Renal Clearance of a Recombinant Granulocyte Colony-Stimulating Factor, Nartograstim, in Rats

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Received December 20, 1995; accepted May 18, 1995

Purpose. To clarify the role of the kidney in the elimination of a recombinant human granulocyte colony-stimulating factor, nartograstim, we have investigated its pharmacokinetics in rats with renal failure.

Methods. The steady-state clearance (CL_{ss}) were determined by the intravenous infusion for 4 hr to unilateral renally-ligated and cisplatin-treated rats, whose renal functions were about 50 and 10 % of controls, respectively.

Results. CL_{ss} of nartograstim (27 ml/hr/kg) in the renally-ligated rats at a high infusion rate was significantly lower (25%) than in control rats ($p<0.05$). CL_{ss} in these rats, at a low infusion rate was 95 ml/hr/kg, 14 % lower than in control rats. The saturable CL_{ss} in these rats, 68 ml/hr/kg, was not significantly different from control rats (75 ml/hr/kg, $p>0.05$). Also, CL_{ss} in cisplatin-treated rats with extensive renal failure, at a high infusion rate, decreased to 57 % of controls. Furthermore, the total body clearances (CL_{tot}) of nartograstim after bolus intravenous administration to renally-ligated and cisplatin-treated rats were reduced to 33-49 % of controls.

Conclusions. These results suggest that the kidney may be responsible for 40- 50 % of the nonsaturable clearance of nartograstim. Thus, the kidney should make a major contribution to the elimination of nartograstim when rats are given a high dose of nartograstim, which saturates the receptor-mediated clearance.

KEY WORDS: granulocyte colony-stimulating factor; nonlinear pharmacokinetics; renal failure.

INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF) stimulates the differentiation and proliferation of the precursor cells to granulocytes (1). Recently, recombinant human G-CSF (rhG-CSF) produced by genetic-engineering techniques has been shown to be an effective drug for promoting neutrophil recovery in patients with chemotherapy- and radiotherapy-induced neutropenia (2,3). Non-linear pharmacokinetics of rhG-CSF or its derivative, nartograstim (rhG-CSFs) has been reported and the plasma clearance of rhG-CSFs in was reduced with increasing dose after intravenous administration (4,5). We have previously reported that the total clearance of nartograstim consists of both a saturable and nonsaturable component in various species (5).

In addition, it has been reported that the kidney plays an important role in the elimination of rhG-CSF (6,7). However, a quantitative analysis of the renal clearance of rhG-CSFs has not been reported. Also, considering that rhG-CSFs are used after chemotherapy and, in some cases, the anticancer drug treatment such as cisplatin, induces nephrotoxicity (8,9), it is important clinically to clarify the role of the kidney in the elimination of rhG-CSFs. In this study, to evaluate quantitatively the renal clearance of nartograstim, we determined the steady-state clearance of nartograstim (CL_{ss}) in renally-ligated and cisplatin-treated rats given nartograstim by intravenous infusion. From previous results (10), we chose the two infusion rates, one a non-saturated infusion rate, below 1 pmol/hr/kg (low infusion rate), and the other a saturated rate, above 500 pmol/hr/kg (high infusion rate).

MATERIALS AND METHODS

Materials

rhG-CSF derivative from *Escherichia coli,* designated nartograstim, was prepared and supplied by Kyowa Hakko Kogyo Co. Ltd. Nartograstim was greater than 99% pure with a specific activity of 4.8-5.1 $\times$ 10^8 U/mg protein (11). Certain N-terminal amino acids are different in nartograstim compared with hG-CSF namely, the Thr-1, Leu-3, Gly-4, Pro-5 and Cys-17 of hG-CSF are replaced by Ala, Thr, Tyr, Arg and Ser, respectively (11). ^{14}C-inulin and ^{3}H-p-aminohippuric acid (PAH) were purchased from Amersham International plc. (Buckinghamshire, United Kingdom.). Cisplatin (Randa®) was purchased from Nippon Kayaku Co. (Tokyo, Japan). Unless otherwise specified, all other reagents were of the highest level of purity commercially available.

Animals

Male Wistar rats were purchased from Japan Charles River (Yokohama, Japan) and were used when they were 7- to 8-weeks-old, weighing 200-300 g. Food and water were available *ad libitum*. To induce severe renal failure, cisplatin (4 mg/kg) was administered to rats via a femoral vein 4 days before the studies on nartograstim were carried out (9). Control rats were given intravenously the same volume of saline. Other rats had their left renal artery and vein ligated under light anesthesia with diethyl ether 1 hr before the studies began. Sham-operated animals were used as controls. Each group was composed of 3 or 4 rats. All pharmacokinetic studies were carried out after recovery from the anesthesia.

Inulin and PAH Clearances

Under light anesthesia with diethyl ether, the right femoral artery and bladder were cannulated using polyethylene tubes (PE-50, Becton Dickinson & Co., Parsipanny, New Jersey) for blood and urine sampling, respectively. The injected solution, containing both ^{14}C-inulin (6 mg/ 0.2 μCi/ml)

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Notation: G-CSF, granulocyte colony-stimulating factor; h, human; r, recombinant; PAH, p-aminohippuric acid; GFR, glomerular filtration rate; C_{ss}, plasma concentration at steady-state; CL_{ss}, plasma clearance at steady-state; CL_{tot}, total body plasma clearance; AUC, the area under the plasma concentration-time curve

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and ^{3}H-PAH (12 mg/ 2 μCi/ ml), was prepared. After intravenous administration of the injected solution 1 ml/kg, rats were then infused with this at a flow rate of 2 ml/hr. After a 30-min equilibration period, the urine was collected for two 15 min. periods. Blood samples were collected into the heparinized tubes at the midpoints of the periods and were centrifuged at 1500 × g for 10 min. to obtain plasma. The plasma and urinary concentrations of inulin and PAH were determined by scintillation counting (4530, Packard Instrumental Co., Downers Gloves, Illinois). The clearance for each drug was calculated from the urinary excretion rate divided by the plasma concentration.

Infusion Studies

Following cannulation of the right femoral artery, rats were infused nartograstim via a left femoral vein at a flow rate of 1 ml/hr for 4 hr. Nartograstim was diluted with saline containing 0.002 % Tween 80 to give the required concentrations. The infusion rates were set at a low (0.4-0.6 pmol/hr/kg) and high rate (740-1100 pmol/hr/kg) in control and ligated rats. Blood samples (0.2 ml) were collected at 0, 0.25, 0.5, 0.75, 1, 2, 3, and 4 hr during the infusion period and these were centrifuged at 1500 × g for 10 min. to obtain plasma. In the cisplatin-treated rats, the infusion rate was set only at a high rate. White blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts before infusion were determined using a microcell counter CC-180A (Sysmex, Toa Medical Electrics, Kobe, Japan). The plasma concentrations of nartograstim were determined by enzyme-linked immunosorbent assay (ELISA) (5). The samples were stored at −20 °C until assayed. The nartograstim clearance at steady-state (CL_{ss}) was calculated as the infusion rate divided by the plasma concentration at steady-state (C_{ss}).

Bolus Studies

Rats were intravenously given 2.6 nmol/kg of nartograstim via a femoral vein. Serial blood samples were collected into heparin-treated tubes from a tail vein at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hr after nartograstim administration. The plasma concentration-time profile of nartograstim in individual animals was analyzed. The estimated parameters included the elimination rate constant (k) obtained by log-linear least squares regression of the terminal data points, the apparent elimination half-life, ($t_{1/2}$), calculated as 0.693/k, the area under the plasma concentration-time curve, (AUC) using the trapezoidal rule with extrapolation to infinity, the total body plasma clearance, (CL_{tot}), calculated as the dose divided by AUC.

Statistical Methods

Comparisons were performed by Student's t-test. For all tests, $p < 0.05$ was taken as statistically significant.

RESULTS

Renal Function in the Treated Rats

The renal clearances of inulin and PAH in the unilateral renally-ligated and cisplatin-treated rats were measured as indicators of glomerular filtration rate (GFR) and tubular secretion, respectively (Table I). These clearances in renally-ligated rats were significantly decreased to 248 and 472 ml/hr/kg ($p<0.05$) respectively, which were 58 and 51 % those in control rats. The cisplatin treatment induced severe nephrotoxicity as reported previously (9), and the renal clearances of inulin and PHA were 9 and 10 %, respectively, of those in control rats.

Effect of the Renal Failure on Nartograstim Clearance at Steady-State

The plasma concentrations of nartograstim during intravenous infusion to control and renally-ligated rats were measured by ELISA. The plasma concentrations were normalized for the corresponding infusion rates to evaluate the infusion-rate dependence of nartograstim pharmacokinetics and correct interindividual differences in infusion rates (Figure 1). The plasma concentrations of nartograstim gradually increased after the start of the infusion then remained almost constant after 3 hr in all groups. Thus, the disposition of nartograstim was at steady-state after that time point. At both high and low infusion rates, the plasma concentration of nartograstim in the renally-ligated rats was slightly higher than those in control rats. The CL_{ss} at the high infusion rate in the renally-ligated rats was 27 ml/hr/kg, which was significantly lower ($p<0.05$) than that in control rats (35 ml/hr/kg, Table II). The CL_{ss} at the low infusion rate in the renally-ligated rats (95 ml/hr/kg) was also lower than in controls (110 ml/hr/kg). However, the saturable CL_{ss} in the renally-ligated rats (75 ml/hr/kg, Table II), which is the difference in CL_{ss} between the two rates, was almost similar to that in controls (68 ml/hr/kg). This indicates that the kidney may contribute to the nonsaturable elimination of nartograstim. To confirm this assumption, cisplatin-treated rats with severe renal failure, were studied. The plasma concentrations during infusion at a high infusion rate to the ciplatin-treated rats were significantly higher than in control rats ($p<0.05$, Figure 1). In addition, the CL_{ss} in the cisplatin-treated rats was 19 ml/hr/kg, which was 43 % lower than in control rats (Table II) as expected. In the cisplatin-treated rats, the white blood cell, red blood cell and platelet counts in peripheral blood were 158±30 $\times 10^2$ cells/μl, 726±44 $\times 10^4$ cells/μl and 6.2±1.5 $\times 10^4$ cells/μl, respectively, which were 92, 100 and 84 % of the values before cisplatin treatment. This suggests that bone marrow function may be intact in cisptain-treated rats.

Table I. The Renal Clearances of Inulin and PAH in Control, Renally-Ligated and Cisplatin-Treated Rats[a]

Rats	Renal clearance			
	Inulin	% of control	PAH	% of control
	ml/hr/kg		ml/hr/kg	
Control	430 ± 87		921 ± 272	
Ligated	248 ± 60[b]	57.7	472 ± 197[b]	51.2
Cisplatin-treated	37 ± 7[b]	8.6	93 ± 19[b]	10.1

[a] Rats were infused ^{14}C-inulin and ^{3}H-PAH for 60 min. Each value represents the mean ± s.d. of 3–5 animals.
[b] Significantly different from the control rats ($p < 0.05$).

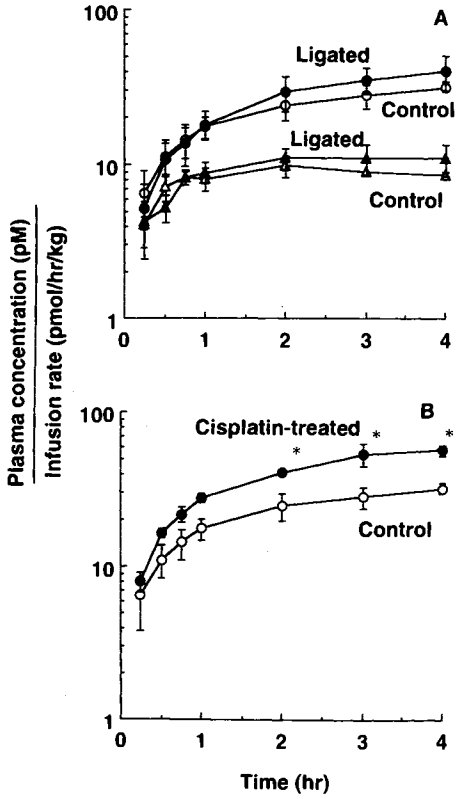

Fig. 1. Infusion rate-normalized plasma concentration of nartograstim in renally-ligated (A) and cisplatin-treated rats (B). A) Control and renally-ligated rats were infused nartograstim at at a high rate (940-1100 pmol/hr/kg, ○, ●) and a low rate (0.4-0.6 pmol/hr/kg, △, ▲) B) Control and cisplatin-treated rats were infused nartograstim at at a high rate (940-1100 pmol/hr/kg, ○, ●). Each point represents the mean ± s.d. of 3 or 4 rats.

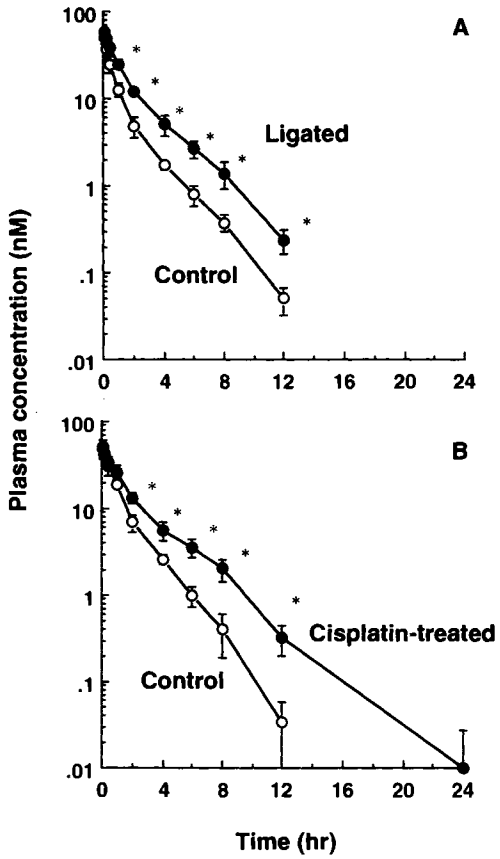

Fig. 2. Plasma concentration-time profiles of nartograstim in renally-ligated (A) and cisplatin-treated rats (B) after a bolus intravenous administration. The open symbols represent the plasma concentration of control rats and the closed symbols represent those of treated rats. Rats were given 2.6 nmol/kg nartograstim intravenously. Each point represents the mean ± s.d. of 4 rats.

Pharmacokinetics of Nartograstim After Bolus Intravenous Administration

The plasma concentrations of nartograstim after bolus administration of a dose of 2.6 nmol/kg to renally-ligated rats were significantly higher ($p<0.05$) than in controls during the elimination phase (Figure 2). The $t_{1/2}$ in the renally-ligated rats was prolonged to 1.8 hr (Table III). The reduction in CL_{tot} following renal ligation was 49 % (Table III). The plasma concentrations of nartograstim after bolus administration to the cisplatin-treated rats were also significantly higher ($p<0.05$) than in controls during the elimination phase (Figure 2). Prolongation of $t_{1/2}$ was also observed (Table III). The CL_{tot} in the cisplatin-treated rats fell by 33 %.

Table II. The NTG Clearances at Steady-State During i.v. Infusion[a,b,c]

Rats	CL_{ss} Low rate	CL_{ss} High rate	Saturable CL_{ss}
	ml/hr/kg	ml/hr/kg	ml/hr/kg
Exp. 1			
Control	110 ± 18	35 ± 5	75 ± 19
Ligated	95 ± 10	27 ± 2[d]	68 ± 10
Exp. 2			
Control	—[e]	33 ± 3	—
Cisplatin-treated	—	19 ± 1[d]	—

[a] Each value represents the mean ± s.d. of 3 or 4 animals
[b] Rats were infused NTG at a low (0.4–0.6 pmol/hr/kg) or high rate (950–1100 pmol/hr/kg) in Exp. 1.
[c] Rats were infused NTG at 740–950 pmol/hr/kg in Exp. 2.
[d] Significantly different vs control ($p < 0.05$).
[e] Not tested.

DISCUSSION

To clarify the role of the kidney in the elimination of nartograstim, the clearances of nartograstim have been investigated in two groups of rats with different types of renal failure. The decrease in the renal clearance of inulin (a marker of GFR) produced by the unilateral renal ligation was 42 %, which was similar to the 48 % decrease in the renal clearance of PAH (a marker of tubular secretion) as expected (Table I). On the other hand, the decrease in both renal functions produced by cisplatin treatment were similar, being 90- 92 %. This could be explained by the "intact nephron theory" (12), i.e., the remaining renal function is due to the surviving nephrons. Thus, the number of functional nephrons was reduced by cisplatin treatment.

The nonsaturable CL_{ss} of nartograstim, which nearly equals the CL_{ss} at a high infusion rate, showed a 25 % re-

duction in the unilateral renally-ligated rats (Table II). The observation that the reduction in the saturable CL_{ss} in these rats was about 10 % (Table II), indicates that the kidneys contribute mainly to the nonsaturable elimination of nartograstim. This hypothesis is supported by our previous results, showing that the renal extraction ratio and initial uptake clearance are independent of the dose (13). From the results, a 50 % reduction in nonsaturable CL_{ss} in the cisplatin-treated rats, which have only 10 % renal function, fell by 43 % (Table II). Thus, the contribution of the kidneys to the nonsaturable elimination can be estimated as 40-50 % of the nonsaturable CLss; i.e., 14-17 ml/hr/kg. This value was comparable with the GFR of nartograstim (14-34 ml/hr/kg), which can be calculated from the free fraction of nartograstim in the plasma (6-8 %) (13) and the GFR (430 ml/hr/kg) (Table I).

Clinically, G-CSFs are usually administered by the subcutaneous or bolus intravenous route (1-3) and not by the sustained infusion used in the above experiments. The pharmacokinetics of nartograstim after bolus administration were investigated at the saturated dose (5) in the rats with renal failure. The CL_{tot} in the cisplatin-treated rats decreased by 35 % compared with the controls, which was similar to the reduction in CL_{ss}. However, the reduction in CL_{tot} following the renal ligation was 50 %, which was larger than the reduction in CL_{ss}. In the case of rhG-CSF, the reduction in CL_{tot} produced by unilateral renal ligation ranged from 35 % to 46 % (6,7), which was comparable with our result. One possible explanation for the discrepancy involving the CL_{tot} is that the accurate estimation of the CL_{tot} of a nonlinear drug such as nartograstim is difficult because the AUC was calculated from the plasma concentration-time profile over a long period (12-24 hr, Figure 2) with a changing contribution of the saturable elimination.

In this paper, we analyzed quantitatively the role of the kidney in the elimination of nartograstim and found that the kidney may account for half the nonsaturable elimination of nartograstim. Thus, the role of the kidney is important in the elimination of nartograstim at high doses, which could saturate the receptor-mediated clearance by bone marrow (10).

Table III. Pharmacokinetic Parameters of NTG After Intravenous Administration of a Dose of 2.6 nmol/kg to Control and Treated Rats[a]

Rats	$t_{1/2}$	AUC	CL_{tot}
	hr	nM · hr	ml/hr/kg
Exp. 1			
Control	1.5 ± 0.1	49 ± 10	55 ± 11
Ligated	1.8 ± 0.1[b]	93 ± 3[b]	28 ± 1[b]
Exp. 2			
Control	1.3 ± 0.2	63 ± 6	42 ± 4
Cisplatin-treated	2.1 ± 0.0[b]	95 ± 15[b]	28 ± 4[b]

[a] Each value represents the mean ± s.d. of 4 animals.
[b] Significantly different from control rats ($p < 0.05$).

ACKNOWLEDGMENTS

The authors wish to thank Yoko Makino and Rika Murofushi for their excellent technical assistance.

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