International Journal of Pharmaceutics, 101 (1994) 89–96
Elsevier Science Publishers B.V.

IJP 03319

Pharmacological activity of tablets containing recombinant human granulocyte colony-stimulating factor (rhG-CSF) in rats

Kanji Takada, Yoshinori Nakahigashi, Toshinori Tanaka, Shu-ichi Kishimoto, Yoshiteru Ushirogawa and Tomohiro Takaya

Department of Pharmaceutics and Pharmacokinetics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607 (Japan)

(Received 12 April 1993)
(Modified version received 20 May 1993)
(Accepted 31 May 1993)

Key words: Recombinant human granulocyte colony-stimulating factor (rhG-CSF); Organic acid; Trypsin inhibitor; Dietary protein; Oral availability; Blood total leucocyte count; Rat

Summary

Several kinds of small tablets containing recombinant human granulocyte colony-stimulating factor (rhG-CSF) were prepared and the pharmacological activities of the tablets were evaluated in in vivo rat experiments. Except for rhG-CSF, organic acids, surfactant, protease inhibitors and/or enteric coating material were formulated as tablets. After a mixture of rhG-CSF was made with organic acid, surfactant and/or protease inhibitor, small tablets were made and then coated with an enteric-coating material. After the intraduodenal administration of several kinds of small test tablets, 3 mm × 0.7 cm, containing 100 and/or 50 μg rhG-CSF/rat, to rats through a gut incision, blood samples were obtained over a 96 h period from the rat tail artery and the blood total leucocyte (BTL) counts were measured as a pharmacological index of rhG-CSF. The results are expressed as a relative increase in BTL count as compared to the pre-dose level, baseline level. The area under the curves (AUC; % BTL increase × h) obtained was used as an index for the pharmacological activity of rhG-CSF when comparing the test with the placebo tablets. (1) The 2.5-fold increase in the formulated amount of surfactant (HCO-60®, polyoxyethylated castor oil derivative), increased the pharmacological activity of rhG-CSF about 2-fold; (2) protease inhibitors, such as soybean trypsin inhibitor and ovalbumin, did not exert any synergistic effect with organic acids with respect to the pharmacological activity of rhG-CSF; and (3) addition of citric acid to tablets mainly composed of tartaric and succinic acids significantly increased the pharmacological activity of rhG-CSF. This study shows the usefulness of both surfactant and organic acid as pharmaceutical additives for the development of an oral delivery system for rhG-CSF.

Introduction

Granulocyte colony-stimulating factor (G-CSF) has been identified as having a stimulatory action on the proliferation of bone marrow precursor cells and their differentiation into granulocyte colonies (Nicola et al., 1987). Recent developments in biotechnology make it possible to produce considerable amounts of recombinant human granulocyte colony-stimulating factor (rhG-CSF) (Nagata et al., 1986). The thus obtained rhG-CSF is also capable of supporting the formation of granulocytic colonies from committed precursor cells (Cohen et al., 1987). Clinically, rhG-

Correspondence to: K. Takada, Department of Pharmaceutics and Pharmacokinetics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan.

CSF has been administered to patients with leukemia (Teshima et al., 1989), Hodgkin's disease, testicular germ cell tumor (Layton et al., 1989), and advanced pulmonary tumor (Eguchi et al., 1989) to accelerate hematologic recovery after high-dose chemotherapy or irradiation with or without autologous bone marrow rescue. The duration of neutropenia was shortened in these patients and it was found that rhG-CSF is a useful drug for the treatment of cancer patients with drug- or irradiation-induced myelosuppression. However, these studies were performed with rhG-CSF injections. For the use of rhG-CSF over a wide variety of patients, a more convenient dosage form such as a tablet or capsule is needed.

To develop an oral dosage form of rhG-CSF, two important problems must be resolved: namely, (1) rapid enzymatic degradation in the gastro-intestinal (GI) tract, and (2) poor membrane permeability. Recently, the usefulness of microspheres of insulin containing protease inhibitors on the pharmacological effect of insulin has been demonstrated (Morishita et al., 1992). This methodology is certainly effective for protection of the protein from enzymatic degradation in the GI tract. However, another disadvantage, i.e., poor membrane permeability, must be overcome in order to obtain the pharmacological activity with a lower oral dose of protein drugs. Our studies (Takada et al., 1989, 1991) showed that intraduodenally (i.d.) administered rhG-CSF is pharmacologically available and that the pharmacological activity of rhG-CSF, as measured by the increase in blood total leucocyte (BTL) count, was affected by several kinds of additives. In particular, organic acids and protease inhibitors such as trypsin inhibitors and dietary protein decreased the rate of rhG-CSF hydrolysis by the GI fluid during in vitro stability experiments (Takada et al., 1991). This study also suggests that decreased rate of degradation of rhG-CSF in the GI tract might be useful for the improvement of the pharmacological activity of orally administered rhG-CSF. To enhance the pharmacological activity of rhG-CSF, both surfactant and organic acids such as tartaric and citric acids were used and increased pharmacological activity of rhG-CSF was confirmed (Ushirogawa et al., 1992).

In the present investigation, several kinds of small tablets containing rhG-CSF and the pharmaceutical additives previously mentioned were prepared. In addition, the pharmacological activities of these small tablets were evaluated by performing an in vivo experiment using rats in which the the increase in BTL was measured after administration.

Materials and Methods

The following materials were used in this study and were obtained from the indicated sources: rhG-CSF solution, 250 μg/ml (Kirin Brewery Co., Ltd, Tokyo, Japan); anhydrous L-tartaric, succinic and citric acids (Wako Pure Chemical Co. Ltd, Osaka, Japan); polyoxyethylated castor oil derivative, HCO-60® (Nikko Chemical Co., Ltd, Tokyo, Japan; crude soybean trypsin inhibitor (TI), type II-S (Sigma Chemical Co. (St Louis, MO, U.S.A.); gelatin and ovalbumin (OA) (Wako Pure Chemicals); and hydroxypropylmethylcellulose phthalate, HP-55® (Shin-etsu Chemical Industry Co., Ltd, Tokyo, Japan). All other reagents were commercial products of reagent grade.

Preparation of small test tablet

Small tablets were initially prepared using rhG-CSF, organic acid, surfactant and/or protease inhibitor. To the rhG-CSF solution, the other components were added, dissolving readily. The resultant mixture was transferred to a mortar. Stirring was continued at room temperature (23°C) until the solvent had almost evaporated. The resultant mixture was dried under vacuum overnight at room temperature. After pulverization in a mortar with a pestle, $NaHCO_3$ was added to the mixture which was then mixed thoroughly. $NaHCO_3$ was used to make an effervescent tablet. By adding one or two drops of an ethanol : water (1 : 1) mixture, the pulverized material was formed into eight small tablets by hand. After drying overnight in a dessicator, the tablet weights were measured. Each tablet was adjusted to contain 100 or 50 μg of rhG-CSF. The dimensions of the tablets were approx. 3 mm outside

diameter and 0.7 cm in length. The tablet obtained was inserted into a small enteric tube (3.2 mm inside diameter and 0.9 cm length) which was prepared with HP-55. Thereafter, the two ends were closed with a drop of concentrated HP-55 solution in a methylene chloride : methanol (7 : 3) mixture. The thickness of the enteric tube was measured with a micrometer, the mean value being determined as 50 μm. Table 1 lists the recipes (Rp.) of the tablets tested in this study. Each column shows the amounts of component used to prepare the tablets in one batch. Two kinds of tablets containing different amounts of surfactant were prepared, designated as Rp. 1 and 2 in Table 1. In addition, eight small placebo tablets were prepared. After being thoroughly dried, the tablet weight was measured and adjusted to be equal to that prepared for the Rp. 1 tablet. The enteric coating with HP-55 was also carried out. Rp. 3–5 tablets contained protease inhibitor or dietary protein. The recipes of tablets containing other organic acids, such as succinic and citric acids, are also referred to as Rp. 6–9. All the enteric tablets were tested by soaking them in 0.1 N HCl solution. Tablets not having a pinhole were used in the following animal study.

Animal study

Three to four male Wistar rats (SLC, Hamamatsu, Japan), weighing 280–320 g, were used in each experimental group. The rats were housed under standardized conditions at a room temperature of 22 ± 1°C. 1 week acclimatization for the rats was allowed. Food and water were freely available. Under anesthesia induced by an intraperitoneal injection of sodium pentobarbital (45 mg/kg), a midline incision was performed. A blank blood sample (100 μl) was collected into a heparinized syringe by puncture into the tail artery. The test tablet was administered into the rat's duodenum through an incision (0.5 mm) in the gut near the pylorus. After administration of the tablet, the gastric incision was sutured and sealed with tissue cement (Aron Alpha®, Sankyo Co., Tokyo). The midline incision was also sutured. Single blood samples (100 μl) were obtained by tail arterial puncture after drug administration. The standard sampling schedule was at 6, 9, 15, 18, 24, 32, 48 and 96 h. The BTL counts were manually determined on gentian violet-stained blood smears. Namely, 0.9 ml of Turk solution was added to 100 μl of the blood sample and the leucocytes were stained. The BTL count was performed using an ultramicroscope. The baseline BTL count was determined as the blood sample prior to dosing. The baseline value was considered as the 100% level and all subsequent BTL count-time data recorded were expressed as a percentage of the baseline. These values denote a percentage pharmacological response (% BTL

TABLE 1

Formulation of tablets used in this study

Test tablet	Amount of component (mg)								
	rhG-CSF	Tartaric acid	Succinic acid	Citric acid	HCO-60	$NaHCO_3$	TI	OA	Gelatin
Placebo	0	800	200	60					
Rp. 1	1	800			200	60			
Rp. 2	1	800			80	60			
Rp. 3	1	800			80	60	10		
Rp. 4	1	800			80	60	2		
Rp. 5	1	800			80	60	10		
Rp. 6	1	200	200	60					100
Rp. 7	0.5	200	200	60					100
Rp. 8	0.5	200	200						100
Rp. 9	0.5	200	200	60				200	

SI, soybean trypsin inhibitor; OA, ovalbumin. Each row shows the amounts of component to prepare test small tablets in one batch (10 tablets/batch). Each tablet contained 100 μg (Rp. 1–6) or 50 μg (Rp. 7–9) rhG-CSF.

count increase). The area under the curve (AUC) of the % BTL count increase-time curve was determined as an index for the total pharmacological activity of rhG-CSF. The AUC values obtained for test tablets were compared to that obtained with the placebo tablet.

Statistics

Statistical differences were assumed to be reproducible when $p < 0.05$ (two-sided t-test).

Results

The main additives used in our test tablets are protease inhibitors and pharmaceutical additives such as surfactant and organic acid. At first, the effect of the surfactant content in the tablets on the pharmacological activity of rhG-CSF was studied after administration into the rat duodenum. Two kinds of tablets, Rp. 1 and 2, were tested. The administered rhG-CSF dose of these tablets was 100 μg/rat. In Rp. 2 tablets, the amount of surfactant (HCO-60) decreased to 40% as compared to Rp. 1 tablets. Fig. 1 shows the results along with those of the control experimental group rats which received the placebo tablets. The dose of each additive in this control group was 80 mg of tartaric acid, and 20 mg of HCO-60.

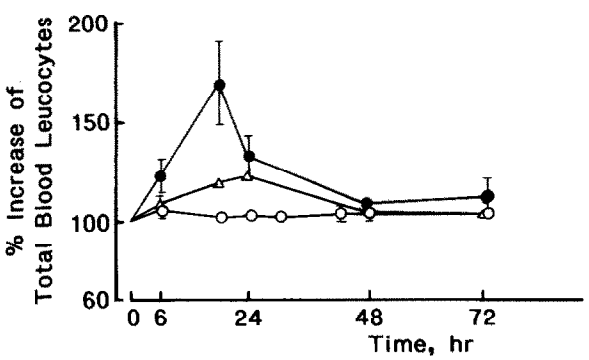

Fig. 1. Effect of the amount of surfactant on blood total leucocyte dynamics after administration of rhG-CSF tablets, 100 μg/rat. (○) Placebo tablet (control group); (●) rhG-CSF tablet containing HCO-60, 20 mg/rat (Rp. 1); (△) rhG-CSF tablet containing HCO-60, 8 mg/rat (Rp. 2). Each point represents three or four individual determinations, and is expressed as the mean ± S.E.

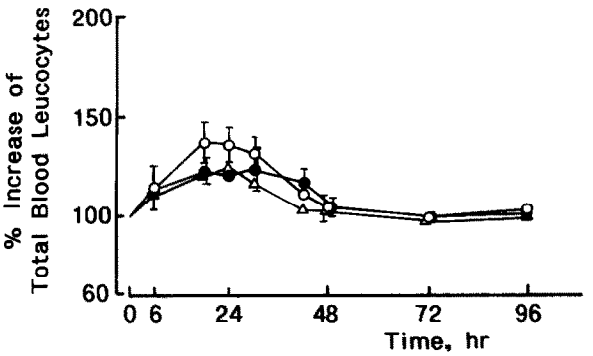

Fig. 2. Effect of protease inhibitors on blood total leucocyte dynamics after administration of rhG-CSF solution, 100 μg/rat. (○) Tablet containing TI, 1 mg/rat (Rp. 3); (●) tablet containing TI, 0.2 mg/rat (Rp. 4); (△) tablet containing OA, 1 mg/rat (Rp. 5). Each point represents three or four individual determinations, and is expressed as the mean ± S.E.

In the case of the control study, the BTL count did not significantly increase as compared to the pre-dose level. This result supports the feasibility of our in vivo evaluation system for intraduodenal (i.d.) administration of the rhG-CSF small tablet. In the group of rats which received Rp. 1 tablets containing a high dose of HCO-60 (20 mg/rat), the maximum increase in BTL count was 170%, which appeared at 18 h after administration of the tablet. The BTL count was reduced to the control level 48 h after drug administration. Therefore, blood sampling was not performed after this sampling time. Rp. 2 tablets containing less surfactant (8 mg/rat) showed lower pharmacological activity (BTL increase) than those with a higher surfactant content (Rp. 1).

Fig. 2 represents the effect of soybean trypsin inhibitor (TI) on the pharmacological activity of i.d. administered rhG-CSF. Since a surfactant affects the pharmacological activity of rhG-CSF, the doses of the surfactant in the following three kinds of tablets (Rp. 3–5) was reduced to 8 mg. The difference in the recipes between Rp. 3–5 tablets and the Rp. 2 tablet is merely the addition of trypsin inhibitor and/or ovalbumin. The increase in BTL count in rats which received Rp. 3 tablets (higher TI content, 1 mg/rat) was greater than that found in those receiving tablets with a lower TI content (0.2 mg/rat, Rp. 4). Therefore, the effect of TI on the pharmacological activity of

rhG-CSF shows dose-dependent behavior. In another group of rats, ovalbumin (OA) was prescribed instead of TI (Rp. 5). The dose of OA was 1 mg/rat. To evaluate the total pharmacological activity of the test tablets, the areas under the curves (AUC; % BTL increase × h) were calculated, the results being listed in Table 2. The first row in Table 2 shows the mean authentic AUC value for each tablet. The value in the second row was determined by dividing the AUC value of each test tablet by that of the placebo tablet group. The AUC observed with Rp. 5 tablets is the same as that found with Rp. 2 tablets. Therefore, we may state that OA does not affect the pharmacological activity of rhG-CSF.

Fig. 3 depicts the effect of additionally prescribed organic acids on the pharmacological activity of i.d. administered rhG-CSF tablets. In these tablets, succinic and citric acids were prescribed (Rp. 6), the doses being 20 and 6 mg/rat, respectively. To examine the effect of the additionally formulated organic acids, a surfactant was not prescribed for the tablets. In the former tablets, i.e., Rp. 1–5, HCO-60 had another function, namely, that of a binder. However, the preparation of stable tablets was very difficult without HCO-60. Therefore, gelatin was used as the binder. Since gelatin is a dietary protein, it behaves as a competitive inhibitor for rhG-CSF against the hydrolytic enzymes which exist in the GI tract (Takada et al., 1991). After the administration of Rp. 6 tablets, the BTL count increased

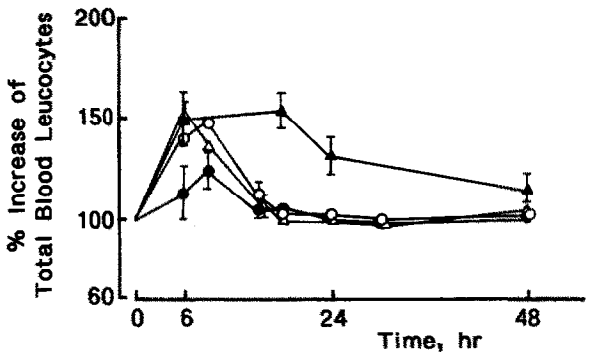

Fig. 3. Effect of organic acid on blood total leucocyte dynamics after administration of rhG-CSF solution, 100 and 50 μg/rat. (▲) Tablet containing citric acid, 6 mg/rat (Rp. 6); (○) tablet containing citric acid, 6 mg/rat (Rp. 7); (●) tablet without citric acid (Rp. 8); (△) tablet containing OA (Rp. 9). Each point represents three or four individual determinations, and is expressed as the mean ± S.E. In the groups of rats receiving Rp. 7–9 tablets, the rhG-CSF dose was 50 μg/rat.

to 150% at 18 h. As shown in Table 2, the AUC determined with Rp. 6 tablets was the maximum value among the test tablets. Since citric acid exerts an absorption enhancing effect on the transepithelial transport of drugs (Cho et al., 1989), Rp. 6 tablets showed a high pharmacological activity. Therefore, the dose was reduced by half in the following groups of rats which received Rp. 7–9 tablets.

At first, the same kinds and amounts of pharmaceutical additives that were used to prepare Rp. 6 tablets were also formulated into tablets in which the prescribed amount of rhG-CSF was

TABLE 2

Comparison of pharmacological activities with several rhG-CSF tablets

Parameter	rhG-CSF test tablets									
	Placebo	Rp. 1	Rp. 2	Rp. 3	Rp. 4	Rp. 5	Rp. 6	Rp.7	Rp. 8	Rp. 9
AUC	132 ± 132	1475 [a] ± 398	705 [a] ± 75	1077 [a] ± 598	885 [a] ± 215	678 [a] ± 233	1822 [a] ± 312	518 [a] ± 23	234 ± 29	437 [a] ± 30
Relative pharmacological activity (%)	100	1117	534	816	670	514	1380	392	177	331

AUC was determined to represent the total pharmacological activities of the test solutions and was calculated by multiplying the % BTL count increase with time (h). Each value represents the mean ± S.E.

[a] Statistically significant difference from the value of the control experiment by Student's *t*-test ($p < 0.05$).

reduced to half, i.e., 500 μg (Rp. 7). Therefore, the dose of rhG-CSF administered to rats receiving Rp. 7 tablets was 50 μg/rat. In the case of Rp. 7 tablets, the maximum increase in BTL count appeared at 9 h after administration and the percentage increase in BTL count was 150%, almost equal to that determined with Rp. 6 tablets. However, the AUC value decreased to about 1/4 as compared to Rp. 6 tablets. Rp. 8 tablets were prepared without citric acid and administered to rats. However, a decreased maximum BTL count was observed as compared to Rp. 7 tablets. The relative pharmacological activity was low (177%). Consequently, we consider citric acid to be an important component in the development of an oral delivery system for rhG-CSF. In Rp. 9 tablets, the effect of a large amount of OA, 200 mg (i.e., 20 mg/rat), on the pharmacological activity of rhG-CSF was examined. Since OA functioned as a binder, gelatin was not formulated in Rp. 9 tablets. However, the pharmacological activity elicited by Rp. 9 tablets was lower than that in the case of Rp. 7 tablets as shown in Fig. 3 and Table 2.

Discussion

Among the protein drugs obtained via biotechnology, rhG-CSF is a unique and potent drug for increasing the granulocyte fraction in the circulating blood. After subcutaneous injection of rhG-CSF into hamsters at 100 μg/kg body weight, the BTL counts increased 230% (Cohen et al., 1987). To develop an oral delivery system for rhG-CSF, we have been investigating the pharmacological activity of i.d. administered rhG-CSF (Takada et al., 1989, 1991; Ushirogawa et al., 1992). Our previous study using several kinds of test solutions administered into the rat duodenum demonstrated that soybean trypsin inhibitor (TI), at 0.80 and 3.20 mg/kg, markedly increased the pharmacological activity of rhG-CSF. Namely, the increases in AUC as compared to rats of the control group were 182% at 0.8 mg/kg and 657% at 3.2 mg/kg, respectively. TI has also been used in the present work at a 5-fold greater dose than that employed in our previous study. However, a considerable increase in the pharmacological activity of rhG-CSF was not detected in this study. On the other hand, the effect of organic acids was clearly observed, particularly in the case where citric acid concomitantly formulated into the tablet, resulting a marked increase in the pharmacological activity of rhG-CSF. According to our previous report (Takada et al., 1991), organic acid decreased the rate of hydrolysis of rhG-CSF by decreasing the pH of the environmental water layer. In addition, surfactant affected the pharmacological activity of rhG-CSF. Therefore, both surfactant (HCO-60) and organic acids are considered to be essential components in the designing of an oral delivery system for rhG-CSF. In an approach to developing an oral delivery system for peptide-proteins, Saffran et al. (1986) reported the azo-polymer to be useful. Tablets containing insulin coated with this polymer were administered orally to two rabbits and a decreasing effect on blood glucose levels was observed. We believe that this unique technology shows considerable promise for potential applications in the near future. However, the azo-polymer has thus far not been used as a pharmaceutical additive. Therefore, much experimentation is needed to develop an oral peptide-protein delivery system using such an new pharmaceutical adjuvant. In particular, thorough safety studies must be performed. Since the report of Saffran et al. (1986), many investigators have shown interest in the oral delivery system for peptide-protein drugs (Pusztai, 1989; Pitt, 1990; Lee, 1990, 1991; Nellans et al., 1991; Ritschel, 1991; Smith et al., 1992; Swenson et al., 1992). Nevertheless, a practically applicable delivery system has yet to be developed. Furthermore, microparticles, nanoparticles and liposomes have been widely studied as an oral delivery system for vaccines. Gilligan et al. (1991) stated that microparticles and nanoparticles will influence the future development of both oral and parenteral vaccines substantially. However, particular emphasis must be placed on the use of biodegradable and biocompatible polymers as in the case of the azo-polymer. In the case of liposomes, the possibility that proteins might be inactivated during the process of preparation liposomes cannot be excluded ow-

ing to the high temperatures and shear pressures required (O'Hagan et al., 1992). For the oral delivery of protein drugs, two kinds of technologies are needed: (i) the protection of protein from attack by hydrolytic enzymes in the GI tract and (ii) enhancement of the absorption of protein. The technologies mentioned above are certainly of value for the protection of protein drugs from hydrolytic enzymes. However, they cannot perform the function of enhancing the absorption of protein. However, our system fulfills the criteria for both functions. In addition, our system consists of pharmaceutical additives which are widely used in pharmaceutical sciences. Therefore, safety considerations do not constitute a barrier to the development of an oral delivery system for rhG-CSF.

Acknowledgements

The present study was supported in part by a grant entitled 'Development of biointelligent materials' from the Research Development Corporation of Japan and also by a grant from the Japan Research Foundation for Clinical Pharmacology. The authors are grateful for their support.

References

Cho, M.J., Scieszka, J.F. and Burton, P.S., Citric acid as an adjuvant for transepithelial transport. *Int. J. Pharm.*, 52 (1989) 79–81.

Cohen, A.M., Zsebo, K.M., Inoue, H., Hines, D., Boone, T.C., Chazin, V.R., Tsai, L., Ritch, T. and Souza, L.M., In vivo stimulation of granulopoiesis by recombinant human granulocyte colony-stimulating factor. *Proc. Natl. Acad. Sci. USA*, 84 (1987) 2284–2288.

Eguchi, K., Sasaki, S., Tamura, T., Sasaki, Y., Shinkai, T., Yamada, K., Soejima, Y., Fukuda, M., Fujihara, Y., Kunitou, H., Tobinai, K., Ohtsu, T., Suemasu, K., Takaku, F. and Saijo, N., Dose escalation study of recombinant human granulocyte-colony-stimulating factor (KRN8601) in patients with advanced malignancy. *Cancer Res.*, 49 (1989) 5221–5224.

Gilligan, C.A. and Li Wan Po, A., Oral vaccines: Design and delivery. *Int. J. Pharm.*, 75 (1991) 1–24.

Layton, J.E., Hockman, H., Sheridan, W.P. and Morstyn, G., Evidence for a novel in vivo control mechanism of granulopoiesis: mature cell-related control of a regulatory growth factor. *Blood*, 74 (1989) 1303–1307.

Lee, V.H.L. and Yamamoto, A., Penetration and enzymatic barriers to peptide and protein absorption. *Adv. Drug Del. Rev.*, 4 (1990) 171–207.

Lee, V.H.L., Yamamoto, A. and Kompella, U.B., Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. *Crit. Rev. Ther. Drug Carr. Sys.*, 8 (1991) 91–192.

Morishita, I., Morishita, M., Takayama, K., Machida, Y. and Nagai, T., Hypoglycemic effect of novel oral microspheres of insulin with protease inhibitor in normal and diabetic rats. *Int. J. Pharm.*, 78 (1992) 9–16.

Nagata, S., Tsuchiya, M., Asano, S., Kaziro, Y., Yamazaki, T., Yamamoto, O., Hirata, Y., Kubota, N., Oheda, N., Nomura, H. and Ono, M., Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. *Nature*, 319 (1986) 415–418.

Nellans, H.N., Mechanism of peptide and protein absorption (1) Paracellular intestinal transport: modulation of absorption. *Adv. Drug Del. Rev.*, 7 (1991) 339–364.

Nicola, N.A., Granulocyte colony-stimulating factor and differentiation-induction in myeloid leukemic cells. *Int. J. Cell Cloning*, 5 (1987) 1–15.

O'Hagan, D.T., Oral delivery of vaccines: Function and clinical pharmacokinetic considerations. *Clin. Pharmacokinet.*, 22 (1992) 1–10.

Pitt, C.G., The controlled parenteral delivery of polypeptides and proteins. *Int., J. Pharm.*, 59 (1990) 173–196.

Pusztai, A., Transport of proteins through the membranes of the adult gastro-intestinal tract – a potential for drug delivery? *Adv. Drug Del. Rev.*, 3 (1989) 215–228.

Ritschel, W.A., Targeting in the gastrointestinal tract: New approaches. *Methods Find. Exp. Clin. Pharmacol.*, 13 (1991) 313–336.

Saffran, M., Kumar, G.S., Savariar, C., Burnham, J.C., Williams, F. and Neckers, D.C., A new approach to the oral administration of insulin and other peptide drugs. *Science*, 233 (1980) 1081–1084.

Smith, P.L., Wall, D.A., Gochoco, C.H. and Wilson, G., Oral absorption of peptides and proteins. *Adv. Drug Del. Rev.*, 8 (1992) 253–290.

Swenson, E.S. and Curatolo, W.J., Intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity. *Adv. Drug Del. Rev.*, 8 (1992) 39–92.

Takada, K., Tohyama, Y., Oh-hashi, M., Yoshikawa, H., Muranishi, S., Shimosaka, A. and Kaneko, T., Is recombinant human granulocyte colony-stimulating factor (G-CSF) orally available in rats? *Chem. Pharm. Bull.*, 37 (1989) 838–839.

Takada, K. and Ushirogawa, Y., Effect of pH, dietary proteins and trypsin inhibitors on the hydrolytic rate of human granulocyte colony-stimulating factor (G-CSF) by rat digestive enzymes. *J. Pharmacobio-Dyn.*, 14 (1991) 363–370.

Ushirogawa, Y., Nakahigashi, Y., Kiriyama, A. and Takada, K., Effect of organic acids, trypsin inhibitors and dietary protein on the pharmacological activity of recombinant human granulocyte colony-stimulating factor (rhG-CSF) in rats. *Int. J. Pharm.*, 81 (1992) 133–141.

Teshima, H., Ishikawa, J., Kitayama, H., Yamagami, T., Hiraoka, A., Nakamura, H., Shibata, H., Masaoka, T. and Takaku, F., Clinical effects of recombinant human granulocyte colony-stimulating factor in leukemia patients: a phase I/II study. *Exp. Hematol.*, 17 (1989) 853–858.