Pharmacokinetics and Pharmacodynamics of Human Chorionic Gonadotropin (hCG) after Rectal Administration of Hollow-Type Suppositories Containing hCG[1,2)]

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To determine the effectiveness of human chorionic gonadotropin (hCG) administered rectally, we studied the pharmacokinetics and pharmacodynamics of hCG using a hollow-type suppository. HCG was not detected in plasma when only hCG was administered rectally, even at a higher dose (4000 IU/kg body weight) than intravenous injection, because of its low bioavailability due to high molecular weight or degradation by proteolytic activity. To enhance the rectal absorption of hCG, the effectiveness of its coadministration with α-cyclodextrin (α-CyD), an absorption-enhancing agent, was investigated in male rabbits. HCG was detected in plasma following coadministration of hCG and α-CyD (10 mg/kg body weight) into the rectum. The plasma hCG concentration increased with increasing dose of α-CyD. The $AUC_{0\rightarrow48}$ observed after coadministration of hCG and α-CyD at 30 mg/kg body weight was approximately four times higher than that of hCG and α-CyD at 10 mg/kg body weight. HCG at a high concentration induced a rapid increase in the plasma testosterone concentration (74.2±3.4 ng/ml) 2 h after intravenous administration. However, the testosterone concentration 24 h after intravenous administration decreased to the physiological level (approximately 20 ng/ml) which had been observed before such administration. On the other hand, the maximum level of testosterone concentration (40.0±12.6 ng/ml) was observed 24 h after rectal administration of hCG (400 IU/kg body weight) in combination with α-CyD (30 mg/kg body weight). Moreover, the plasma testosterone concentration (31.0±11.4 ng/ml) obtained 72 h after rectal administration tended to be maintained at a higher level than that (14.4±0.9 ng/ml) observed before the administration. These results suggest that the hollow-type suppository as a rectal delivery system of hCG is promising as a new mode of hCG therapy.

Key words human chorionic gonadotropin; rectal administration; α-cyclodextrin; hollow-type suppository; plasma human chorionic gonadotropin concentration; plasma testosterone concentration

Human chorionic gonadotropin (hCG) is a gonad-stimulating polypeptide[3)] (MW, approximately 40000; sugars, 30—33%) hormone produced by the placenta and extracted from the urine of pregnant women. The action of hCG is almost identical to that of pituitary luteinizing hormone (LH). It is generally used as a substitute for LH. Exogenous gonadotropin regimens, consisting of treatment with hCG, are frequently used for patients with pituitary deficiency or corpus luteum insufficiency.[4)] Unfortunately, the administration of hCG has been limited to intramuscular injection (JP13, USP24).[5)] To improve the quality of life (QOL) of these patients who require long-term therapy, a new method of hCG administration *via* non-oral and parenteral routes is desirable for its systemic administration. Therefore, rectal administration of hCG was investigated to reduce the inconvenience associated with injections.

We studied the pharmacokinetics and pharmacodynamics of hCG administered using the hollow-type suppository developed by Watanabe *et al.*[6)] to evaluate the effectiveness of the drug when administered rectally. The advantage of using the hollow-type suppository is that it can eliminate the effect of the heating process on the properties of polypeptide drugs during preparation, and can minimize differences in the release rate of a drug from the dosage vehicle. Previously, we successfully applied these suppositories for the rectal delivery of insulin,[7)] recombinant human granulocyte colony-stimulating factor (rhG-CSF)[8,9)] and elcatonin.[1)] However, to our knowledge, no studies on hollow-type suppositories for hCG delivery have been reported to date. In the present investigation, the effects of the coadministration of hCG and α-cyclodexirin (α-CyD), an absorption-enhancing agent,[10)] on rectal absorption of hCG were examined in rabbits, and the usefulness of the hollow-type suppository for hCG administration was evaluated.

MATERIALS AND METHODS

Materials HCG (4066 IU/mg per polypeptide, Puberogen™, Sankyo Yell) and α-CyD (Nihon Shokuhin Kako, Tokyo, Japan) were used. The suppository base, Witepsol H-15 (Hüls Trosidorf, Germany) was kindly supplied by Mitsuba Trading Co., Tokyo. All other reagents used were of analytical grade.

Preparation of Suppositories Two formulations of hollow-type suppositories (approximately 2 g), one containing an aqueous hCG solution alone (suppository I), and the other an aqueous hCG solution with α-CyD (suppository II), were prepared using Witepsol H-15 by the fusion method described by Watanabe *et al.*[6)] For the control experiments, a suppository containing α-CyD without hCG was prepared using Witepsol H-15. The amounts of hCG and α-CyD used are listed in Table 1. An aqueous hCG solution was prepared by dissolving hCG at an appropriate concentration in isotonic phosphate buffer solution (pH 7.4). The prepared solution at an hCG dosage volume of 100 μl per kg body weight of rabbit was placed in the cavity (approximately 0.4 cm^3) of the suppository, and then the opening at the end of the suppository was sealed by melting the base. A combination of hCG

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Table 1. Amounts of hCG and α-CyD in Administered Preparations

Preparation	Dose	
	hCG (IU/kg body weight)	α-CyD (mg/kg body weight)
Intravenous injection	40	—
Suppository I (aqueous solution)	4000	—
Suppository II (aqueous solution)	4000	10
		30

and α-CyD in phosphate buffer solution was used in a suspension form because of the solubility of α-CyD. After preparation, all suppositories were refrigerated overnight prior to use.

Animal Experiments Male albino rabbits weighing 2.8—3.2 kg were used. They received food and water *ad libitum* and were housed individually in cages in a forced-air facility that was maintained at 23±1 °C and 55% relative humidity, under a 12-h light/dark cycle. Animals with free access to water were fasted for one night prior to each experiment. For intravenous bolus administration, the hCG (1000 IU/ml, for dose, see Table 1) in physiological saline solution was injected into the marginal vein of one ear. The suppositories were administered into the lower part of rectum according to the method described in our previous papers.[6—10] Following the intravenous or rectal administration of hCG, 2 ml of blood samples was collected from the auricular vein using a syringe containing edetate disodium (EDTA-2Na) at predetermined time intervals. These samples were centrifuged at 3000 rpm for 15 min to separate the plasma. Each plasma sample was stored at −30 °C until assayed for hCG. The plasma hCG concentration was determined by the HCG-CPT Test Wako Kit (Wako Pure Chemical Co., Tokyo, Japan). This kit is a sandwich-type immunoassay[11] for hCG determination in serum and urine which uses a highly specific anti-hCG-β Carboxyl Terminal Peptide (hCG-βCTP) antibody in solid phase that specifically recognizes the amino acid sequence located between the 123rd and 145th hCG β-subunits. Therefore, its cross reactivity with similar hormones such as human LH (hLH) is very low, enabling a highly precise and sensitive determination of low amounts of hCG.[12] The plasma testosterone concentration was determined by the immunoassay method employing a Testosterone Enzyme Immunoassay kit (Cayman Chemical, U.S.A.).[13]

Pharmacokinetic and Pharmacodynamic Data Analysis As pharmacokinetic parameters, the peak plasma hCG level (C_{max}) and the time required to reach the peak plasma hCG concentration (t_{max}) were obtained from individual plasma hCG concentration–time curves. The area under the individual plasma hCG concentration–time curves from 0 to 48 h after rectal administration ($AUC_{0\to48}$) was calculated using the trapezoidal rule.[14] As an index of pharmacodynamic activity, changes in the plasma testosterone concentration were examined. Statistical analyses were conducted using one-way analysis of variance and Dunnett's tests. A significant difference was estimated using $p=0.05$ as the minimal level of significance.

RESULTS AND DISCUSSION

The hollow-type suppository I or II containing hCG with or without α-CyD in isotonic phosphate buffer solution (pH 7.4) was inserted into the rectum of the rabbits to determine the time courses of plasma hCG concentration following administration. For the control experiment, hCG in physiological saline was administered intravenously to the animals. The mean semi-log plasma hCG concentration-time curves and the changes in the plasma testosterone concentration are shown in Figs. 1 and 2, respectively. The mean values of the pharmacokinetic parameters of hCG are shown in Table 2. Evaluation of the pharmacodynamic activity of hCG (as indicated by the increase in plasma testosterone level) was done by administering aqueous hCG solution (40 or 400 IU/kg body weight) intravenously. As shown in Table 2, $AUC_{0—48}$ of hCG increased with increasing dose of hCG. When hCG at a dose of 40 IU/kg body weight was administered intravenously, the plasma testosterone concentration significantly increased from 2 to 12 h after administration compared with the testosterone concentration before administration (physiological level). Thus, an hCG dose of 40 IU/kg body weight is sufficient to increase the testosterone concentration. However, hCG was not detected in plasma when it alone was administered rectally, even at a higher dose (4000 IU/kg body weight) than intravenous injection, because of low bioavailability due to its high molecular weight or degradation by proteolytic activity.

To enhance rectal absorption of hCG, coadministration of hCG and α-CyD, an absorption-enhancing agent, was investigated. We found previously that natural CyDs, such as α-CyD, significantly enhanced rectal absorption of polypeptide drugs.[8—10] Moreover, the CyD effect of enhancing nasal absorption of the same kinds of polypeptide drugs has been reported.[15,16] These findings led us to investigate whether hCG absorption in the rectum might be enhanced when hCG was administered together with a CyD, for example, α-CyD. As shown in Fig. 1, hCG was detected in plasma following coadministration of hCG and α-CyD (10 mg/kg body weight) into the rectum. The plasma hCG concentration increased with increasing dose of α-CyD. The $AUC_{0\to48}$ observed after rectal administration of hCG and α-CyD at 30 mg/kg body weight was approximately four times higher than that after administration of hCG and α-CyD at 10 mg/kg body weight. Generally, absorption-enhancing agents are believed to act through three mechanisms: compromising the integrity of the mucosal membrane, inhibiting proteolytic activity, or increasing thermodynamic activity of the peptide and protein.[17] It has been reported that CyDs possibly affect rectal membrane integrity after its coadministration with morphine in rabbits.[18] CyDs may extract lipid from the gastrointestinal mucosa, facilitating drug absorption.[19] Although the level of proteolytic activity is generally lower in the rectum than in the jejunum and ileum, other absorption-enhancing mechanisms of CyDs that affect the enzymatic barrier of hCG in the rectum may also be involved. Concerning the effect of CyDs on proteolytic activity, Arima *et al.*[20] reported that the degradation of protein drugs such as insulin was retarded by CyDs when incubated with rat nasal membrane homogenates. α-CyD is probably partially associated with reducing metabolic hCG degradation in the rectal mucosa.

The plasma testosterone concentration did not increase rapidly after rectal administration as compared with that observed after intravenous administration, but interestingly, the increase was sustained after rectal administration. HCG at a high concentration induced a rapid increase in plasma testosterone concentration (74.2±3.4 ng/ml) 2 h after intravenous administration. However, 24 h after intravenous administration the concentration had decreased to the physiological level (approximately 20 ng/ml), which was determined before the administration. Incontrast, the maximum testosterone concentration (40.0±12.6 ng/ml) was observed 24 h after rectal administration of hCG (4000 IU/kg body weight) in combination with α-CyD (30 mg/kg body weight). Moreover, 72 h after rectal administration the plasma testosterone concentration (31.0±11.4 ng/ml) tended to be maintained at a higher level than that (14.4±0.9 ng/ml) before administration. Veijola and Kellokumpu[21] reported that the peak concentrations of serum testosterone in systemic circulation gradually shift earlier after intravenous injections with increase in the dose of hCG. Hodgson and deKretser[22] reported that different time patterns are required to detect the absolute maximal response to various low doses of hCG. It is interesting to note that the steroidogenic response was greatly varied depending on the hCG dose. In this investigation, we observed a time delay to reach a high plasma testosterone concentration, and found that the plasma hCG concentration after rectal administration was lower than that after intravenous administration. These results are in agreement with the findings of Veijola and Kellokumpu[21] and Hodgson and deKretser.[22]

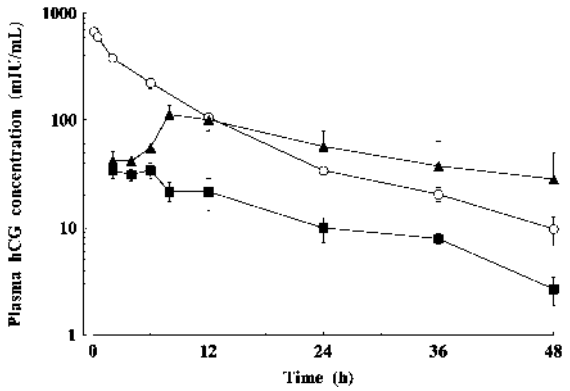

Fig. 1. Plasma hCG Concentration after Intravenous Bolus Injection of hCG (40 IU/kg Body Weight) and Rectal Administration of hCG (4000 IU/kg Body Weight) with α-CyD in Rabbits

Each point represents the mean±S.E. of three experiments. ○, Intravenous injection; ■, rectal administration of hCG with α-CyD 10 mg/kg body weight; ▲, with α-CyD 30 mg/kg body weight.

Rectal absorption of polypeptides of various molecular weights, such as elcatonin (MW 3300), insulin (MW 6000) and rhG-CSF (MW 19000) is enhanced by α-CyD. In this study, we found that α-CyD enhanced rectal absorption of hCG, which has a high molecular weight (approximately 40000), and an efficient pharmacodynamic action (as indicated by the increase in testosterone concentration) was induced. We report for the first time the enhanced rectal absorption of hCG by its coadministration with α-CyD. These results suggest the possibility of hCG therapy by rectal administration. In conclusion, the hollow-type suppository as a

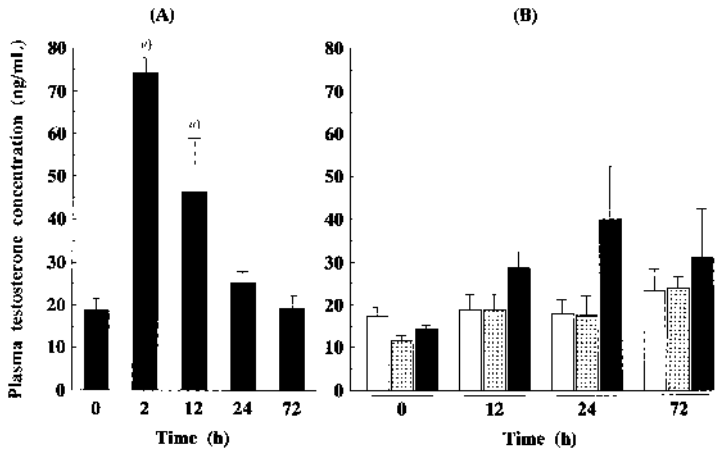

Fig. 2. Plasma Testosterone Concentration after Intravenous Bolus Injection of hCG (40 IU/kg Body Weight) (A) and Rectal Administration of hCG (4000 IU/kg Body Weight) with or without α-CyD (B) in Rabbits

Each column represents the mean±S.E. of three experiments. Statistically significant differences: *a*) $p<0.05$ at 2 and 12 h *vs.* 0 h. Columns in Fig. 2 (B): □, hCG without α-CyD; ▨, hCG with α-CyD 10 mg/kg body weight; ■, hCG with α-CyD 30 mg/kg body weight.

Table 2. Pharmacokinetic Parameters of hCG after Intravenous or Rectal Administration of hCG with or without α-CyD

Administration	hCG (IU/kg body weight)	α-CyD (mg/kg body weight)	C_{max} (mIU/ml)	t_{max} (h)	$AUC_{0\to48}$ (h·mIU/ml)
Intravenous (bolus)	40	—	—	—	4260±340
	400	—	—	—	63000±6400
Rectal	4000	None	N.D.	N.D.	N.D.
	4000	10	39.3±5.9	3.2±1.4	671±139
	4000	30	114.8±25.5[a)]	9.3±1.3[a)]	2733±869[a)]

Each value represents the mean±S.E. of three experiments. *a*) $p<0.05$ in α-CyD 30 mg/kg body weight *vs.* α-CyD 10 mg/kg body weight. N.D.

rectal delivery system for hCG is promising as a new mode for hCG therapy.

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