Pulmonary Absorption of Recombinant Human Granulocyte Colony-Stimulating Factor (rhG-CSF) after Intratracheal Administration to Rats

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Pulmonary absorption of recombinant human granulocyte colony-stimulating factor (rhG-CSF) was examined in the rat. The relative bioavailabilities of rhG-CSF after intratracheal administration to intravenous and subcutaneous means were 11.6% and 27.4%, respectively, which were evaluated from the ratio of area under the curve (AUC) of the plasma rhG-CSF concentration versus time for 8 h. The pharmacological availability after the pulmonary absorption was determined from the AUC for the increase ratio in total blood leukocyte numbers versus time curve. The availability was equal to or more than the availabilities after intravenous or subcutaneous administration. Comparison of the two different parenteral administration routes for rhG-CSF, intratracheal and intranasal showed the pharmacological availability after the intratracheal administration to be superior. The pulmonary absorption of rhG-CSF is thus an effective parenteral route of administration.

Key words rhG-CSF; pulmonary absorption; intratracheal administration; total leukocyte number; relative bioavailability; pharmacological availability

Human granulocyte colony-stimulating factor (hG-CSF), a hydrophilic glycoprotein of 20000 Da is a hematogenic factor which stimulates granulocyte precursor cells in the bone marrow and specifically promotes their differentiation and proliferation into granulocytes. Recombinant hG-CSF (rhG-CSF) has recently been produced from Chinese hamster ovary cells with high purity and in large quantities.^{1,2)} rhG-CSF is used therapeutically for the treatment of neutropenia that occurs during tumor chemotherapy, for various blood myelodysplasias and for bone marrow transplantation.

Most peptides are so rapidly hydrolyzed and poorly absorbed in the gastrointestinal tract that they are usually administered intravenously or subcutaneously for therapeutic use. As alternative administration routes for physiologically active protein of comparatively low molecular weight such as insulin or calcitonin are nasal,³⁾ buccal,⁴⁾ rectal⁵⁾ and ocular⁶⁾ routes. For proteins of more than 10000 Da, however, few trials have been made to develop new routes of administration.

rhG-CSF is currently administered intravenously and subcutaneously, but an other convenient route would be preferable. The authors earlier examined the nasal absorption of rhG-CSF, and reported that the nasal route gave 5–10% pharmacological availability of the substance,⁷⁾ that the bioavailability was enhanced three times by Laureth-9 (nonionic surfactant) and that it depended on hydrophilic lipophilic balance (HLB) values of the nonionic surfactants added.⁸⁾ Niven *et al.*⁹⁾ examined the intratracheal route as an alternative and reported that it showed higher availability than the nasal route using nonglycosylated methionyl, *Escherichia coli*-derived rhG-CSF. In this study, the authors report the results after intratracheal administration of glycosylated rhG-CSF, which has higher pharmacological activity than nonglycosylated one,¹⁰⁾ to examine the greater efficacy

of the pulmonary route than the nasal.

MATERIALS AND METHODS

Chemicals The chemicals and bulk solution of rhG-CSF used in this study were as follows: Tween 20 from Nikko Chemicals Co., Ltd., Tokyo, Japan; sodium pentobarbital (Nembutal® injection, 50 mg/ml) from Sankyo Co., Ltd., Tokyo and sodium phenobarbital (Phenobar®, 0.1 g/ml) from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan. A bulk solution of rhG-CSF (phosphate buffer solution, 440 µg/ml, specific activity 10⁸ U/mg protein) was supplied by Chugai Pharmaceutical Co., Ltd., Tokyo. Other reagents were of analytical grade or better.

Preparation of rhG-CSF Solution for Intravenous, Subcutaneous and Intratracheal Administration The pH and osmotic pressure of rhG-CSF solution administered were adjusted to pH 4–8 with 100 mM NaH₂PO₄ or 1 N HCl and to 180–1140 mOsm/kg with NaCl. Tween 20 was added to the solution to make a final concentration of 0.01% (w/v), and final rhG-CSF concentration was 250 µg/ml.

Animal Experiments for Pharmacokinetic Study Male SD rats (8 weeks old, 250–300 g; Clea Japan, Inc., Tokyo) were used.

Femoral artery and vein were cannulated with polyethylene tubing (PE-50, Clay-Adams, U.S.A.). Under the combined anesthesia of sodium phenobarbital (100 mg/kg) and sodium pentobarbital (50 mg/kg) administered intraperitoneally, the rhG-CSF solution was administered into lung by the method of Enna and Schanker.¹¹⁾ Intravenous (i.v.) and subcutaneous (s.c.) administration were performed through the femoral vein cannula and into the back skin, respectively, after the animals awakened. Blood samples were collected from the femoral artery for 24 h after i.v. and s.c. administration,

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but only for 8 h after intratracheal (i.t.) administration according to Enna and Schanker.¹¹⁾

Animal Experiments for Pharmacodynamic Study The pharmacological activity was determined by the increase of total leukocyte number as reported previously.⁷⁾ All administration and blood sampling for i.v. and s.c. routes were performed as reported previously.⁷⁾ For i.t. route, drug solution was injected directly into the trachea under ether anesthesia. About 50 μ l of blood was collected periodically from the tail vein for 72 h following all three types of administration at 9 a.m. Twenty μ l of the collected sample was used in the determination of leukocytes.

Assay The plasma concentration of rhG-CSF was determined by enzyme immunoassay.¹²⁾ Total leukocyte numbers were counted with a microcellcounter (Sysmex F-500, Toa Medical Electronics, Tokyo).

RESULTS

The plasma concentration time-courses after i.v., s.c. and i.t. administration of 100 μ g/kg rhG-CSF are shown in Fig. 1.

The area under the plasma concentration-time curve of rhG-CSF until 8 h after administration (AUC_{0-8}), and bioavailability are listed in Table 1. The relative bioavailability of i.t. administration to i.v. and s.c. were 11.6% and 27.4%, respectively. The effects of pH and

Fig. 1. Plasma Concentration of rhG-CSF Following Intravenous (●), Subcutaneous (○) and Intratracheal (▲) Administration

Dose of rhG-CSF is 100 μ g/kg. Values represent the mean $\pm$ S.E. of more than four rats.

Table 1. AUC and Relative Bioavailability after rhG-CSF Administration in Rat

Administration route	AUC_{0-8} (ng h ml ⁻¹) ^{a)}	Relative bioavailability (%) ^{b)}
Intravenous (i.v.)	3150.8 $\pm$ 386.2	—
Subcutaneous (s.c.)	1340.1 $\pm$ 209.9	—
Intratracheal (i.t.)	366.6 $\pm$ 106.1	11.6 $\pm$ 3.4 (to i.v.) 27.4 $\pm$ 7.9 (to s.c.)

Data are the mean $\pm$ S.E. of more than four rats. a) AUC for 0–8 h after administration of 100 μ g/kg of rhG-CSF. b) Calculated using AUC_{0-8} .

osmotic pressure of rhG-CSF solution on the bioavailability after i.t. administration are listed in Table 2.

Under physiological conditions as pH 6.5 or 285 mOsm/kg, the relative bioavailability tended to be less than that under other conditions, but the significant difference was found only in osmotic effects. The reason for this remains to be further investigated. The time-course of increase ratio of total leukocyte numbers in plasma after i.t. administration with that after i.v. and s.c. administration are shown in Fig. 2 to examine the pharmacological effect of rhG-CSF. The increased number of total leukocytes was determined as a ratio to these numbers before administration of rhG-CSF.

The leukocyte number after saline administration as control varied chronologically but remained constant to a certain extent, showing that the surgical procedure did not affect the leukocyte number. The time-courses of leukocyte number were not as different among the three administration routes as those of rhG-CSF plasma con-

Table 2. Effects of pH and Osmotic Pressure of rhG-CSF Solution on Relative Bioavailability of Intratracheally Administered rhG-CSF to Intravenous (i.v.) or Subcutaneous (s.c.) Administration in Rat

pH	Osmotic pressure (mOsm/kg)	Relative bioavailability (%) ^{a)}
4.0	285	15.3 $\pm$ 5.9 (to i.v.) 36.1 $\pm$ 13.8 (to s.c.)
5.0	285	13.6 $\pm$ 6.3 32.0 $\pm$ 14.8
6.5	180	20.2 $\pm$ 5.2* 47.4 $\pm$ 12.1*
6.5	285	11.6 $\pm$ 3.4 27.4 $\pm$ 7.9
6.5	560	16.7 $\pm$ 7.3 39.3 $\pm$ 17.2
6.5	1140	23.3 $\pm$ 8.0* 54.7 $\pm$ 18.9*
8.0	285	18.6 $\pm$ 4.4 43.8 $\pm$ 10.5

Data are the mean $\pm$ S.E. of more than four rats. a) Calculated by the ratio of AUC_{0-8} after intratracheal administration to that after intravenous (i.v.) and subcutaneous (s.c.) administration of rhG-CSF solution at the corresponding pH and osmotic pressure. * 0.01 $< p < 0.05$ vs. the relative bioavailability at pH 6.5 and 285 mOsm/kg.

Fig. 2. Increase Ratio of Total Leukocyte Numbers Following Intravenous (●), Subcutaneous (○), and Intratracheal (■) Administration of rhG-CSF

In this control (Δ), saline solution was administered intratracheally. Values on the ordinate represent the increase ratios of total leukocyte numbers, and 100% indicates the initial value before administration. Dose of rhG-CSF is 100 μ g/kg. Data are the mean $\pm$ S.E. of more than four rats.

Fig. 3. Relation between *AUC* for Increase Ratio of Total Leukocyte Numbers and Dose of rhG-CSF Following Intravenous (●), Subcutaneous (○) and Intratracheal (▲) Administration of rhG-CSF

Data for three different routes are the mean $\pm$ S.E. of more than four rats.

Fig. 4. Change in the Total Leukocyte Numbers Following Intratracheal Administration of rhG-CSF

Doses are 10 (○), 50 (●), 100 (□) and 200 μ g/kg (■). In the control (Δ), saline solution was administered intratracheally. Data are the mean $\pm$ S.E. of more than four rats.

centration. The pharmacological effect appeared more slowly and was sustained longer than rhG-CSF plasma concentration by all routes. The pharmacological availabilities after s.c. administration which was calculated as the *AUC* for the increase ratio vs. time curve to that after i.v. and i.t. administration were $111.1 \pm 15.1\%$ and $89.9 \pm 12.2\%$ at 100 μ g/kg, respectively. The dose dependence of rhG-CSF on leukocyte number differed among the three administration routes as shown in Fig. 3. The i.t. administration showed similar availability to that by s.c. and i.v. at 10–100 μ g/kg, but at 200 μ g/kg, the availability after i.t. administration was greatest.

Figure 4 shows the dose effect of rhG-CSF on leukocyte number after i.t. administration. The peak number was observed about 8 h after the administration of 10 μ g/kg rhG-CSF, but at 50–200 μ g/kg administration, the peaks appeared later.

DISCUSSION

The i.t. administration was shown to be a more effective route for rhG-CSF than intranasal administration, since the relative bioavailability of rhG-CSF after i.t. administration (11.6% to i.v. administration and 27.4% to s.c. administration in Table 1) was more than ten times larger than that after intranasal administration (0.92% to i.v. administration and 1.95% to s.c. administration), as reported previously.⁷⁾ The bioavailability of rhG-CSF after i.t. administration obtained in this report does not contradict those of insulin after i.t. administration (7–10%) reported by Wigley *et al.*¹³⁾ and (40%) by Yoshida *et al.*¹⁴⁾ considering the relation between molecular weight and bioavailability after i.t. administration of FITC-dextran with molecular weight of 4000–70000.¹⁵⁾ These improved values for bioavailability show that i.t. administration is an effective route for peptide and protein. Figure 3 shows that although the pharmacological effect of rhG-CSF increased dose-dependently, i.t. administration had the highest effect among the three routes. This means that the plasma concentration of rhG-CSF does not correspond to its pharmacological effect, although a dose-dependent pharmacological effect was observed by this route (Fig. 4). Although of the three routes plasma appearance and apparent elimination of rhG-CSF was slowest after i.t. administration (Fig. 1), this tended to result in higher pharmacological activity of rhG-CSF, especially at 200 μ g/kg, the activity was significantly greater than that after i.v. or s.c. administration (Fig. 3). These results suggest that the length of time in which rhG-CSF remains in plasma has some relation with the activity, though the mechanism for this is not yet known. From the pharmacological point of view, i.t. administration is suggested to be effective route for current clinical use. Administration by this route is probably worthy of further study, not only to obtain an even greater pharmacological effect but also to develop an invasive administration route assuring a better quality of life of patients.

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