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Note

# Absorption and blood leukocyte dynamics of recombinant human granulocyte colony-stimulating factor (rhG-CSF) from intranasally administered preparations containing rhG-CSF and cyclodextrins in rabbits ☆

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**Abstract**

The absorption of recombinant human granulocyte colony-stimulating factor (rhG-CSF) and blood leukocyte dynamics following intranasal (i.n.) administration of aqueous preparations containing rhG-CSF with or without cyclodextrins (CyDs) were investigated in conscious rabbits by measuring the serum G-CSF concentration and the total number of leukocytes in peripheral blood. Serum G-CSF concentration was rapidly increased by coadministration of rhG-CSF and CyD, particularly, $\alpha$ and $\beta$ types. The total number of leukocytes in peripheral blood also increased (the maximum level of approx. 20000/$\mu$l was attained 24 h after administration) and exceeded physiological levels (5200–12000/$\mu$l) in rabbits. The increase in the number of leukocytes was observed between approx. 8 and 48 h after i.n. administration. These results suggest that rhG-CSF is efficiently absorbed through the nasal mucosa. It may be concluded that the development of a rhG-CSF delivery system could be achieved in the form of the nasal administration of rhG-CSF with CyDs.

*Key words:* Granulocyte colony-stimulating factor; Nasal absorption; Cyclodextrin; Serum G-CSF concentration; Blood leukocyte count; Intranasal administration; Conscious rabbit

Biologically active (granulopoietic) human granulocyte colony-stimulating factor, which is purified and molecularly cloned by means of recombinant DNA technology (rhG-CSF; Nagata et al., 1986; Souza et al., 1986), has been successfully administered clinically to patients with aplastic anemia, neutropenia induced by chemo-

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therapy with or without autologous bone marrow rescue. However, the clinical application of rhG-CSF is limited to only subcutaneous (s.c.) or intravenous (i.v.) administration. Generally, a number of problems, including the inconvenience of injection dosage form, are associated with the use of peptide drugs such as rhG-CSF for treatment of patients, particularly children. Therefore, the development of acceptable alternative methods of rhG-CSF administration should be investigated. The nasal route is an attractive alternative to parenterals due to the ease of administration and rich vascularization (Chien et al., 1989). Although attempts to achieve nasal absorption of various peptide drugs promoted by absorption-enhancing agents (absorption enhancers) have been reported, only a few investigations on the nasal administration of rhG-CSF have been carried out in the field of drug delivery systems. It is, therefore, necessary and desirable to develop an alternative dosage form which allows for the nasal absorption of rhG-CSF, contributing to the improvement of the quality of life in patients.

The aim of the present study was to evaluate the nasal absorption of rhG-CSF in rabbits following administration of a preparation containing rhG-CSF and cyclodextrin (CyD), which is classified under a new category of absorption enhancers (Verhoef et al., 1992; Merkus et al., 1993). Recently, it has been reported that CyDs enhance the nasal absorption of certain drugs, such as steroid hormones (estradiol (Hermens et al., 1990), progesterone (Schipper et al., 1990)) and polypeptides (insulin (Merkus et al., 1991; Irie et al., 1992; Watanabe et al., 1992)). Furthermore, CyD, which is a biocompatible polymer, would be a safe material for practical preparations. If such CyD-containing rhG-CSF preparations were to be formulated, the application of these preparations as novel nasal rhG-CSF delivery systems would be possible. We investigated the absorption of rhG-CSF and blood leukocyte dynamics following intranasal (i.n.) administration of aqueous preparations containing these compounds in rabbits.

A lyophilized rhG-CSF (filgrastim, Kirin Brewery Co., Tokyo, Japan), which consists of 175 amino acids without *O*-glycoside (Mol. Wt approx. 19000), was used. Three kinds of natural cyclodextrins ($\alpha$-, $\beta$-, and $\gamma$-CyD; obtained from Nihon Shokuhin Kako Co., Tokyo, Japan) were used in the formulation. Aqueous preparations (freshly prepared, pH 4.0) for i.n. administration were made by dissolving appropriate amounts of rhG-CSF with or without CyD in 167 mM acetate buffer solution since rhG-CSF is physicochemically stable at a pH of approx. 4. The placebo preparation contained the same amount of CyDs without rhG-CSF in buffer solution. The method of i.n. administration as described in our previous paper (Watanabe et al., 1992) in conscious rabbits was employed. Briefly, male rabbits (Japan White, weighing 3.0–3.5 kg) were not allowed food but did have access to tap water for one night prior to each experiment. The next day, aqueous preparations with the administered volume calibrated to 50 $\mu$l per kg body weight were instilled into one nostril with a micropipette (Bio-Bik, Osaka, Japan). For the control experiments (i.v. bolus injection), rhG-CSF solution was injected into the marginal vein of an ear. Blood samples were taken from the auricular vein of the other ear at predetermined intervals after administration. The G-CSF concentration in serum was determined according to the enzyme immunoassay method of Tanaka and Tokiwa (1990). The total number of leukocytes in peripheral blood was measured by the Coulter counter method (Model ZM, Nikkaki, Tokyo, Japan). Pharmacokinetic parameters, such as the peak serum G-CSF level ($C_{max}$), time of peak serum G-CSF concentration ($t_{max}$) and area under the serum G-CSF concentration-time curve (AUC), were determined from the individual serum G-CSF concentration curve. The $AUC_{0-12}$ was estimated using the trapezoidal rule for measured serum G-CSF levels from 0 to 12 h after i.n. administration. Statistical analysis of the results was conducted by ANOVA and the least-significant-difference tests.

The mean serum G-CSF concentration-time curves and levels of the total number of leukocytes in peripheral blood following i.n. administration of the rhG-CSF preparation with or without $\beta$-CyD are shown in Fig. 1 and 2, respectively. The serum concentration of G-CSF increased rapidly ($C_{max}$, $231 \pm 35$ ng ml$^{-1}$; $t_{max}$,

1.3 ± 0.3 h) on coadministration (indicated by filled circles) of rhG-CSF (100 μg kg$^{-1}$) and β-CyD (10 mg kg$^{-1}$). The mean values of serum G-CSF levels attained on coadministration were significantly ($p < 0.05$) higher than those for administration of rhG-CSF without CyD (designated by unfilled circles in Fig. 1). As shown in Fig. 2, the mean values of the total number of leukocytes in blood (indicated by filled circles) also increased (the maximum level of approx. 21000/μl was attained 24 h after coadministration of rhG-CSF and β-CyD) significantly ($p < 0.05$) and exceeded physiological levels (5200–12000/μl) in rabbits (Tajima, 1972) between 8 and 48 h after administration. However, the total number of leukocytes (maximum level, 17000/μl) on administration of rhG-CSF without CyD (denoted by unfilled circles) was lower compared with that for coadministration. The total number of leukocytes (indicated by the dotted line) did not exceed physiological levels when the placebo preparation containing β-CyD without rhG-CSF was administered. Similar levels of leukocytes were observed when the vehicle solution without CyD was instilled. The extent of bioavailability (EBA), calculated as the ratio of $AUC_{0-12}$ values of an i.n. dosage form to that of an i.v. injection,

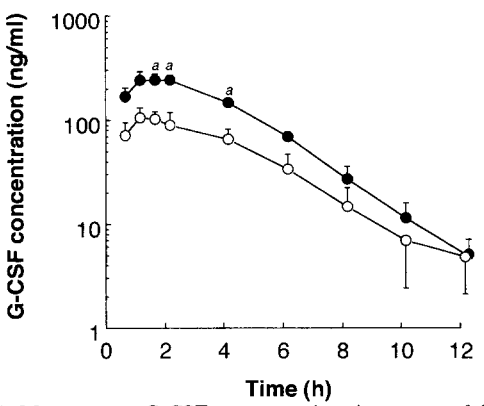


Fig. 1. Mean serum G-CSF concentration-time curves following intranasal (i.n.) administration of rhG-CSF preparations in rabbits. Dose: rhG-CSF, 100 μg kg$^{-1}$ with 10 mg kg$^{-1}$ of β-CyD (●———●); rhG-CSF, 100 μg kg$^{-1}$ without CyD (○———○). Each point represents the mean ± S.E. of at least three rabbits. Statistically significant differences: (a) $p < 0.05$ in rhG-CSF with β-CyD vs rhG-CSF without CyD.

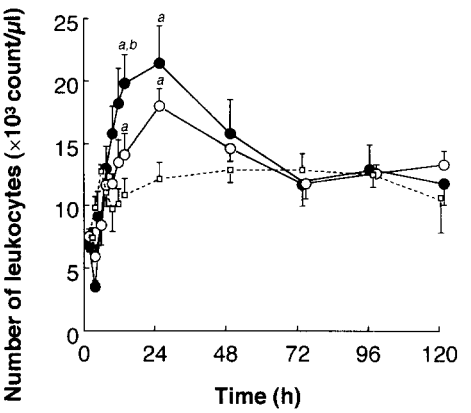


Fig. 2. Mean total number of leukocytes in peripheral blood following intranasal (i.n.) administration of rhG-CSF preparations in rabbits. Dose: rhG-CSF, 100 μg kg$^{-1}$ with 10 mg kg$^{-1}$ of β-CyD (●———●); rhG-CSF, 100 μg kg$^{-1}$ without CyD (○———○). Solid and dashed lines indicate rhG-CSF preparation with or without CyD and placebo preparation containing β-CyD without rhG-CSF, respectively. Each point represents the mean ± S.E. of at least three rabbits. Statistically significant differences: (a) $p < 0.05$ in rhG-CSF with β-CyD vs placebo preparation; (b) $p < 0.05$ in rhG-CSF with β-CyD vs rhG-CSF without CyD.

was approx. 11% when rhG-CSF was administered with β-CyD. Thus, it was confirmed that rhG-CSF was efficiently absorbed in the case of coadministration of rhG-CSF and β-CyD. The serum G-CSF levels and total number of leukocytes observed for coadministration of rhG-CSF and α-CyD were equivalent to those evaluated by use of β-CyD.

Fig. 3 illustrates the mean values of the $AUC_{0-12}$ observed following coadministration of rhG-CSF and various CyDs. A high mean value of the $AUC_{0-12}$ (1030 ± 95 h ng ml$^{-1}$) was obtained with β-CyD. When rhG-CSF was administered with α-CyD, a similar level of mean $AUC_{0-12}$ was found. However, it appears that the use of γ-CyD does not significantly increase $AUC_{0-12}$ values compared with the rhG-CSF preparation without CyD. From Fig. 3, we can safely conclude that the use of α- and β-CyD, both natural forms, is preferable over the use of DM-β-CyD, a chemically modified form, in the rhG-CSF formulation described in a previous report (Watanabe et al., 1993).

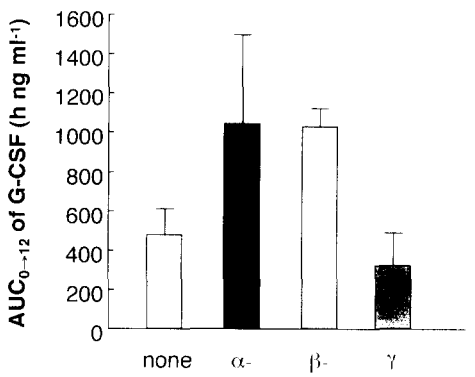


Fig. 3. Mean $AUC_{0-12}$ of rhG-CSF obtained after i.n. administration of rhG-CSF and various CyDs in rabbits. Dose: rhG-CSF, 100 $\mu g\ kg^{-1}$; CyD, 10 mg $kg^{-1}$. Each value represents the mean ± S.E. of at least three experiments.

In our preliminary experiments, however, only a slight increase was found in serum G-CSF concentration following intranasal coadministration of rhG-CSF and proteolytic enzyme inhibitors such as aprotinin and nafamostat mesilate. Therefore, it seems that a decrease in the extent of metabolic rhG-CSF degradation does not play an essential role in the enhancement of rhG-CSF absorption. On the other hand, in the case of the biophysical barrier, it has been reported that CyDs may extract lipids from the gastrointestinal mucosa (Uekama and Otagiri, 1987) and solubilize membrane lipids of human erythrocytes, with various CyDs possessing different solubilizing abilities (Ohtani et al., 1989).

It is probable that the solubilization of lipids from the nasal mucosa by CyDs operates via a mechanism similar to that above, and that the perturbation of the nasal epithelium by CyDs may contribute substantially to the enhanced membrane permeability to rhG-CSF. In our experiments, the absorption-enhancing effects achieved by using $\alpha$- and $\beta$-CyD are in general consistent with those of Shao et al. (1992).

We have found that when rhG-CSF was intranasally administered with CyDs, serum G-CSF levels and total number of leukocytes in blood significantly increased following the use of aqueous preparations containing both compounds. These results are indicative of the efficient absorption of rhG-CSF through the nasal mucosa in rabbits. It may be concluded that a promising rhG-CSF delivery system instead of the parenteral form could be developed in the form of the nasal administration of rhG-CSF with CyDs.

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