Influence of Renal and Hepatic Failure on the Pharmacokinetics of Recombinant Human Granulocyte Colony-stimulating Factor (KRN8601) in the Rat

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ABSTRACT

The pharmacokinetics of recombinant human granulocyte colony-stimulating factor (rhG-CSF) was studied in rats experiencing renal and hepatic failure. The serum concentration of rhG-CSF after i.v. administration to male Sprague-Dawley rats at a dose of 10 µg/kg was investigated by a sandwich enzyme immunoassay. Total-body clearance of rhG-CSF was 44.48 ml/h/kg in sham-operated rats compared with 9.429 ml/h/kg in bilaterally nephrectomized rats. In sham-operated rats, the half-life ($\beta$) of rhG-CSF was 1.512 h, and it increased to 5.333 h after nephrectomy. The volumes of distribution were identical in both rats. In rats with acute renal failure induced by uranyl nitrate, the clearance and volume of distribution were identical to those of control rats, but the half-life ($\beta$) was slightly shorter. In partially (70%) hepatectomized rats, the clearance of rhG-CSF decreased from 42.08 ml/h/kg to 31.93 ml/h/kg. Similar half-lives were observed in rats in both the sham-operated and hepatectomized groups. However, the volume of distribution decreased after hepatectomy. In rats with hepatic failure induced by CCL₄, the pharmacokinetic changes were similar to those observed in hepatectomized rats. These results suggest that renal clearance makes a major contribution to total-body clearance compared with hepatic clearance.

INTRODUCTION

The generation of granulocytes from immature hematopoietic progenitor cells depends on the presence of several colony-stimulating factors (1, 2). Colony-stimulating factors are acidic glycoproteins required for the survival, proliferation, and differentiation of hematopoietic progenitor cells. G-CSF² is identified by its ability to stimulate bone marrow precursor cell proliferation and differentiation into granulocyte colonies (3, 4). The gene encoding human G-CSF has been isolated (5, 6). Characterization of the gene encoding G-CSF has led to the production of the protein by recombinant techniques. rhG-CSF is also capable of supporting the formation of granulocytic colonies from committed precursor cells (5-7). Recently rhG-CSF has been applied clinically in patients with various cancers to accelerate the hematological recovery after high-dose chemotherapy with and without autologous bone marrow rescue, and it has been found to markedly shorten the duration of neutropenia (8-13). Hence, rhG-CSF may be very useful in the treatment of cancer patients with drug- or radiation-induced myelosuppression.

We have previously reported the pharmacokinetics and pharmacodynamics of rhG-CSF in normal male SD rats (14). However, the metabolism and excretion of rhG-CSF are not well understood. It is well known that the liver and kidneys play an important role in drug elimination from the blood. In this study, we investigated the role of the kidneys and liver in rhG-CSF pharmacokinetics in the rat.

MATERIALS AND METHODS

Animals. Male SD rats were obtained from Japan SLC Co., Ltd. (Shizuoka, Japan). The animals were housed for at least 1 wk before experimentation with free access to food and water. All animals were used when they were 8 wk old.

Reagents. rhG-CSF (KRN8601, lot G.22028) was produced by Kirin Brewery Co., Ltd., as reported previously (5). All reagents were of analytical grade and were obtained commercially.

Biochemical Examination. Blood was obtained from the abdominal aorta of animals under light ether anesthesia immediately after the final sampling time point for the pharmacokinetic studies. Blood was centrifuged at 18,000 $\times$ g for 5 min to separate serum, and the obtained serum was frozen in liquid nitrogen and stored at $-80^\circ$C until used. The development of renal and hepatic dysfunctions was monitored by measurement of serum creatinine, BUN, GOT, and GPT concentrations using clinical test kits (Wako Pure Chemicals Industries, Ltd., Osaka, Japan).

Surgical Procedures. After light ether anesthesia, the animals were laparotomized. Experimental bilateral functional nephrectomy was achieved by bilateral renal artery and vein ligation using thread. Unilateral functional nephrectomy was performed by left renal artery and vein ligation. Functional partial (70%) hepatectomy was performed by the ligation of the median and left lateral lobes of the liver. Sham-operated control animals were laparotomized, but arteries and veins were not ligated. The procedures took 5 to 10 min, and the rats recovered consciousness quickly because of the short duration of action of the anesthetic.

Experimental Renal or Hepatic Failure Induced by Chemicals. Acute renal failure was induced in male SD rats by an i.v. injection of 5 mg/kg of uranyl nitrate via the tail vein. Animals given injections of an equal volume of saline served as controls. Animals were used on the fifth day after treatment. Hepatic failure was induced in the rat by p.o. administration of 2 ml/kg of 50% carbon tetrachloride (CCL₄) in olive oil. Olive oil was administered p.o. to animals as controls. The experiment using animals with hepatic failure was started 24 h after treatment.

Collection of Serum Samples for Sandwich Enzyme Immunoassay. The experimental design provided three or four animals in each group. Ten µg/kg of rhG-CSF were administered through the tail vein. At subsequent intervals, 200-µl samples of blood were collected into ice-cold polypropylene tubes (Falcon Labware, Oxnard, CA) from the tail vein. Blood was centrifuged at 10,000 $\times$ g for 2 min to separate the serum, and the obtained serum was transferred into a microtube (Assist Trading Co., Ltd., Tokyo, Japan). The serum was then frozen in liquid nitrogen and stored at $-80^\circ$C until used.

Urine Collection for Sandwich Enzyme Immunoassay. The urethra of three rats were catheterized with a polyethylene tube (inner diameter, 0.86 mm; outer diameter, 1.27 mm) under light ether anesthesia. The animals were placed in a Bollman cage after the animals recovered consciousness. Urine was collected in a polypropylene tube at 4°C for 24 h after 10 µg/kg of rhG-CSF were administered via the tail vein. A sample of 0.5 ml of 16% BSA solution in PBS was added to the polypropylene tube to prevent the adsorption of rhG-CSF onto the tube surface before use. The collected urine was diluted 2-fold with 16% BSA solution in PBS, and the concentration of rhG-CSF in urine was measured using a sandwich enzyme immunoassay.
Buffer (pH 9.2) to a concentration of 400 μg/ml. Diluted immunoglobulin, anti-rhG-CSF immunoglobulin was diluted with 50 mM carbonate of rhG-CSF was evaluated using a sandwich enzyme immunoassay.1 In SD.

Subsequent intervals by enzyme immunoassay. Points, mean of four animals; bars, SD.

Male SD rats (8 wk old) after sham operation (O), unilateral nephrectomy (A), and bilateral nephrectomy (D) were given injections of 10 μg/kg of rhG-CSF, and concentrations of rhG-CSF in serum were measured at subsequent intervals by enzyme immunoassay. Points, mean of four animals; bars, SD.

**PHARMACOKINETICS OF rhG-CSF IN DISEASE**

The development of renal and hepatic dysfunction was monitored by measurement of creatinine, BUN, GOT, and GPT levels. Statistical comparisons of two means were performed between sham operation and nephrectomy and between saline and uranyl nitrate injection.

**Table 1 Biochemical measurements in rats experiencing renal failure**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Creatinine (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>GOT (IU/liter)</th>
<th>GPT (IU/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>0.626 ± 0.027ab</td>
<td>15.0 ± 1.4</td>
<td>74.5 ± 14.3</td>
<td>33.0 ± 5.6</td>
</tr>
<tr>
<td>Unilateral nephrectomy</td>
<td>0.810 ± 0.049a</td>
<td>24.5 ± 2.2</td>
<td>77.8 ± 8.5</td>
<td>29.0 ± 10.1</td>
</tr>
<tr>
<td>Bilateral nephrectomy</td>
<td>2.46 ± 0.04a</td>
<td>83.6 ± 5.5a</td>
<td>101.3 ± 13.1f</td>
<td>32.5 ± 7.5</td>
</tr>
<tr>
<td>Saline</td>
<td>0.650 ± 0.047</td>
<td>16.2 ± 1.5</td>
<td>56.0 ± 13.7</td>
<td>37.0 ± 12.6</td>
</tr>
<tr>
<td>Uranyl nitrate</td>
<td>4.19 ± 1.24a</td>
<td>208.0 ± 38.3</td>
<td>70.5 ± 1.3</td>
<td>27.3 ± 2.8</td>
</tr>
</tbody>
</table>

* Mean ± SD (n = 4).
+ P < 0.01
° Mean ± SD (n = 4, but n = 3 for CC14 treatment).

**Table 2 Biochemical measurements in rats experiencing hepatic failure**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Creatinine (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>GOT (IU/liter)</th>
<th>GPT (IU/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>0.654 ± 0.043a</td>
<td>14.0 ± 1.34</td>
<td>66.3 ± 5.9</td>
<td>28.0 ± 2.9</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>0.863 ± 0.041a</td>
<td>34.0 ± 3.5</td>
<td>1785 ± 350a</td>
<td>1098 ± 420f</td>
</tr>
<tr>
<td>Olive oil</td>
<td>0.555 ± 0.042</td>
<td>14.2 ± 1.5</td>
<td>50.3 ± 8.2</td>
<td>34.8 ± 6.3</td>
</tr>
<tr>
<td>CCl4</td>
<td>0.734 ± 0.023</td>
<td>21.8 ± 1.0</td>
<td>210.7 ± 20.5f</td>
<td>170.7 ± 45.9f</td>
</tr>
</tbody>
</table>

* Mean ± SD (n = 4, but n = 3 for CCl4 treatment).
+ P < 0.01
° Mean ± SD (n = 4).
° Mean ± SD (n = 3 for CCl4 treatment).
° 0.01 < P < 0.05.

**Fig. 2. Serum concentrations of rhG-CSF via i.v. administration to rats after uranyl nitrate administration.** Male SD rats (8 wk old) after uranyl nitrate (●) or saline treatment (○) were given injections of 10 μg/kg of rhG-CSF, and concentrations of rhG-CSF in serum were measured at subsequent intervals by enzyme immunoassay. Points, mean of four animals; bars, SD.

**RESULTS**

Biochemical Analysis. The development of renal and hepatic dysfunction was monitored by measurement of creatinine,
Comparisons of two means were performed between sham operation and nephrectomy and between saline and uranyl nitrate treatment (Table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC (ng h/ml)</th>
<th>CL (ml/h/kg)</th>
<th>Vc (ml/kg)</th>
<th>Vdss (ml/kg)</th>
<th>t1/2α (h)</th>
<th>t1/2β (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>231.5 ± 42.0*</td>
<td>44.48 ± 9.46</td>
<td>44.29 ± 14.95</td>
<td>62.18 ± 15.79</td>
<td>0.4474 ± 0.0916</td>
<td>1.512 ± 0.265</td>
</tr>
<tr>
<td>Unilateral nephrectomy</td>
<td>415.5 ± 23.5a</td>
<td>24.13 ± 1.42c</td>
<td>36.90 ± 3.80</td>
<td>55.90 ± 7.86</td>
<td>0.4176 ± 0.1303</td>
<td>2.037 ± 0.536</td>
</tr>
<tr>
<td>Bilateral nephrectomy</td>
<td>1075.8 ± 153.9</td>
<td>9.429 ± 1.25c</td>
<td>42.75 ± 5.23</td>
<td>65.15 ± 6.37</td>
<td>0.4142 ± 0.1417</td>
<td>5.333 ± 1.591</td>
</tr>
<tr>
<td>Saline</td>
<td>247.4 ± 24.0</td>
<td>40.69 ± 3.79</td>
<td>34.43 ± 2.57</td>
<td>54.95 ± 3.62</td>
<td>0.3078 ± 0.0608</td>
<td>1.315 ± 0.0625</td>
</tr>
<tr>
<td>Uranyl nitrate</td>
<td>243.8 ± 19.1</td>
<td>41.20 ± 3.20</td>
<td>32.52 ± 2.32</td>
<td>51.86 ± 1.24</td>
<td>0.2102 ± 0.077</td>
<td>1.085 ± 0.027</td>
</tr>
</tbody>
</table>

* Mean ± SD (n = 4).

# Table 3 Pharmacokinetic parameters of rhG-CSF obtained after i.v. injections of 10 μg/kg to rats experiencing renal failure

![Graph 1](image1)

**Fig. 3.** Serum concentrations of rhG-CSF via i.v. administration to rats after hepatectomy. Male SD rats (8 wk old) after sham operation (C) and partial hepatectomy (A) were given injections of 10 μg/kg of rhG-CSF, and concentrations of rhG-CSF in serum were measured at subsequent intervals by enzyme immunoassay. Points, mean of four animals; bars, SD.

BUN, GOT, and GPT levels in serum. Treatment of functional nephrectomy caused appreciable elevations of creatinine and urea nitrogen levels in serum. The elevations appeared to be related to the number of kidneys ligated. Uranyl nitrate injection also caused severe renal dysfunction. Uranyl nitrate injection did not significantly influence the hepatic function investigated here. After bilateral nephrectomy, GOT levels increased but the change was small (Table 1).

Partial hepatectomy led to marked increases in GOT and GPT levels. In addition, functional hepatectomy resulted in increases in creatinine and urea nitrogen levels in serum. Administration of CCl4 caused hepatic dysfunction and also influenced renal function (Table 2).

Pharmacokinetics of rhG-CSF in Rats Determined by Enzyme Immunoassay. The pharmacokinetics of rhG-CSF administered i.v. to male SD rats was studied after various treatments. While the serum rhG-CSF concentration-time curves after unilateral and bilateral nephrectomy or sham operation were best described by a biexponential equation, the serum concentrations increased after nephrectomy (Fig. 1). A similar time course of mean serum rhG-CSF concentrations was observed between the control rats and those in renal failure induced by uranyl nitrate injection (Fig. 2). Pharmacokinetic analysis of the serum rhG-CSF concentration-time data resulted in the following disposition characteristics for rhG-CSF. As shown in Table 3, the total-body clearance of rhG-CSF decreased from 44.48 ml/h/kg in sham-operated rats to 24.13 ml/h/kg in unilaterally nephrectomized rats, and to 9.429 ml/h/kg in bilaterally nephrectomized rats. No difference in the volume of distribution, Vc, and Vdss was observed in both animal groups after nephrectomized or sham operation. The terminal disposition half-life (t1/2β) differed between sham-operated and bilaterally nephrectomized rats. Renal dysfunction induced by uranyl nitrate injection had no effect on the total-body clearance and volume of distribution of rhG-CSF but shortened the t1/2β as shown in Table 3.

The mean serum concentration-time curves of rhG-CSF after partial hepatectomy and sham operation are shown in Fig. 3. After partial hepatectomy, higher serum rhG-CSF concentrations were observed early after administration, and after 2 h a significant difference was not found. A similar phenomenon was observed in the rats with hepatic failure induced by CCl4 (Fig. 4). Induction of hepatic failure by CCl4 resulted in higher serum rhG-CSF concentrations than in control rats early after administration, but the differences between the rhG-CSF concentrations in CCl4-treated rats and those in control rats were smaller. Pharmacokinetic analysis (Table 4) shows a 24% and 18% decrease in total-body clearance after partial hepatectomy and CCl4 treatment, respectively. No changes in half-lives were observed, but the volume of distribution decreased after partial...
The pharmacokinetics of rhG-CSF in animals and humans has been reported. Various assay techniques for rhG-CSF determination in blood, plasma, or serum have been used in these studies. The assay procedures were categorized into three techniques, i.e., bioassays (9, 14), immunological assays (17–20), and assays using radiolabeled compounds (21). Iodination of protein often causes a decrease in biological activity, and it has been reported that the iodination of rhG-CSF is difficult (22). Methods of metabolic radiolabeling of rhG-CSF have been reported previously but entailed great expense. The concentrations of rhG-CSF in rat serum after induced renal and hepatic failure were not accurately determined by a [3H]thymidine uptake assay, because rat serum after the treatments described above inhibited the biological activities of rhG-CSF (data not shown). Therefore, we used a sandwich enzyme immunoassay for this rhG-CSF pharmacokinetic study in the rat after renal or hepatic failure was induced.

Evidence has been accumulated showing that the pharmacokinetics and pharmacodynamics of many drugs such as phenytoin, diazepam, and propranolol are altered in renal or hepatic failure (23–26). In most cases, the causes of pharmacokinetic changes have been attributed to impaired serum protein binding, metabolism, or blood flow changes in disease. The pharmacokinetic analyses of several peptide or protein drugs such as epidermal growth factor (27), interferon (28), and plasminogen activator (29) are also influenced by renal and hepatic failure, so in many cases liver and kidney dysfunction caused marked decreases in total-body clearance. Such information is clinically very important because patients suffer from various disorders.

The pharmacokinetics of rhG-CSF administered to male SD rats by i.v. injection after functional nephrectomy was studied. Unilateral and bilateral nephrectomy caused decreases in total-body clearance, i.e., a 46% and 79% reduction, respectively. Urinary excretion of rhG-CSF injected into normal rats was negligible, so the decrease in total-body clearance of rhG-CSF after nephrectomy was due to reductions in metabolism. It has been reported that uranyl nitrate causes necrosis of proximal renal tubules (30). The renal dysfunction index, creatinine, and BUN levels showed severe damage to the kidneys by uranyl nitrate, but uranyl nitrate injection had little influence on the pharmacokinetics of rhG-CSF in rats. Therefore the clinical index of kidney function, such as creatinine and BUN levels, cannot be used to estimate the changes in rhG-CSF metabolism. A similar phenomenon was reported in the pharmacokinetic studies of erythropoietin (31, 32).

Partial hepatectomy and CCl₄ administration caused a 24% and 18% reduction in total-body clearance of rhG-CSF in rats. As shown in Table 4, the reduction in clearance was due to the change in volume of distribution rather than to changes in metabolism.

To understand the fate of drugs in animals, it is important to determine the main organs of elimination. The contribution of an organ, such as the liver or kidneys, to total-body clearance of drugs can be determined by measuring the extraction ratio of the organ. In vivo or in situ (perfusion), the extraction ratio of an organ is determined from blood concentration differences between an artery (input perfusate) and vein (output perfusate) (27, 33). The total-body clearance of rhG-CSF is low, as shown in Tables 3 and 4. Therefore the extraction ratio might be very small in any one organ, and it is difficult to determine the extraction ratio of rhG-CSF from artery-vein concentration differences. The contribution of kidney clearance to total-body clearance obtained from these renal nephrectomy experiments showed that the main elimination organ for rhG-CSF in rats was the kidney. However, the obtained values were not accurate, because renal dysfunction influenced the blood flow and the metabolic activity of other organs (34, 35).

We conclude that renal metabolism is a major route of rhG-CSF elimination. The reduction of total-body clearance of rhG-CSF in patients with renal dysfunction cannot be estimated from an ordinary clinical index, such as creatinine and BUN levels. Therefore, it is necessary to take precautions against overdosing rhG-CSF in patients with renal dysfunction.

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**REFERENCES**

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