Is Recombinant Human Granulocyte Colony-Stimulating Factor (G-CSF) Orally Available in Rats?

Kanji TAKADA,*[,a] Yoshinori TOHYAMA,[a] Mamoru OOHASHI,[a] Hiroshi YOSHIKAWA,[a] Shozo MURANISHI,[a] Akihiro SHIMOSAKA[b] and Tatsuhiko KANEKO[b]

Department of Biopharmaceutics, Kyoto Pharmaceutical University,[a] Yamashina-ku, Kyoto 607, Japan, and Pharmaceuticals Department, Research and Development Division, Kirin Brewery Co., Ltd.,[b] Shibuya-ku, Tokyo, Japan. *Received August 5, 1988*

Oral availability of recombinant human granulocyte colony-stimulating factor (G-CSF) was investigated in rats by measuring the blood total leucocyte (BTL) counts. Oral test G-CSF solution was prepared with 10% HCO-60® (polyoxyethylated, 60 μmol, castor oil derivative), 1% DK ester (sugar ester) or 10% MYS-40® (polyethyleneglycol monostearate), in which the G-CSF concentration was 500 or 250 μg/ml. Each test solution was injected into the duodenum of three rats at the G-CSF dose level of 300 or 600 μg/kg, and BTL counts were monitored for 48 h. All of the test G-CSF solution raised the BTL levels within 24 h after injection. In particular, the HCO-60 solution increased the BTL levels over 2 times as compared to the predose level at 600 μg/kg dose and the effect was apparently dose-dependent. A short-term study suggested that the effect of G-CSF on the BTL level appeared at the fastest at about 5 h after administration of HCO-60 test solution, 300 μg/kg. In view of the pattern of BTL dynamics obtained after i.v. injection of HCO-60 solution at 25 and 50 μg/kg, the increase of BTL levels observed after oral administration of the HCO-60 solution is considered to be due to the orally supplied G-CSF.

Keywords recombinant human granulocyte colony-stimulating factor (G-CSF); orally available; blood leucocyte dynamics; pharmaceutical additive; rat

Introduction

The human granulocyte colony-stimulating factor (G-CSF), a hematopoietic glycoprotein controlling the proliferation of granulocytes and macrophages, has recently been purified, molecularly cloned and expressed as recombinant protein.[1-3] Several studies showed the efficiency of this hormone to augment leukocyte counts *in vivo*.[4] The administration of G-CSF by i.v. infusion to normal and pancytopenic (virus-infected) non-human primates resulted in significant increases in eosinophil, functionally active neutrophil and monocyte counts.[5,6] Moreover, continuous i.v. infusion of the factor (0.3 to 4.5 μg/kg/d) produced a significant increase in the leucocyte counts of sixteen neutropenic acquired immunodeficiency syndrome (AIDS) patients.[7] Thus, G-CSF dramatically affects the systemic level of eosinophils. However, its clinical use is limited to the i.v. route. On the other hand, some papers have suggested that orally administered peptides or proteins such as insulin[8-10] and vasoactive peptide[11] show pharmacological activities in *in vivo* rat experiments. However, the components used to increase the oral availability of the peptide/proteinous drugs are not permitted for general use as pharmaceutical additives. Therefore, to use such peptide/protein delivery systems clinically, many studies concerning the safety of the additives (toxicity, carcinogenesis, *etc.*) are needed. On the other hand, we have developed an orally available enteric solid dispersion system for a cyclic peptide drug, cyclosporin A (CyA),[12] in which a pharmaceutical additive such as HCO-60® (polyoxyethylated, 60 μmol, castor oil derivative) is used to improve the oral availability of CyA. To examine whether our oral peptide/protein delivery system works well with other peptide/proteinous drugs, G-CSF was chosen as another large molecular weight model peptide, and this report presents evidence supporting the availability of G-CSF from an oral dosage form.

Experimental

Materials Recombinant human granulocyte colony-stimulating factor (G-CSF) was kindly supplied by Kirin Brewery Co., Ltd. (Tokyo, Japan). Polyoxyethylated, 60 μmol, castor oil derivative (HCO-60®) and polyethyleneglycol monostearate (MYS-40®) were obtained from Nikko Chemicals Co., Ltd. (Tokyo, Japan). Sugar ester (DK ester F-160®) was obtained from San-ei Chemicals Co., Ltd. (Toyonaka, Japan). All other reagents were commercial products of reagent grade.

Preparation of Test Solution The HCO-60 solution was prepared by dissolving G-CSF in 10% (w/v) HCO-60 solution in water. The DK ester solution and MYS-40 solution were also prepared with 1% (w/v) and 10% (w/v) solution, respectively. The concentrations of G-CSF in test solution were 250 μg/ml for the 300 μg/kg dose level and 500 μg/ml for the 600 μg/kg dose level.

Animal Study Three male Wistar rats, weighing 300—400 g, were used in each experimental group. Under anesthesia induced by an intraperitoneal injection of sodium pentobarbital, 45 mg/kg, midline incision was performed. Test drug solution was administered to rats by an injection into the duodenum of the rat. Group I-1, I-2 and I-3 rats are the control experimental groups. These groups of rats received 1.2 ml/kg of 10% HCO-60, 1% DK ester or 10% MYS-40 solutions wich did not contain G-CSF, respectively. Group II rats received HCO-60 test solution at the CSF dose level of 300 μg/kg. Group III, IV and V rats received HCO-60, DK ester and MYS-40 test solutions at the dose level of 600 μg/ml, respectively. Before drug administration, 0.2 ml of the blank blood sample was obtained by a puncture into the tail artery. A 1.2 ml aliquot of each test solution per kilogram of rat body weight was injected into the rat duodenum. After administration, the pore made in the duodenum was closed with a drop of tissue cement, Aron Alpha® (Sankyo Co., Ltd., Tokyo). Single blood samples, 200 μl, were obtained by rat tail arterial puncture after drug administration at 6, 18, 24, 30 and 48 h. In group VII rats, a short-term study was performed at the G-CSF dose level of 300 μg/kg. Group VI rats were the control group. The experimental method was the same as described above. However, the blood sampling time was pre-dosing and after that at 1, 2, 3, 4, 5, 6 and 7 h. Moreover, in two rats, an i.v. study was performed at dose level of 25 and 50 μg/kg. The i.v. test solution was prepared by diluting the 10% HCO-60 test solution with saline.

The blood total leucocyte (BTL) counts were determined manually on gentian violet-stained blood smears. The BTL count was expressed as the relative value, which was obtained by dividing the BTL count by the respective control BTL count, namely the pre-dosing BTL count.

Results and Discussion

The BTL dynamics after intraduodenal injection of several G-CSF test solutions are represented in Fig. 1. The BTL level of the blood sample obtained just before the administration of test solution was set to unity and all of the measured BTL levels after drug administration were represented as a relative value to the starting value. As the

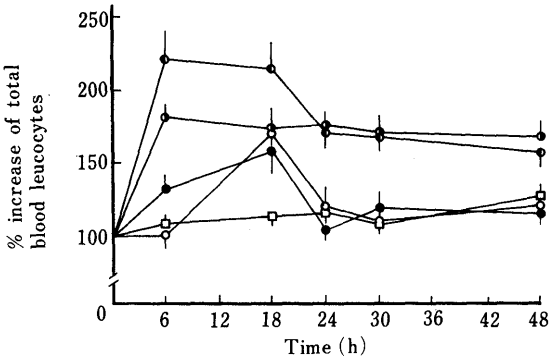

Fig. 1. Blood Total Leucocyte Dynamics Following Intraduodenal Injection of G-CSF Solutions

□, controls; ◑, 10% HCO-60 group, 300 μg/kg (group II); ◐, 10% HCO-60 group, 600 μg/kg (group III); ●, 1% DK ester group, 600 μg/kg (group IV); ○, 10% MYS-40 group, 600 μg/kg (group V). Each point represents three individual determinations, and is expressed as the mean ± S.E.

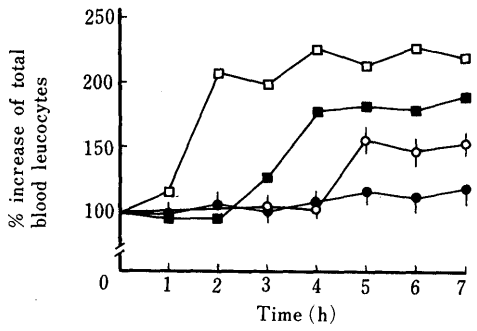

Fig. 2. Short-Term Blood Total Leucocyte Dynamics Study Following Intraduodenal (i.d.) and Intravenous (i.v.) Injection of G-CSF Solution

●, control, i.v. injection of solvent (group VI); ○, 10% HCO-60 group, i.d. injection of 300 μg/kg (group VII); ■, 10% HCO-60 group, i.v. injection of 25 μg/kg; □, 10% HCO-60 group, i.v. injection of 50 μg/kg. For groups VI and VII, each point represents three individual determinations, and is expressed as the mean ± S.E. For the i.v. study of 25 and 50 μg/kg G-CSF dosing, each point represents a single datum.

three groups, which received merely the solvent, 10% HCO-60 (group I-1), 1% DK ester (group I-2) or 10% MYS-40 solution (group I-3), showed almost the same BTL dynamics pattern, the results are inclusively represented by a single line. On the other hand, BTL levels were greatly increased in group III rats which received a high dose of G-CSF in 10% HCO-60 solution, 600 μg/kg. The maximum BTL count was obtained at 6 h after administration and high BTL levels continued until 48 h. Moreover, dose-dependency in BTL dynamics was observed at 6 and 18 h between the two doses, 600 and 300 μg/kg, in 10% HCO-60 solution. On the other hand, the effect of the other two surfactants, DK ester (group IV) and MYS-40 (group V), was not so evident as compared to HCO-60 (group III). However, the BTL levels of group IV and V rats at 18 h after injection were almost the same as that of group II. These results suggest that the BTL dynamics are dependent on both the quality of the solvent and the quantity of the dose. As this study was a preliminary one, BTL dynamics were monitored for only 2 d and not many blood samplings were performed. Namely, the first blood sampling point was at 6 h after administration. However, these results strongly support the pharmacological availability of G-CSF after intraduodenal (i.d.) dosing. We were next interested in how fast the BTL level increases after the i.d. administration of G-CSF. Thus, a short-term study was performed with six more rats (groups VI and VII). The results are represented in Fig. 2, with the additional data obtained after i.v. injection of G-CSF at two dose levels, 25 and 50 μg/kg. In the case of i.d. dosing, the BTL level started to rise at 5 h after dosing, though the BTL level rose earlier after i.v. injection. This result suggests that the BTL response obtained after i.d. administration of G-CSF is not so fast as that after i.v. dosing.

G-CSF is a glycoprotein and its molecular weight was estimated to be 17600.[13] Like other proteinous drugs, G-CSF is subject to enzymatic digestion in the gastrointestinal (GI) tract after oral administration. However, the lowest protease activity was reported to be found in the duodenal region of the small intestine.[14,15] This implies that an orally administered peptide/proteinous drug may be absorbed intact in the upper region of the GI tract.[16] As a pharmacokinetic study was not performed in this report, it is difficult to know (1) whether G-CSF is absorbed intact and transported to the systemic circulation, and (2) how much oral G-CSF is available to the systemic circulation. However, we may clearly state that G-CSF is pharmacologically available after oral administration with pharmaceutical additives. By comparing the BTL dynamics obtained after i.v. and i.d. administrations of G-CSF, the i.d. 500 μg/kg dose is almost comparable to the i.v. 50 μg/kg dose. Therefore, the results presented in this report strongly support the usefulness of an oral G-CSF dosage form in rats.

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