Hemofiltration of Recombinant Hirudin by Different Hemodialyzer Membranes: Implications for Clinical Use

Kerstin Benz,* Matthias A. Nauck,† Joachim Böhler,‡ and Karl-Georg Fischer§

*Universitätsklinikum Erlangen, Kinder- und Jugendklinik, Erlangen, †Universitätsklinikum Greifswald, Institut für Klinische Chemie und Laboratoriumsmedizin, Greifswald, ‡Deutsche Klinik für Diagnostik, Wiesbaden, and §Universitätsklinikum Freiburg, Medizinische Klinik, Abteilung Nephrologie und Allgemeinmedizin, Freiburg, Germany

Recombinant hirudin (lepirudin) is a potent direct thrombin inhibitor that is used particularly for treatment of immune-mediated heparin-induced thrombocytopenia. Because hirudin is almost exclusively eliminated by the kidneys, its half-life is markedly prolonged in patients with severe renal insufficiency. Therefore, these patients are at risk for bleeding, particularly because no antidote is available. To use hirudin safely in patients who are on renal replacement therapy, knowledge of hirudin-sieving characteristics of different hemodialyzers is required. Data on this issue are sparse and in part contradictory. Eight different conventional low-flux and high-flux hemodialyzers were tested in an in vitro circuit with ultrafiltrate reinfusion. In each experiment, lepirudin concentration was repetitively measured during 3 h in the prefilter, the postfilter, and the filtration line using a chromogenic assay. On the basis of these data, sieving coefficients were calculated. All high-flux hemodialyzers tested allowed filtration of hirudin yet with marked differences in steady-state sieving (sieving coefficients in whole blood: polysulfone [PS] 0.97 ± 0.03; polymethylmethacrylate [PMMA] 0.75 ± 0.02; polyarylethersulfone 0.73 ± 0.02; polyamide 0.49 ± 0.02). None of the low-flux hemodialyzer membranes tested (cuprophane, hemophane, PS, and PMMA) showed significant hirudin filtration. Owing to marked differences in hirudin-sieving characteristics, choice of the appropriate hemodialyzer membrane is an important determinant of bleeding risk in dialysis-dependent patients who are treated with hirudin. In case of overdosage or bleeding complications, hemofiltration via PS membranes is recommended to reduce plasma hirudin concentration. Hirudin dosage should be adapted not only to the clinical situation but also to the hirudin-sieving characteristics of the assigned dialyzer.


R

ecombinant hirudin (r-hirudin; lepirudin, Refludan, Pharmion, Hamburg, Germany) is a potent direct thrombin inhibitor that is approved and increasingly used for alternative anticoagulation in immune-mediated heparin-induced thrombocytopenia (HIT). By forming a stable, noncovalent stoichiometric 1:1 complex with thrombin, (r-)hirudin is capable of inhibiting the numerous thrombin actions (e.g., promotion of fibrin formation; activation of clotting factors V, VIII, XIII; thrombin-induced platelet activation) (1). In contrast to heparin, (r-)hirudin does not require co-factors and inhibits also clot-bound thrombin (2). Furthermore, endogenous inhibitors do not exist (3), and an antidote is not available.

Elimination of hirudin (hirudin refers to recombinant hirudin throughout the subsequent text) largely depends on renal function. For healthy individuals with normal renal function, a total hirudin clearance of 174.0 ± 37.6 ml/min was reported, corresponding to an elimination half-life of 1.7 ± 1.5 h (4). In contrast, patients with ESRD and a residual creatinine clearance <7 ml/min showed a total hirudin clearance of 2.7 ± 1.2 ml/min only. In these patients, hirudin half-life was prolonged to 51.8 ± 15.6 h (5). After bilateral nephrectomy, hirudin half-life of >300 h has been reported (6).

Given these data, adequate hirudin anticoagulation in patients with severe renal insufficiency obviously is a demanding task (7). The issue is even more complex when extracorporeal blood purification procedures are required. In this regard, knowledge of hirudin-sieving properties of different artificial hemodialyzer membranes is mandatory. Available systematic data are limited, in part contradictory, or not easily interpreted for methodologic reasons (5,8–10). Furthermore, elimination characteristics have been investigated at hirudin concentrations that markedly exceed those that are required to run regular hemodialysis (HD) procedures (3.5 to 50 µg/ml [8–10] versus 0.6 – 1.5 µg/ml [11]. This prompted us to investigate systematically hirudin-sieving properties of eight different hemodialyzer membranes at hirudin concentrations of approximately 1 µg/ml (within the therapeutic range). To this end, in vitro studies were performed using a closed hemofiltration circuit with complete reinfusion of the ultrafiltrate. By this approach, steady-state conditions were achieved, allowing for accurate determination of sieving coefficients for each hemodialyzer membrane tested. On the basis of these data, conclusions are drawn as to the clinical application of hirudin in patients who require HD procedures. In addition, the results add further
systematic evidence concerning the suitability of different hemodialyzer membranes to rapidly remove hirudin in case of hirudin overdose or hirudin-associated bleeding.

Materials and Methods

Hemodialyzers

The hemodialyzers used in this study are listed in Table 1.

Recombinant Hirudin

Throughout the study, lepirudin (Refludan) was used. The lyophilisate was diluted with 0.9% NaCl solution according to the instruction of the manufacturer.

Carrier Solutions Used in the In Vitro Hemofiltration Circuit

Hirudin was added to 500 ml of the various carrier solutions (0.9% NaCl to characterize hirudin permeability of high-flux hemodialyzers without protein interaction, 5% human albumin, human compositional whole blood) each filled in standard plastic bags to yield a final concentration of 1 μg/ml. The following solutions were used: 0.9% NaCl (Braun Schiwa, Glandorf, Germany) and 5% human albumin (Baxter, Unterschleißheim, Germany). Human compositional whole blood was produced from human packed red blood cells and blood group–identical fresh-frozen plasma by standard protocols. The final hematocrit was standardized to 35%.

Hemofiltration Circuit

Figure 1 shows a scheme of the hemofiltration circuit used for in vitro hemofiltration of hirudin. The circuit consisted of shortened blood tubing systems (Hospal, Nürnberg, Germany) connected with the respective adult-size hemodialyzer. The solutions were circulated by a conventional HD blood pump (Fresenius, Homburg/Saar, Germany) integrated in the prefiler line. Within the hemofiltration circuit, flow was 100 ml/min for 0.9% NaCl and 5% human albumin. Human whole blood, the flow was adjusted to 200 ml/min to avoid hemococoncentration. An identical pump was integrated in the filtrate circuit, ensuring a constant filtrate flow of 35 ml/min. The filtrate line was connected to the postfilter line in front of the fluid reservoir to allow recirculation. This setup met the requirements of Euronorm EN1283 (12), which regulates the determination of ultrafiltration coefficients for hemodialyzers.

Experimental Protocol

After the bags that contained the carrier solutions were connected to the in vitro circuit, carrier solutions were circulated with 300 ml/min for 10 min (human whole blood 200 ml/min for 15 min to minimize mechanical hemolysis). After pump speed in the blood compartment was reduced to 100 ml/min (whole blood 200 ml/min as mentioned), controlled ultrafiltration was begun by starting the pump within the filtrate line (35 ml/min). Pump velocities were not changed throughout the whole protocol (start = time point 0 min). Samples (1 ml) were drawn from the prefiler line, the postfilter line, and the filtrate line at the following time points: 15, 30, 45, 60, 90, 120, 150, and 180 min. Reduction of total circulating volume was <5%.

Determination of Hirudin Concentration

Samples from 0.9% NaCl and 5% human albumin were directly transferred to the coagulometer (Behring Coagulation System; Dade Behring, Liederbach, Germany). Blood samples were centrifuged (5000 U/min for 15 min), and plasma was transferred to the Behring Coagulation System coagulometer. Hirudin was determined by a chromogenic assay (Hirudin Activity Assay; Dade Behring) applying standard protocols. The assay was validated for all carrier solutions used.

Table 1. Hemodialyzersa

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Abbreviation</th>
<th>Hemodialyzer</th>
<th>Ultrafiltration Coefficient (ml/h per mmHg)</th>
<th>Surface Area (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low flux</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hemophane</td>
<td>HP</td>
<td>Idemsa 25H</td>
<td>5.2</td>
<td>1.3</td>
</tr>
<tr>
<td>cuprophane</td>
<td>CP</td>
<td>Allwall GFS 12</td>
<td>6.5</td>
<td>1.3</td>
</tr>
<tr>
<td>polysulfone</td>
<td>PS (LF)</td>
<td>Fresenius F6HPS</td>
<td>8.5</td>
<td>1.3</td>
</tr>
<tr>
<td>polymethylmethacrylate</td>
<td>PMMA (LF)</td>
<td>Filtryzer B3-1.3A</td>
<td>8.8</td>
<td>1.3</td>
</tr>
<tr>
<td>High flux</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>polysulfone</td>
<td>PS (HF)</td>
<td>Fresenius F60S</td>
<td>40</td>
<td>1.3</td>
</tr>
<tr>
<td>polymethylmethacrylate</td>
<td>PMMA (HF)</td>
<td>Filtryzer BK-1.3P</td>
<td>26</td>
<td>1.3</td>
</tr>
<tr>
<td>polyacrylethersulfone</td>
<td>PAES</td>
<td>Arylane H4</td>
<td>62</td>
<td>1.35</td>
</tr>
<tr>
<td>polyamide S</td>
<td>PA</td>
<td>Polyflux 14H</td>
<td>50</td>
<td>1.4</td>
</tr>
</tbody>
</table>

aCharacteristics of the various hemodialyzers are given as specified by the respective manufacturer.
Calculation of Sieving Coefficients

Calculation of sieving coefficients (SC) was performed at each time point according to the following equation:

\[
SC = \frac{2 \times \text{hirudin concentration filtrate}}{\text{hirudin concentration pre-filter line} + \text{hirudin concentration post-filter line}}
\]

Eight SC values were calculated for each experiment. Unless otherwise indicated, SC values given in the text or tables refer to the mean of all experiments performed with the respective hemodialyzer and carrier solution.

Results

Filtration of Hirudin by High-Flux Hemodialyzers

To characterize hirudin permeability of high-flux hemodialyzers without protein interaction, we performed a set of experiments using 0.9% NaCl as carrier solution. As shown in Table 2, the polysulfone (PS), polyarylethersulfone (PAES), and polyamide (PA) high-flux hemodialyzers were hirudin-permeable under these conditions. In another set of experiments, hirudin filtration was tested using 5% human albumin as carrier solution. The respective data are depicted in Table 2. Hirudin permeability of high-flux hemodialyzers was not significantly reduced by albumin. In contrast, the PS and PAES hemodialyzers showed increased hirudin filtration in the presence of albumin in the carrier solution.

The polymethylmethacrylate (PMMA) high-flux hemodialyzers showed marked hirudin adsorption in physiologic saline. In fact, despite priming of the solution with hirudin up to a concentration of 30 μg/ml, no hirudin was detected after circulation was started within the circuit. Using 5% human albumin as carrier solution, this prominent hirudin adsorption by PMMA high-flux hemodialyzers was no longer observed, and hirudin SC could be determined.

In a third series of experiments, the different hemodialyzer membranes were tested in human whole blood. Under these conditions, all high-flux hemodialyzers were hirudin-permeable. Figure 2A depicts an original experiment with a PS high-flux hemodialyzer. Figure 2B shows the respective mean hirudin concentration during 3 h of controlled filtration in the prefilter, postfilter, and filtrate line of this single experiment.

Figure 3 summarizes the experiments that were performed with high-flux hemodialyzers, five of each type, using human compositional whole blood as carrier solution. With respect to the PS (SC mean ± SEM 0.97 ± 0.03), PMMA (SC 0.75 ± 0.02), and PAES (SC 0.73 ± 0.02) hemodialyzer membrane, sieving in whole blood was slightly reduced compared with hirudin sieving in albumin. The PA hemodialyzer showed a more pronounced reduction of hirudin filtration under these conditions (SC 0.49 ± 0.02). Figure 4 shows a comparison of mean SC of the different series.

No Filtration of Hirudin by Low-Flux Hemodialyzers

It has been reported that some low-flux hemodialyzers allow for hirudin filtration as well (10,11). For this reason, we additionally investigated four low-flux hemodialyzer membranes. Hirudin filtration of these low-flux membranes was negligible, irrespective of the carrier solution used (Table 3).

Discussion

Pharmacokinetics of recombinant hirudin closely correlates with residual renal function (7). In patients who have ESRD and require HD, elimination half-life is markedly prolonged (5).
This prompted us to determine hirudin sieving properties of eight different hemodialyzer membranes at hirudin concentrations within the therapeutic range. These studies were performed using an in vitro recirculation model with constant filtrate flow according to the requirements of Euronorm 1283 for determination of SC (12).

Regarding pharmacology of hirudin, a distribution volume of 0.16 to 0.28 L/kg body wt and only slight protein binding have been reported (3,5). Given its molecular weight of approximately 7 kD, these data should favor its elimination by hemofiltration via high-flux hemodialyzer membranes with a cutoff >40 kD. Indeed, all high-flux hemodialyzer membranes were permeable for hirudin. However, significant differences among the various membranes were observed. Within the 3-h course of the experiments using human compositional whole blood as carrier solution, the PS high-flux hemodialyzer membrane showed best hirudin sieving, whereas hirudin sieving of the PA high-flux hemodialyzer was markedly reduced (mean 3-h SC: PS 0.97 ± 0.03; PMMA 0.75 ± 0.02; PAES 0.73 ± 0.02; PA 0.49 ± 0.02). Comparable SC were recently reported for PS and PA high-flux hemodialyzer membranes in a similar setup, albeit at markedly higher hirudin concentrations (15 to 18 µg/ml) based on an in vitro
tein solutions such as 5% human albumin or whole blood, this capacity in 0.9% NaCl as carrier solution. However, using pro-
membrane indeed showed an enormous hirudin adsorption
tested, adsorption of hirudin could have occurred. The PMMA
branes tested.

markedly hinder hirudin sieving by the hemodialyzer mem-
pared with 0.9% NaCl, indicating that plasma proteins do not
significant reduction of hirudin SC in 5% human albumin com-
NaCl and 5% human albumin as carrier solutions. There was no
sieving of the various hemodialyzer membranes and used 0.9%
filtration, all high-flux hemodialyzer membranes tested showed a
reduction of hirudin sieving by approximately 20%.

Contact of blood to the artificial surfaces of the hemodialyzer
membranes results in rapid formation of a proteinaceous
boundary layer (14), which reduces their respective SC, espe-
cially for high molecular weight solutes. Because molecular
weight of hirudin is approximately 7 kD, reduction of its siev-
ing over time is to be awaited. Indeed, after 3 h of hemofiltration,
all high-flux hemodialyzer membranes tested showed a
reduction of hirudin sieving by approximately 20%.

We further tested the impact of plasma proteins on hirudin
sieving of the various hemodialyzer membranes and used 0.9%
NaCl and 5% human albumin as carrier solutions. There was no
significant reduction of hirudin SC in 5% human albumin com-
pared with 0.9% NaCl, indicating that plasma proteins do not
markedly hinder hirudin sieving by the hemodialyzer mem-
branes tested.

On the large surface area of the hemodialyzer membranes
tested, adsorption of hirudin could have occurred. The PMMA
membrane indeed showed an enormous hirudin adsorption
capacity in 0.9% NaCl as carrier solution. However, using pro-
tein solutions such as 5% human albumin or whole blood, this
adsorption was no longer observed and hirudin concentrations
remained constant throughout the 3-h experiments.

Given the pharmacologic properties of hirudin, significant
dieving of hirudin by low-flux hemodialyzers with an ultrafil-
tration coefficient of <10 ml/h per mmHg at first glance seems
unlikely. Indeed, none of the low-flux hemodialyzers tested
hirudin-permeable within our setup. This is in contrast to re-
results from the previous study cited (10), in which a considerable
number of low-flux hemodialyzers were found to be hirudin-
permeable. In our view, the methodologic aspects discussed
should lead to cautious interpretation of those data. For a more
detailed discussion of sieving properties of hemodialyzer mem-
branes, the reader is referred to recent review articles, which
give an in-depth evaluation of the respective literature (7,15).

Our in vitro data could have been confirmed in part in se-
lected patients (16), leading to the following cautious clinical
conclusions:

1. Choice of the Membrane and Dialysis Procedure. This
decision depends on the actual patient condition (e.g., risk for
bleeding versus thrombosis, residual renal function, scheduled
or imminent surgery or invasive interventions) and comorbid-
ities. If the patient requires anticoagulation with hirudin after
dialysis to prevent thrombus formation (e.g., as in acute HIT),
then not removing much hirudin may be appropriate and the
use of a low-flux membrane or performing HD instead of
hemofiltration is feasible. In contrast, in a patient who is at high
risk for bleeding or already experiencing bleeding complica-
ions, removing as much hirudin as possible should be the goal.
In this situation, a high-flux hemodialyzer and high-volume
hemofiltration are the appropriate choice. In a patient who has
a history of HIT and receives hirudin anticoagulation as pre-
dialytic bolus to avoid rechallenge with heparin, use of low-flux
hemodialyzers may be an inadequate choice, because the use of
a hirudin-impermeable dialyzer will result in the patient’s be-
ing anticoagulated into the interdialytic interval with only a
small decay of hirudin plasma concentration over time. This is
of specific relevance in patients who are referred to emergency
interventions or surgery. For these reasons, we do not support
the general recommendation to use non–hirudin-permeable
hemodialyzers (10).

2. Initial and Maintenance Dosing of Hirudin in Renal
Failure. Obviously, this is a delicate task. In patients with a
residual creatinine clearance <15 ml/min, the initial dosage
required to achieve systemic anticoagulation is minimal. We
advise the use of repeated bolus instead of continuous appli-

**Table 3. Sieving coefficient of low-flux hemodialyzers**

<table>
<thead>
<tr>
<th>Hemodialyzer</th>
<th>0.9% NaCl</th>
<th>5% Human Albumin</th>
<th>Human Whole Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>0.09 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>HP</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>PS (LF)</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>PMMA (LF)</td>
<td>NP</td>
<td>0.01 ± 0.01</td>
<td>NP</td>
</tr>
</tbody>
</table>

*NP, not performed.*
ation because hirudin may accumulate. In intensive care patients with declining or low residual renal function, intermittent hirudin application is strongly suggested (17–19), and hirudin dosage has to be cautiously individualized on a day-to-day basis depending on renal and dialysis elimination of the drug (17). As long as continuous HD is performed with high-flux hemodialyzer membranes, hirudin application may also be continuous. The risk is high, however, that despite interruption of hemofiltration, the application of the drug may inadvertently continue, resulting in accumulation and bleeding risk. Irrespective of the risk for hirudin accumulation, in acute HIT or other strongly prothrombotic conditions, thrombin formation must be sufficiently inhibited.

3. Monitoring of Hirudin Therapy in Renal Failure. For safe performance of hirudin anticoagulation in HD patients, repetitive monitoring is mandatory, which should at least rely on activated partial thromboplastin time (aPTT) measurements. There is a sufficient correlation of aPTT prolongation and hirudin plasma concentration up to 300 ng/ml plasma (20). If available, chromogenic assays or ecarin clotting time also should be used to measure r-hirudin plasma concentrations accurately (21,22).

4. Hirudin Removal before Surgery. In case of scheduled or emergency interventions, utmost caution is warranted. To avoid bleeding episodes within or after invasive procedures, we recommend HD with a high-flux, hirudin-permeable dialyzer membrane to allow reduction of hirudin blood concentration and thus bleeding risk. Because aPTT may stay prolonged for several days after dialysis, it has to be repetitively checked before surgery.

5. Hirudin Removal in Case of Overdose or Bleeding. Hirudin-permeable hemodialyzers allow significant reduction of hirudin plasma concentration when used in hemofiltration mode. In a patient with a body weight of 70 kg, the distribution volume of hirudin is approximately 14 L. Assuming this patient is without residual renal function, hemofiltration (ultrafiltration rate 2.5 L/h; SC 0.97) will result in a hirudin half-life of approximately 4 h. With hemodialyzers showing an SC of 0.75, hirudin half-life would be 5 h. With an SC of 0.49, elimination half-life would be increased up to 8 h. These calculations show how decisive the choice of hemodialyzers is for fast elimination of hirudin (i.e., elimination within hours). This has been impressively demonstrated in two recent case reports (16,23). In the first case, the HD-dependent patient with HIT received a hirudin dosage 30-fold higher than normal. After 6 h of hemodiafiltration with a PS high-flux hemodialyzer, hirudin plasma concentration was reduced by approximately 60% (23). Bleeding did not occur. Another HIT patient with ESRD owing to bilateral nephrectomy was treated with hirudin-anticoagulated HD using a PS low-flux hemodialyzer. Hours after elective resection of a lung metastasis and >24 h after the single predialysis hirudin bolus, the patient developed hemodynamically relevant bleeding within “therapeutic” ranges of hirudin blood levels at an aPTT of 66 s. A 3-h hemofiltration treatment with a PS high-flux hemodialyzer reduced hirudin plasma concentration by 50%, and the patient was stabilized (16). Of note, the elimination of hirudin in this case exactly reproduced the values from our in vitro study. Especially in the latter case, HD with a hirudin-permeable high-flux hemodialyzer before surgery would have reduced intra- and postoperative bleeding risk.

6. Hirudin Removal in Patients with Anti-hirudin Antibodies. Anti-hirudin antibodies frequently occur upon prolonged hirudin treatment and are detectable by rising aPTT values or hirudin concentration while hirudin dosage remains unchanged (24,25). In this case, hemofiltration may no longer be sufficient (26), and plasmapheresis may be the only remaining feasible option to rapidly reduce hirudin plasma concentration (26).

Conclusion

At hirudin concentrations within the therapeutic range, hirudin sieving was found to be significant with high-flux hemodialyzers but absent with low-flux hemodialyzer membranes. However, marked differences in hirudin sieving were also observed between different high-flux hemodialyzers. The PS high-flux hemodialyzer membrane was most effective in removing hirudin. Conclusions that are drawn for hirudin dosing and removal depending on the clinical situation should facilitate use of hirudin anticoagulation in HD-dependent patients. However, use of hirudin in dialysis patients remains a demanding task.

Acknowledgments

We gratefully acknowledge the expert help of the HD technician R. Mörder. We are indebted to the staff of the Hemodialysis Unit and the Department of Transfusion Medicine, University Hospital Freiburg, for support of the study.

Data were previously presented in abstract form (Ann Hematol 79[Suppl 1]: A26, 2000).

Disclosures

None.

References


