staff of the Acute Dialysis Unit, Department of Medicine III, Division of Nephrology and Dialysis, at the Vienna General Hospital, Austria, for active support of the study. We also thank Thomas Kainrath for his excellent graphical layout.

Reprint requests to Dr. Roland Hofbauer, Department of Medical and Chemical Laboratory Diagnostics, Level 51, University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria.
E-mail: roland.hofbauer@akh-wien.ac.at

REFERENCES