Pharmacodynamics and pharmacokinetics of polyethylene glycol-hirudin in patients with chronic renal failure

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Background. Hirudin selectively inhibits thrombin without cofactors and is eliminated via the kidneys. Recombinant hirudin (r-hi) has a terminal elimination half-life (t1/2) of about 50 to 100 minutes. Coupling of polyethylene glycol (PEG) to r-hi, giving PEG-hirudin (PEG-Hi), prolongs its t1/2 while enhancing efficacy. We looked at the pharmacodynamic and pharmacokinetic behavior of PEG-Hi in patients with impaired renal function.

Methods. Anticoagulant activity and the pharmacokinetic parameters of a single intravenous bolus injection of 0.05 mg/kg body weight PEG-Hi were studied in 38 subjects. They were assigned to five groups: group IA, creatinine clearance (Ccr) ≥ 80 mL/min, 8 healthy volunteers; group IB, Ccr ≥ 80 mL/min, 8 patients with normal renal function; group II, Ccr 79 to 50 mL/min, 7 patients with mild chronic renal failure (CRF); group III, Ccr 49 to 20 mL/min, 10 patients with moderate CRF; and group IV, Ccr ≤ 19 mL/min, 5 patients with severe CRF. Plasma and urine samples were collected from patients for up to 120 hours after dosing and from healthy volunteers for up to 24 hours.

Results. PEG-Hi was well tolerated in all groups. No serious adverse events were noted. Cmax values were similar in all groups; area under the curve (AUC) increased in patients from 2.9 ± 1.0 µg·h/mL (IB) to 21.3 ± 5.0 µg·h/mL (IV). According to the severity of renal function, t1/2 was prolonged from 2 hours (IB) to 38.4 hours (IV), while total body clearance (Ctb), renal clearance (Crenal), and recovery of PEG-Hi in the urine (Feo-t) decreased as follows: Ctb from 23.3 ± 6.6 (IB) to 2.9 ± 0.6 mL/min (IV), Crenal from 7.8 ± 5.0 (IB) to 0.8 ± 0.5 mL/min (IV), and Feo-t from 40.2 ± 18.9% (IB) to 12.6 ± 13.0% (IV). Total plasma clearance of PEG-Hi was well correlated with Ccr. Anti-IIa activity of PEG-Hi showed a closer linear relationship to ecarin clotting time than to activated partial thromboplastin time.

Conclusion. Hence, PEG-Hi is considered safe in patients with CRF, but dosing and/or dose intervals should be adjusted according to the severity of renal impairment. Ecarin clotting time is well suited for safe and reliable monitoring of PEG-Hi.

Unfractionated and low molecular weight heparins are the most common anticoagulants in use today and are given to treat a wide range of indications. Patients with end-stage renal failure on maintenance hemodialysis in particular are exposed to substance-specific side effects, such as lipid metabolism disorders, increased bleeding tendency, osteoporosis, alopecia, and, increasingly, heparin-induced thrombocytopenia and heparin-induced thrombosis [1–7]. Although there are several alternative antithrombotic agents, their long-term use is not recommended for a variety of reasons.

Hirudin, a naturally occurring anticoagulant polypeptide obtained from the peripheryngeal glands of the medical leech, Hirudo medicinalis, is a potent and highly selective thrombin inhibitor. Unlike heparin, it does not require the presence of antithrombin III (AT III) or other endogenous cofactors. Furthermore, hirudins inhibit free and clot-bound thrombin, whereas heparins inhibit only free thrombin. The recombinant equivalent of hirudin has proved to be an effective anticoagulant and antithrombotic agent, allowing a new approach to the prevention and treatment of thromboembolic diseases in both the arterial and venous systems [8–11]. With a terminal elimination half-life of about 50 to 100 minutes (abstract; Esslinger et al. Thromb Haemost 65: 1291, 1991) [12, 13], hirudins are rapidly eliminated from the circulation via the kidneys. When using recombinant hirudin (r-hi) in patients with renal insufficiency, a change in the pharmacokinetics of the substance is to be expected [14]. Since the establishment of ecarin clotting time as a reliable bedside monitoring tool, r-hi has been given as an anticoagulant in single dosing [15–17] and regular hemodialysis treatment [18, 19].

To achieve a prolongation of the elimination half-life,
thus enhancing the anticoagulant efficacy of hirudin, a specially designed hirudin-mutein was covalently bound to two polyethylene glycol (PEG)-5000 residues. The resulting compound, PEG-hirudin (PEG-Hi), exhibits similar inhibitory activity and selectivity toward thrombin as its nonconjugated equivalent both in vitro and in vivo [abstract; Iorio et al, Ann Hematol 66(Suppl I):A18, 1993]. Furthermore, with the exception of polymethylmethacrylate membranes, PEG-Hi does not flow through any of the high- and low-flux membranes. Moreover, prolongation of the elimination half-life of PEG-Hi entails a dose reduction and, consequently, lower costs. PEG-Hi is well tolerated by healthy volunteers, and no immunological reactions have been observed as described by Esslinger et al [20]. Since PEG-Hi is eliminated exclusively by renal excretion, impaired renal function might have a significant impact on its pharmacokinetics. The purpose of this study, therefore, was to determine the single-dose pharmacokinetics of intravenous PEG-Hi in patients with varying degrees of chronic renal failure (CRF) in comparison to healthy volunteers.

## METHODS

### Subjects

Eight volunteers between the ages of 22 and 60 and 30 patients between the ages of 26 and 73 with varying degrees of CRF were enrolled in the study. The subjects were assigned to the following groups: group IA, creatinine clearance ($C_{\text{Cr}}$) ≥ 80 mL/min, healthy volunteers; group IB, $C_{\text{Cr}}$ ≥ 80 mL/min, patients with normal renal function; group II, $C_{\text{Cr}}$ 79 to 50 mL/min, patients with mild CRF; group III, $C_{\text{Cr}}$ 49 to 20 mL/min, patients with moderate CRF; and group IV, $C_{\text{Cr}}$ ≤ 19 mL/min, patients with severe CRF.

The patients’ characteristics are shown in Table 1. The final grouping of renal function and the subsequent analyses of the pharmacokinetic data were based on $C_{\text{Cr}}$ values, as determined on the basis of 24-hour urine collection during the in-house study period after PEG-Hi dosing. The study was approved by the Ethics Committee of the Faculty of Medicine at the University of Jena (Jena, Germany). Written informed consent was obtained from each participant prior to enrollment.

### Trial drug and drug administration

Polyethylene glycol-hirudin is a chemically defined conjugate of a recombinant hirudin mutein (rm-hirudin) and two molecules of PEG-5000 with a mean molecular mass of 17 kD. The drug substance, a product of genetic engineering, was supplied by Knoll AG (Ludwigshafen, Germany) in lyophilisate form. Its specific antithrombin activity is about 12,600 antithrombin units per milligram protein. Lyophilized PEG-Hi was reconstituted with 0.5 mL of sterile water for injection and was diluted with physiological saline to a concentration of 1 mg/mL. Thereafter, the solution was administered intravenously as a single bolus injection of 0.05 mg/kg body weight.

### Blood and urine sampling

Blood samples for the evaluation of anti-IIa activity and clotting parameters were collected in tubes containing 1:10 vol/vol 3.8% sodium citrate from HVs and patients before dosing and at 5, 10, 20, and 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 24 hours after dosing. Additional blood samples were collected from patients at 36, 48, 60, 72, 84, 96, 108, and 120 hours after dosing. After separation of the citrated blood, the samples were immediately centrifuged at 1500 × g for 15 minutes at 4°C. The resulting plasma was divided into aliquots and then shock frozen and stored at −75°C until analyzed. Urine was collected from all subjects immediately before dosing and during the following intervals: 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after dosing.

### Anti-IIa activity and pharmacokinetic evaluation

Pharmacokinetic evaluation based on PEG-Hi anti-IIa activity was performed using compartment model-independent procedures. Anti-IIa activity in plasma and

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>M/F</th>
<th>Diagnosis</th>
<th>Age years median (range)</th>
<th>Serum creatinine μmol/L</th>
<th>Creatinine clearance mL/min</th>
<th>UPE mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>8</td>
<td>7/1</td>
<td>HV</td>
<td>30 (22-60)</td>
<td>81.9 ± 11.4</td>
<td>144.7 ± 22.0</td>
<td>125 ± 18</td>
</tr>
<tr>
<td>IB</td>
<td>8</td>
<td>7/1</td>
<td>GN 5, IN 2, PKD 1</td>
<td>40 (26-65)</td>
<td>96.1 ± 27.7</td>
<td>111.0 ± 32.0</td>
<td>725 ± 1084</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>5/2</td>
<td>GN 4, IN 2, HN 1</td>
<td>40 (41-68)</td>
<td>167.1 ± 23.4</td>
<td>58.3 ± 9.6</td>
<td>567 ± 1084</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>6/4</td>
<td>GN 2, IN 6, DN 2</td>
<td>56 (37-66)</td>
<td>269.2 ± 126.1</td>
<td>35.7 ± 10.9</td>
<td>1452 ± 2488</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>4/1</td>
<td>GN 1, IN 4</td>
<td>56 (45-73)</td>
<td>742.2 ± 238.8</td>
<td>10.8 ± 2.5</td>
<td>1257 ± 371</td>
</tr>
</tbody>
</table>

Abbreviations are: HV, healthy volunteers; GN, glomerulonephritis; IN, interstitial nephritis/analgesic nephropathy; DN, diabetic nephropathy; HN, hypertensive nephropathy; PKD, polycystic kidney disease; UPE, urinary protein excretion; M, males; F, females. Data are median (range) or mean ± SD.
urine was measured using chromogenic substrate S-2238 (Chromogenix, Moelndal, Sweden) according to the method originally described by Spannagl et al [21]. Peak plasma anti-IIa activity (C\text{max}) and time to C\text{max} (t\text{max}) were recorded as observed. The slowest hybrid rate constant (λz) and, hence, terminal half-life (t_{1/2} = 0.693/λz) were calculated from the slope of the terminal portion of log plasma anti-IIa activity versus time course. No descriptive statistics were provided if more than two thirds of the measurements at the respective sampling times were below the limit of quantitation (LOQ = 100 ng/mL).

Coagulation assays and bleeding time

Dade® reagent (Baxter, Columbia, MD, USA) was used to determine the activated partial thromboplastin time (aPTT). Monitoring of PEG-Hi concentrations consisted of measuring the ecarin clotting time in the citrated whole blood and plasma using a standardized reagent manufactured by Pentapharm (Basel, Switzerland). The ecarin clotting time is a specific test for the determination of hirudin in blood, plasma, or other body fluids. The starting reagent, made of Echis carinatus snake venom, is ecarin. Ecarin splits the prothrombin molecule at the arginine 320 peptide bond. This limited proteolysis produces a thrombin intermediate called meizothrombin. Hirudin is able to inhibit meizothrombin with the same extent as thrombin. Meizothrombin also converts fibrinogen into fibrin, but with considerably lower activity than thrombin. Fibrin conversion follows reaction kinetics of the first order. Therefore, the calibration curve for hirudin is linear over a wide concentration range (50 to 5000 ng/mL) [22]. The quantitative estimation of fibrinogen was determined as the protein concentration by the biuret method. Template bleeding time was measured at a standardized horizontal cut on the volar aspect of the forearm made with a Surgicutt® device after inflating a sphygmomanometer cuff on the upper arm to 40 mm Hg [23].

Safety assessment

Routine clinical chemistry (including serum creatinine and liver enzyme determinations) was performed, and hematological parameters were determined during a two-week prestudy period, shortly before drug administration and after the study. The urinary protein pattern was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) during the prestudy period. Urine samples were checked repeatedly for hematuria using reagent test strips (Combur®-Test®) as were the feces for occult blood with the hemo-Care® test. ECG recordings, blood pressure, heart rate, and body temperature were determined before dosing, and after the study. Specific anti–PEG-Hi antibody assays were performed using enzyme immunoassays with peroxidase-labeled goat immunonjugates against human IgM and IgG (Nordic, Bochum, Germany), normal limits <2\(^\text{nd}\), for up to six-weeks postdose. The “Total IgE” kit (Immuno-tech, Marseille, France) was used to determine total IgE class antibodies.

Statistical analysis

Statistical analysis was performed with descriptive methods. Dependency of AUC\text{0-\text{t}}, t\text{\text{1/2}}, total body clearance (C\text{TB}) and renal clearance (C\text{\text{Renal}}) on creatinine clearance (C\text{\text{Cr}}), as well as the relationship between PEG-Hi plasma levels and coagulation parameters was analyzed by linear regression.

RESULTS

Tolerability

Polyethylene glycol-hirudin was very well tolerated without any serious adverse events. There were no signs of bleeding in the gastrointestinal tract or urogenital system. The platelet count as well as red and white blood counts (RBC, WBC) were not affected by PEG-Hi. In 18 subjects, IgE class prestudy antibody levels were above 120 ng/mL, with maximum levels of up to about 1600 ng/mL. However, no allergic reactions were observed after injecting PEG-Hi, and IgE levels measured after the study and at six weeks’ follow-up were similar to the baseline values. As a rule, there were no measurable IgG and IgM antibody titers against PEG-Hi. Existing titers between 2\(^{\text{nd}}\) and 2\(^{\text{\text{1/2}}}\) in eight subjects remained unchanged for up to six weeks after PEG-Hi treatment. Clinical chemistry [total serum protein, triglycerides, alanine aminotransferase (ALAT), gamma-guanidinium thiocyanate (γ-GT), and alkaline phosphatase (APH)] values did not significantly change for up to 120 hours after the administration of PEG-H. Bilirubin increased within the normal range, whereas aspartate aminotransferase (ASTAT) and cholesterol displayed a falling tendency. In all patient groups, serum creatinine values declined after PEG-Hi dosing; however, these differences were not statistically significant. Concomitant treatment of underlying diseases was continued and consisted primarily of drugs against hypertension, hyperuricemia, hyperlipidemia, hyperparathyroidism, hypocalcemia, and acidosis. None of the medications were considered to interfere with the study objectives.

Pharmacodynamics and pharmacokinetic parameters

The mean plasma concentration time profiles and pharmacokinetic parameters for each study group are shown in Figure 1 and Table 2, respectively. C\text{\text{max}} values were similar in all groups. AUC increased distinctly in patients with moderate and severe renal failure. Total body clearance from the plasma (C\text{\text{TB}}) and renal clearance (C\text{\text{\text{Renal}}}) values as well as recovery of PEG-Hi from the urine decreased, whereas the elimination half-life (t\text{\text{1/2}}) increased along with the severity of renal function impairment. Total plasma clearance of PEG-Hi correlated
well with $C_r$, as can be seen in Figure 2. Plasma concentration time profiles based on ecarin clotting time measurements are shown in Figure 3. Ecarin clotting time displayed a closer linear relationship with anti-IIa activity of PEG-Hi than aPTT (Figs. 4 and 5). PEG-Hi administration did not prolong bleeding time in either study group. Baseline fibrinogen levels in the patients increased parallel to the severity of renal failure, but these values were not affected by PEG-Hi treatment (Fig. 6).

**DISCUSSION**

Polyethylene glycol-hirudin proved to be a safe, direct thrombin inhibitor in healthy volunteers [20]. Its prolonged duration of action compared with nonconjugated r-hi is characterized by a fivefold reduction in total plasma clearance [20]. Because it is predominantly eliminated via the kidneys and to ensure safety in the patients who will be treated with PEG-Hi in clinical studies (for example, unstable angina, hemodialysis), we investigated the pharmacokinetics and pharmacodynamics of PEG-Hi in patients with various degrees of CRF after a single bolus injection of 0.05 mg/kg PEG-Hi and compared these results with data obtained in healthy volunteers and control patients. PEG-Hi clearance correlates well with $C_r$ ($r^2 = 0.86$). It accounts for about 20% of $C_r$ and therefore reflects well the degree of renal failure. Because of the intravenous administration of PEG-Hi, the peak plasma concentrations were similar (about 1000 ng/mL) in all study groups. These results are in good agreement with data previously obtained from healthy volunteers [20]. At four hours postdose, plasma PEG-Hi levels had already fallen to below 200 ng/mL in healthy volunteers compared with 540 ng/mL in patients with severe renal function.

**Table 2. Pharmacokinetic parameters of PEG-hirudin**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group IA</th>
<th>Group IB</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_r$, mL/min</td>
<td>144.7 ± 22.0</td>
<td>111.0 ± 32.0</td>
<td>58.3 ± 9.6</td>
<td>35.7 ± 10.9</td>
<td>10.8 ± 2.5</td>
</tr>
<tr>
<td>$C_{TB}$, ng/mL</td>
<td>974 ± 119</td>
<td>1000 ± 169</td>
<td>1107 ± 288</td>
<td>1113 ± 103</td>
<td>931 ± 74</td>
</tr>
<tr>
<td>$t_{max}$, hours</td>
<td>0.08 (0.08–0.17)</td>
<td>0.13 (0.08–0.17)</td>
<td>0.08 (0.08–0.08)</td>
<td>0.08 (0.08–0.17)</td>
<td>0.17 (0.08–0.50)</td>
</tr>
<tr>
<td>$t_{1/2}$, hours</td>
<td>1.6 (1.3–2.1)</td>
<td>2.0 (1.1–4.6)</td>
<td>4.1 (3.0–10.0)</td>
<td>12.0 (4.1–32.2)</td>
<td>38.4 (26.0–87.2)</td>
</tr>
<tr>
<td>AUC, μg·h/mL</td>
<td>1.85 ± 0.5</td>
<td>2.48 ± 0.8</td>
<td>4.57 ± 1.2</td>
<td>9.95 ± 4.9</td>
<td>13.54 ± 2.2</td>
</tr>
<tr>
<td>$AUC_{TB}$, μg·h/mL</td>
<td>2.15 ± 0.5</td>
<td>2.86 ± 1.0</td>
<td>5.42 ± 1.2</td>
<td>12.91 ± 6.3</td>
<td>21.31 ± 5.0</td>
</tr>
<tr>
<td>$C_{TB}$, mL/min</td>
<td>30.7 ± 5.6</td>
<td>23.3 ± 6.6</td>
<td>12.1 ± 4.2</td>
<td>6.5 ± 3.4</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>$C_{renal}$, mL/min</td>
<td>10.6 ± 4.8</td>
<td>7.8 ± 5.0</td>
<td>3.0 ± 0.9</td>
<td>2.2 ± 1.4</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>$V_c$, L</td>
<td>4.3 ± 0.8</td>
<td>4.0 ± 0.7</td>
<td>4.9 ± 1.4</td>
<td>6.6 ± 2.8</td>
<td>10.9 ± 4.7</td>
</tr>
<tr>
<td>MRT, hours</td>
<td>2.3 ± 0.4</td>
<td>3.3 ± 1.6</td>
<td>6.8 ± 2.8</td>
<td>23.4 ± 14.3</td>
<td>59.0 ± 27.5</td>
</tr>
<tr>
<td>FE, %</td>
<td>42.6 ± 18.8</td>
<td>40.2 ± 18.9</td>
<td>30.1 ± 17.8</td>
<td>27.9 ± 10.9</td>
<td>12.6 ± 13.0</td>
</tr>
</tbody>
</table>

Values as mean±SD or median and range. Abbreviations are: $C_r$, creatinine clearance; $t_{1/2}$, elimination half-life; AUC, area under the plasma concentration-time curve from time zero to time infinity; $C_{TB}$, total body clearance from the plasma; $C_{renal}$, renal clearance of PEG-hirudin; $V_c$, apparent volume of the control or plasma compartment; MRT, mean residence time; FE, fraction of PEG-hirudin dose recovered in urine up to the last concentration above the lower limit of quantification.
failure. The elimination half-life and AUC values correlated negatively with PEG-Hi and C Cr. In view of these results, PEG-Hi doses have to be adjusted according to the extent of CRF. As there is a linear relationship between C Cr and PEG-Hi, the normal starting dose of 0.08 mg/kg body weight PEG-Hi has to be reduced according to the actual C Cr measured. The therapeutic range of PEG-Hi during hemodialysis is expected to be between 600 and 800 ng/mL [19]. This range was found in an early clinical study investigating the efficacy of r-hi in chronic dialysis patients undergoing five or ten consecutive dialysis sessions. Ecarin clotting time, used to optimize drug monitoring of PEG-Hi, was compared with aPTT and anti-IIa activity methods. A good correlation between ecarin clotting time and anti-IIa activity of the PEG-conjugated r-hi was found. This is consistent with the results reported by Bode et al [abstract: J Am Coll Cardiol 29/2(Suppl A):411, 1997], who found a close linear relationship of ecarin clotting time to PEG-Hi
concentrations from 100 to 3000 ng/mL in unstable angina patients.

Our results support evidence that anti-IIa activity and ecarin clotting time assays are optimal tools for monitoring the anticoagulant effect of PEG-Hi, whereas aPTT seems to be less reliable especially in the presence of higher PEG-Hi concentrations. PEG-Hi did not prolong bleeding time in either study group. This is in keeping with earlier study results in healthy volunteers [20]. Age and gender or special renal disease of the patients, such as diabetic nephropathy, were not of primary interest in this study, which focused on the influence of the degree of renal impairment on the pharmacokinetic and pharmacodynamic parameters of PEG-Hi.

The nature of the renal disease did not influence the pharmacokinetics of PEG-Hi. PEG-Hi was well tolerated in all patient groups. Clinical parameters such as echocardiogram, pulse rate, and body temperature were not affected by the administration of PEG-Hi. No serious adverse events and no signs of bleeding in the gastrointestinal tract or urogenital system were noted. No influence of clinical relevance on blood counts or clinical chemistry values was observed. There were also no differences in anti-PEG-Hi antibody levels (IgM, IgG) or IgE antibodies before dosing or six weeks thereafter. In all patient groups, serum creatinine levels declined after PEG-Hi administration; however, these decreases were not statistically significant. This observation has already been reported by Nowak et al using r-hi [18]. The improvement in renal function observed in our patients during PEG-Hi anticoagulation might be related to enhanced renal perfusion and inhibition of fibrin-bound thrombin [24]. In patients with acute and CRF, higher fibrinogen levels reflecting hypercoagulable status should be taken into consideration. In our study, the direct thrombin inhibitor PEG-H had no influence on fibrinogen serum levels after single bolus administration. Finally, it should be pointed out that so far no specific antidote to PEG-Hi is available. However, dialysis with polymethylmethacrylate-type dialyzers may be used to remove PEG-Hi from the system if bleeding or adverse effects occur (G. Nowak, personal communication).

In summary, a single intravenous bolus injection of 0.05 mg/kg body weight PEG-Hi proved to be safe in patients with CRF. Impaired renal function results in higher AUC values, prolonged elimination half-lives, and reduced Cmax and Cl Renal. Values. In view of its pharmacokinetic properties, PEG-Hi dosing and/or dose intervals should be adjusted according to the severity of renal impairment. Because of the close correlation between ecarin clotting time and PEG-Hi plasma concentrations, this assay is considered to be well-suited for safe and reliable monitoring of PEG-Hi treatment in patients with renal impairment.

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