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Absorption Enhancement of Polypeptide Drugs by Cyclodextrins. I.[1] Enhanced Rectal Absorption of Insulin from Hollow-Type Suppositories Containing Insulin and Cyclodextrins in Rabbits[2]

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The absorption of insulin (from porcine pancreas) from the rectum of rabbits after the administration of hollow-type suppositories containing insulin and five kinds of cyclodextrins (CyDs) was investigated. Three types of suppositories were employed: suppository I containing insulin (approximately 26 IU/mg) and various amounts of each CyD in citric buffer solution at pH 3.0 or powder in its cavity, suppository II containing CyD without insulin, and suppository III containing insulin without CyD. Without CyD, the insulin and glucose levels in plasma were unchanged, whereas a significant increase in the plasma insulin concentration and a marked decrease in the glucose levels were found following simultaneous administration of insulin and CyDs by suppository I. The enhancing effect of CyD on rectal insulin absorption (absorption-enhancing effect) by chemically modified CyDs (heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) and 2-hydroxypropyl-β-CyD (HP-β-CyD)) was higher than those by natural CyDs (α-, β-, and γ-CyD). The area under the plasma concentration–time curve (AUC) and C_{max} of insulin significantly decreased with the preadministration (administration of CyD 6, 24 and 48 h before rectal insulin administration) of DM-β-CyD. The absorption-enhancing effect disappeared 24 h after preadministration. These results suggest that CyDs enhance insulin absorption from the rectum, and that attenuation of the membrane transport barrier function in the rectum recovers at a maximum of 24 h after administration of CyDs.

Keywords cyclodextrin; insulin rectal absorption enhancement; absorption-enhancing effect duration; hollow-type suppository; rabbit

Introduction

Cyclodextrins (CyDs), which are biocompatible polymers, can form a molecular inclusion complex with various drugs and have been successfully used to improve the bioavailability of drugs, such as poorly soluble materials in the pharmaceutical field.[3] Generally, the improvement of bioavailability by means of CyDs complexation is based on the increase in the dissolution rate and solubility of drugs. On the other hand, CyDs are found to interact with lipids and proteins in biological membranes such as human erythrocytes.[4,5] Furthermore, the effect of CyDs on biological membranes at the absorption site of the drug in the intestine has been reported.[6] From these viewpoints, CyDs may provide a potential means of improving mucosal membrane permeability of poorly absorbed peptide drugs. However, only a few experiments on the effect of CyDs on rectal absorption of polypeptide drugs have been reported.

Recently, we have shown that glyceryl-1-monooctanoate (GMO), a medium-chain monoacylglycerol, enhanced the rectal absorption of insulin, a model drug of polypeptides, using hollow-type suppositories[7] in rabbits.[8] Hollow-type suppositories can eliminate the effect of the heating process on the nature of insulin during preparation and can minimize the difference in the release rate from the dosage vehicle between hydrophilic and hydrophobic CyDs. In the present study, to investigate the effect of various CyDs on the rectal absorption of insulin in rabbits, we chose a new insulin formulation which uses a hollow-type suppository containing insulin and amounts of each CyD in either the aqueous solution or the powdered form. The enhancement of the rectal absorption of insulin by CyDs from these suppositories and the effect of formulation factors involving the concentration and dosage form (powder, solution, *etc.*) of these compounds on the absorption enhancement were evaluated.

Materials and Methods

Materials Porcine insulin (crystalline, 26.1 IU/mg containing approximately 0.5% zinc) was obtained from Sigma Chemical, St. Louis, MO, U.S.A. α-CyD and β-CyD were gifts from Sanraku Inc., Tokyo Japan. γ-CyD, heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) and 2-hydroxypropyl-β-CyD (HP-β-CyD: average degree of substitution of hydroxypropyl group, 4.6) were donated by Nihon Shokuhin Kako Co., Tokyo, Japan. A suppository base, Witepsol H-15 (H-15), was kindly supplied by Hüls Troisdorf, Troisdorf, Germany. All other reagents used were of analytical grade.

Preparation of Suppositories Formulations comprising the three types of hollow suppositories (approximately 2 g), containing insulin and amounts of each CyD in a solution or powdered form in the cavity (suppository I), containing CyD in a solution or powdered form in the cavity (suppository II), and containing insulin in a solution or powdered form in the cavity (suppository III), were prepared using Witepsol H-15 by the fusion process method reported by Watanabe *et al.*[9,10] For the control experiments, a hollow-type suppository without insulin or CyD (blank suppository) was prepared using Witepsol H-15. The insulin and CyD contents are listed in Table I. When insulin and CyD were used in a powdered form, the doses of the two materials were accurately weighed and added to each cavity of the suppository. For the freshly prepared solution containing insulin and amounts of each CyD, except for β-CyD, both materials were dissolved at the appropriate concentration in an isotonic citrate buffer solution[11] (CBS) at pH 3.0. A combination of insulin

TABLE I. Amounts of Insulin and CyD added in Hollow-Type Suppositories

Insulin		CyD (mg)	Volume (μl)
Form	Dose[b] (IU)		
Aqueous solution[a]	26	0	200
	26	30	200
	26	100	200
	5.2	100	200
Powder	26	0	—
	26	100	—

a) Insulin was dissolved in an isotonic citrate buffer solution (pH 3.0) containing CyD. *b*) Insulin doses of 26 and 5.2 IU correspond to amounts of 1 and 0.2 mg, respectively.

and β-CyD in CBS was used as a suspension form since β-CyD has low solubility. Two-hundred μl of each prepared solution was added to each cavity of the suppository. The opening at the hind part of the suppository was sealed with the base material, by melting. All suppositories which had been refrigerated overnight after preparation were examined.

Animal Experiments Male Japan White rabbits weighing 3.0 to 4.0 kg were used. They freely received food with water and were housed individually in cages in a forced-air facility that was maintained at 23±1 °C and 55% relative humidity with a 12-h light/dark cycle. Animals with free access to water were fasted for one night prior to each experiment. Each suppository was administered into the rectum according to the method described in our previous reports.[8,9]

Simultaneous Administration Method: Suppository I containing insulin and amounts of each CyD was inserted into the rectum.

Preadministration Method: The CyDs using suppository II were administered into the rectum, then insulin using suppository III was administered at 0.5, 6, 24 or 48 h after CyD administration.

After rectal administration of the suppository, 2 ml blood samples were taken from the auricular vein by a syringe containing ethylendiaminetetraacetic acid disodium salt (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 assays could be performed for insulin.

Determination of Insulin and Glucose Levels in Plasma The plasma insulin concentration was determined by the enzyme immunoassay (EIA) method employing an EIA Insulin test-S kit (Medical & Biological Laboratories, Nagoya, Japan) and the assay of glucose in plasma was performed using a Glucose-test kit (Wako Pure Chemicals, Tokyo, Japan) described in our previous paper.[8]

Pharmacokinetic Analysis The peak plasma insulin level (C_{max}) and the peak concentration time (t_{max}) were obtained from individual plasma insulin concentration–time curves. The area under the individual plasma insulin concentration–time curves from 0 to 6 h after rectal administration (AUC_{0-6}) was calculated using the trapezoidal rule.[12]

Statistical analysis of the results was conducted by the 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

Plasma Insulin Concentrations Following Rectal Administration of Hollow-Type Suppositories Containing Insulin in Aqueous Solution To examine the enhancing effects of natural CyDs (α-, β- and γ-CyD) and chemically modified CyDs (DM-β- and HP-β-CyD) on the rectal absorption of insulin after the simultaneous administration of insulin and CyD, 1 mg of porcine insulin (26 IU) in each suppository was used. To determine the dose of insulin in the suppository, 26 IU (approximately 8 IU/kg), we referred to the reports of Liversidge *et al.*[13] and Aungst *et al.*[14]

Figure 1 shows the mean semilog plasma insulin concentration–time curves after the rectal administration of suppository I or III. The changes in the plasma glucose levels are shown in Fig. 2. The mean values of pharmacokinetic parameters of insulin are summarized in Table II. When suppository III containing insulin without CyD was administered, plasma insulin and glucose concentrations (represented by the dotted lines in Figs. 1 and 2) did not change from the physiological levels (endogenous levels) observed after administration of the blank suppository.[8] Therefore, the rectal absorption of insulin without CyD was negligible.

On the other hand, plasma insulin concentrations were rapidly increased and t_{max} was reached within 1 h after the administration of suppository I containing insulin (26 IU) and amounts of each CyD (30 mg) in 200 μl of CBS in the cavity of the suppository. As shown in Table II, all of the

TABLE II. Pharmacokinetic Parameters of Insulin Following Rectal Administration of Hollow-Type Suppositories Containing Insulin and CyD in Rabbits

CyD	AUC_{0-6} (h·μIU/ml)	C_{max} (μIU/ml)	t_{max} (min)
None	38± 7	14± 2	—
α-CyD	107±23	141±108	30± 6
β-CyD	110±40	85± 33	26± 7
γ-CyD	141±36	166± 49	41± 7
HP-β-CyD	197±31[a]	209± 22	53±23
DM-β-CyD	290±42[b,c]	556±233[d]	49±24

Insulin: 26 IU, CyD: 30 mg. Each value represents the mean±S.E. of three or four experiments. Statistically significant difference: *a*) $p<0.05$ in HP-β-CyD *vs.* none, *b*) $p<0.05$ in DM-β-CyD *vs.* γ-CyD, *c*) $p<0.01$ in DM-β-CyD *vs.* none, α-CyD and β-CyD, *d*) $p<0.05$ in DM-β-CyD *vs.* none and β-CyD.

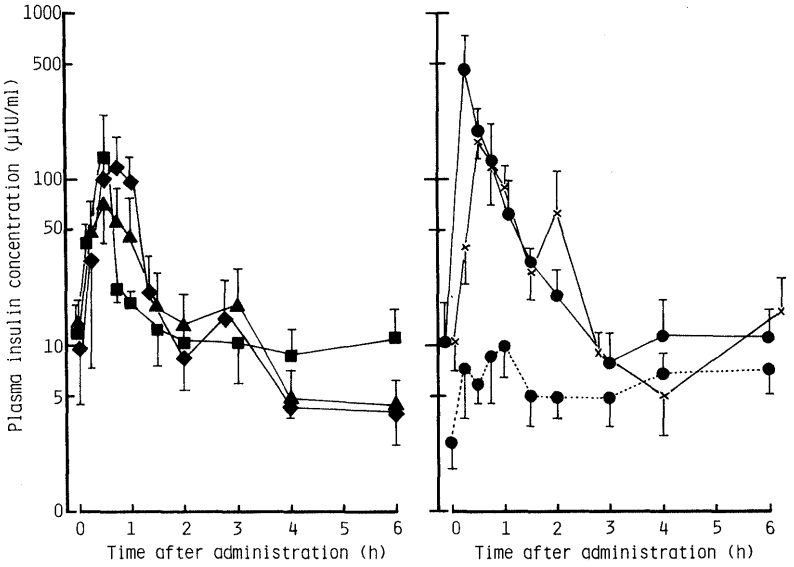

Fig. 1. Mean Plasma Concentrations of Insulin Following the Rectal Administration of Hollow-Type Suppositories Containing Insulin and Various CyDs in Citrate Buffer Solution at pH 3.0 (Simultaneous Administration) in Rabbits

Each point represents the mean±S.E. (vertical bar) of three or four rabbits. Amounts of porcine insulin and CyD in suppositories are 26 IU and 30 mg, respectively. Key: ---●---, insulin without CyD; —■—, α-CyD; —▲—, β-CyD; —◆—, γ-CyD; —×—, HP-β-CyD; —●—, DM-β-CyD.

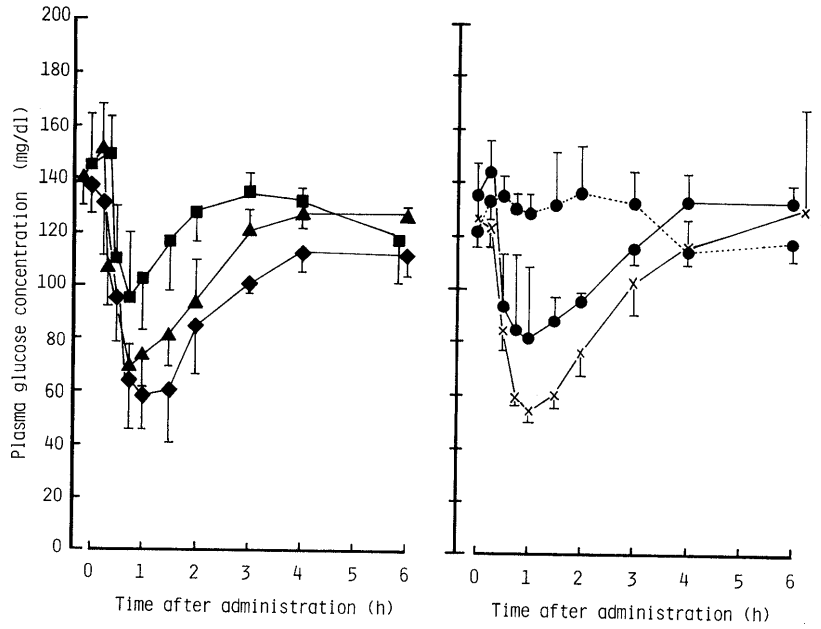

Fig. 2. Mean Plasma Glucose Concentrations Following the Rectal Administration of Hollow-Type Suppositories Containing Insulin and Various CyDs in Citrate Buffer Solution at pH 3.0 (Simultaneous Administration) in Rabbits

Each point represents the mean ± S.E. (vertical bar) of three or four rabbits. Amounts of porcine insulin and CyD in suppositories are 26 IU and 30 mg, respectively. Key: ---●---, insulin without CyD; —■—, α-CyD; —▲—, β-CyD; —◆—, γ-CyD; —×—, HP-β-CyD; —●—, DM-β-CyD.

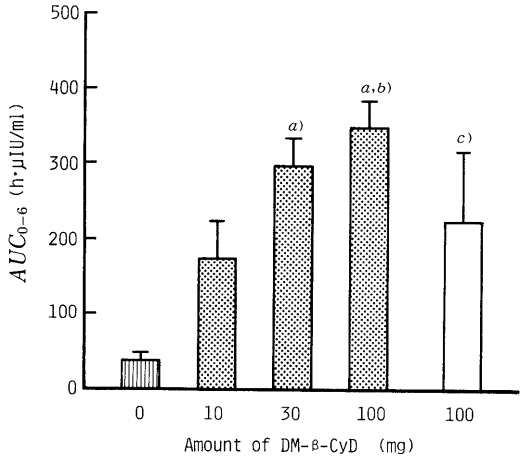

Fig. 3. Mean *AUC* of Insulin Following Rectal Administration of Hollow-Type Suppositories Containing Insulin and Various Amounts of DM-β-CyD in Citrate Buffer Solution (pH 3.0) or Powdered Form in Rabbits

Insulin: 26 IU. Column: ▨, aqueous solution (200 μl); □, powdered form; ▥, without CyD. Each point represents the mean ± S.E. (vertical bar) of 3—5 rabbits. Statistically significant differences: *a*) $p<0.01$ in 30 and 100 mg (aqueous solution) *vs.* 0 mg; *b*) $p<0.05$ in 100 mg (aqueous solution) *vs.* 10 mg; *c*) $p<0.05$ in 100 mg (powder) *vs.* 0 mg.

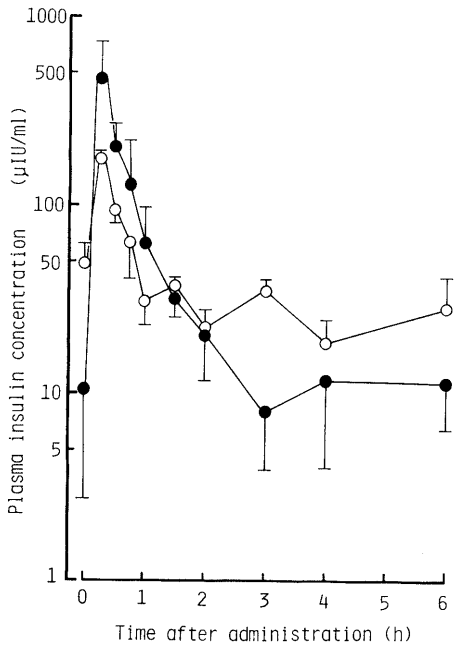

Fig. 4. Mean Plasma Concentrations of Insulin in Rabbits Following Rectal Administration of Hollow-Type Suppositories Containing Insulin (26 or 5.2 IU) and 30 mg of DM-β-CyD in Citrate Buffer Solution

Insulin dose: —●—, 26 IU (1.0 mg); —○—, 5.2 IU (0.2 mg). Each point represents the mean ± S.E. (vertical bar) of four rabbits.

mean values of AUC_{0-6} and C_{max} were increased by CyDs. These mean values obtained by chemically modified CyDs (DM-β-, and HP-β-CyD) were higher than those obtained by natural CyDs. Plasma glucose concentrations decreased with increasing plasma insulin levels, but the order of the maximum levels of decrease in plasma glucose concentration (hypoglycemic effect) obtained after administration of insulin with DM-β- or HP-β-CyD did not correspond to the order of values of AUC_{0-6} or C_{max}. This difference in the decrease of plasma glucose levels observed after administration of insulin with two CyDs is probably related to the individual differences of hypoglycemic response in rabbits.

The absorption-enhancing effect of CyD, for instance, DM-β-CyD, should have increased with increases in the amount of CyD in the suppository (Fig. 3). However, no significant difference in AUC_{0-6} was obtained using DM-β-CyD at amounts between 30 and 100 mg. This is because the absorption-enhancing effect of DM-β-CyD at a dose of 100 mg reaches the supramaximal level.

High levels of insulin in plasma were obtained after

the simultaneous administration of insulin (26 IU) and DM-β-CyD. To observe the plasma insulin concentrations after the administration of insulin in lesser amounts, suppository I containing insulin (5.2 IU) and DM-β-CyD (30 mg) in 200 μl of CBS were administered into the rectum. As shown in Fig. 4, even though the amount of porcine insulin was decreased to 5.2 IU (one-fifth the original amount), high insulin levels were still obtained (C_{max}: 172±17 μIU/ml). With regard to a comparison of the AUC_{0-6} values observed at amounts of insulin between 26 and 5.2 IU, the mean of AUC_{0-6} (223±24 h·μIU/ml) obtained with 2.5 IU of insulin with 30 mg of DM-β-CyD was higher than those (shown in Table II) obtained with 26 IU of insulin with the other CyDs at amounts of 30 mg. Even low levels of insulin in these suppositories, for instance 5.2 IU (0.2 mg), were effective. Consequently, it is possible to regulate plasma insulin levels using hollow-type suppositories containing insulin at various doses combined with at least 30 mg of DM-β-CyD.

Plasma Insulin Concentration Following Rectal Administration of Hollow-Type Suppositories Containing Insulin and CyD in Powdered Form The hollow-type suppository can contain insulin and CyDs in powdered form instead of aqueous solution. To evaluate the applicability of insulin and CyDs in the rectal delivery system, studies concerning the effect of formulation factors, such as the form of these compounds in the suppository, on the absorption of insulin are important. Rectal insulin absorption using a powdered form was examined. The plasma insulin concentrations after administration of suppository I containing powdered insulin and DM-β-CyD at the maximal dose of 100 mg in their cavities were determined. The dose of 1 mg of insulin (porcine, 26 IU) in suppositories was employed. Suppositories containing less than 1 mg of insulin in powdered form were not examined because of the difficulty of weighing out the insulin accurately into each cavity. Without CyD, no increase in plasma insulin concentration occurred with the administration of the suppository containing powdered insulin in the same amount as in suppository I.[8]

As shown in Fig. 3, the AUC_{0-6} values after the simultaneous administration of insulin and DM-β-CyD in powdered form (shown as unfilled column) was also increased, and insulin was efficiently absorbed with DM-β-CyD (100 mg). It is also possible to enhance insulin absorption following rectal administration using the powdered form of the two compounds. However, the mean value of AUC_{0-6} (223±92 h·μIU/ml) after administration in powdered form was lower than that (347±34 h·μIU/ml) by solution form. In all probability, this difference in absorption enhancement by CyDs between solution and powdered form is caused by the difference in the dissolution process in the rectum, because powdered forms of CyDs have different solubilities.

Rectal Insulin Absorption with the Preadministration of CyD CyDs significantly enhanced insulin absorption following simultaneous rectal administration in rabbits. To better understand the absorption-enhancing effect of CyDs, it is necessary to elucidate the duration of this effect. The duration of the enhancing effect by DM-β-CyD on rectal

Table III. Pharmacokinetic Parameters of Insulin Following Rectal Administration of Insulin after Preadministration of DM-β-CyD in Rabbits

Preadministration time[a] (h)	AUC_{0-6} (h·μIU/ml)	C_{max} (μIU/ml)
0[b]	290±42	556±233
0.5	188±34	174± 58
6	116±44[c]	96± 44[d]
24	70±21[c,e]	27± 4[c]
48	33± 9[c,e]	30± 10[c]

Insulin: 26 IU, DM-β-CyD: 30 mg. Each value represents the mean±S.E. of three or four experiments. *a*) Preadministration time 0.5—48 h is the time interval between CyD (suppository II) and insulin (suppository III) administration. *b*) Preadministration time 0 h represents the simultaneous administration of insulin and CyD (suppository I). Statistically significant differences: *c*) $p<0.01$ in 6, 24 and 48 h *vs.* 0 h, *d*) $p<0.05$ in 6 h *vs.* 0 h, *e*) $p<0.05$ in 24 and 48 h *vs.* 0.5 h.

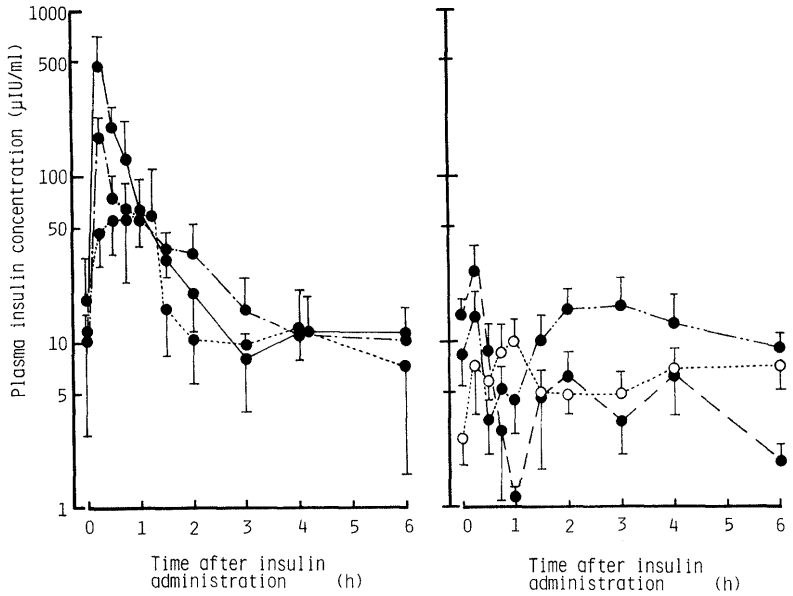

Fig. 5. Mean Plasma Concentrations of Insulin Following Rectal Administration of Insulin after the Preadministration with DM-β-CyD in Rabbits

Doses of insulin and DM-β-CyD in each suppository are 26 IU and 30 mg, respectively. Preadministration time (h): —●—, 0 (simultaneous administration); --●--, 0.5; ---●---, 6; —·—●—·—, 24; ——●——, 48. ---○---, insulin without CyD. Each point represents the mean±S.E. (vertical bar) of three or four rabbits.

absorption of insulin using the preadministration method (administration with only CyD before insulin administration) was investigated.

Figure 5 illustrates the plasma insulin concentration–time curves after insulin administration with DM-β-CyD preadministration. The mean values of C_{max} and AUC_{0-6} are shown in Table III. A significant decrease in plasma insulin concentration was shown to occur when the time interval between preadministration with DM-β-CyD and insulin administration was prolonged. The values of C_{max} and AUC_{0-6} obtained by pretreatment with DM-β-CyD at 6 h and longer time intervals were significantly lower ($p<0.01$, $p<0.05$) than those obtained by the simultaneous administration of insulin and DM-β-CyD. The plasma insulin concentrations obtained by DM-β-CyD pretreatment at 24 and 48 h were not significant compared to the concentrations observed following insulin administration without DM-β-CyD. No decrease in plasma glucose concentration following the rectal administration of insulin was recognized 24 or 48 h after the preadministration of DM-β-CyD.

Discussion

Considerable attention has been paid to the effect of CyDs on the absorption characteristics of drugs in the rectum. It is generally accepted that the improvement in drug absorption by means of CyDs is based on the increase in the dissolution rate of a drug-formed inclusion complex with CyDs. Concerning the enhancing mechanism of drug absorption due to CyDs, the effect of CyDs on physicochemical and biological determinants governing the rectal absorption of drugs should be considered. In this investigation, we found that insulin rectal absorption was significantly enhanced by CyDs, particularly DM-β-CyD. In our preliminary experiments, the interaction between insulin and CyDs was not observed by UV spectroscopic determination. This result is in general agreement with that of Arima *et al.*[15] on the observation of the interaction between these compounds. Therefore, the absorption-enhancing effect of CyDs should be explained by the modification of biophysical determinants for the rectal absorption of insulin, *i.e.*, some interaction between CyDs and mucosal membrane would be expected.[15] Absorption enhancers are believed to act by one of three mechanisms: comprising the integrity of the mucosal membrane, inhibition of proteolytic activity, or increasing thermodynamic activity of the peptide and protein.[16,17] Recently, it has been shown that a possible mechanism of CyDs affects rectal membrane integrity after the coadministration of morphine and CyDs in rabbits.[18]

Uekama and Otagiri[3] suggested that CyDs may extract lipids from the gastrointestinal mucosa, thereby leading to facilitated drug absorption from the intestinal tract. Furthermore, it has been demonstrated that CyDs solubilize membrane lipids of human erythrocytes through rapid and reversible formation of inclusion complexes.[5] Similar mechanisms would also be implicated in the solubilization of lipids from the rectal mucosa by CyDs, and the perturbation of the rectal epithelium by CyDs may contribute substantially to the enhanced membrane permeability to insulin.

Although the level of proteolytic activity is generally lower in the rectum than in the jejunum and ileum, other absorption-enhancing mechanisms of CyDs which affect the enzymatic barrier of insulin in the rectum may also be involved. With reference to the effect of CyDs on the proteolytic activity, it has been reported that the degradation of insulin, when incubated with rat nasal membrane homogenates, was found to be retarded by CyDs in the following order: α- < DM-α- < HP-α- < β- < HP-β- < DM-β-CyD.[15] The order of increase in AUC_{0-6} values observed in this study is in general agreement with the order of retardation of insulin degradation by CyDs, as stated above. The enhancement of rectal insulin absorption by CyDs, particularly HP-β- and DM-β-CyD, is partially related to decreasing metabolic insulin degradation in the rectal mucosa.

We found, by the preadministration method, that the absorption-enhancing effect due to CyDs decreased with increasing time intervals of administration between CyD and insulin. However, it was demonstrated that the absorption-enhancing effect of DM-β-CyD remains for several hours. The retention of a minimal effective concentration of CyDs at absorption sites seems to be necessary for them to exert an absorption-enhancing effect. The enhancing effect of CyDs should therefore decrease as they are absorbed from the rectal lumen. The decrease in the absorption-enhancing effect of CyDs may be related to the decreasing effective concentration of CyDs due to their absorption. Generally, the absorption of intact CyDs from the gastrointestinal tract is rather restricted.[3] However, it is conceivable that CyDs could be absorbed in the rectum when they act as enhancing agents for the rectal absorption of polypeptides. Although confirmation concerning the exact amount of absorption of CyDs with insulin and the amount of CyDs on the rectal lumen is important, we could not determine these residues because of interference from the suppository base materials.

CyD itself may alter the lipid barrier of the absorption site, which may eventually facilitate drug absorption. It is very important to determine the rate at which mucosal membranes recover from the acute irritating effect of a given CyD. The recovery rate appears to be related to the extent of membrane irritation that occurs. With respect to the recovery of barrier function in the rectum after the administration of absorption enhancers, Nakanishi *et al.*[19] reported that the permeability of the rectal membrane was still higher than the control value 24 h after the pretreatment with absorption enhancers such as sodium deoxycholate, sodium lauryl sulfate and EDTA-2Na. The recovery rate is slow in the case of strong absorption enhancers,[20] as stated above, while it is more rapid in the case of CyDs, which are less aggressive. Although deliberate attempts to accelerate repair of mucosal damage by CyDs have not been reported, the recovery of membrane barrier function in the rectum is probably achieved about 24 h after the administration of at least 30 mg of CyD, because AUC_{0-6} values obtained in control experiments (listed as none in Table II) and observed 24 h after preadministration (listed in Table III) were not significant.

Conclusions

We have found that when CyD was administered with insulin, plasma insulin concentrations significantly in-

creased following the use of the hollow-type suppositories containing porcine insulin. These results demonstrate that the insulin is efficiently absorbed through the rectum of rabbits when either the aqueous solution or the powdered form of insulin and CyD is administered. Insulin could be absorbed even when only CyD was given 6 h before insulin administration. However, the attenuation of the membrane transport barrier function is recovered at a maximum of 24 h after the rectal administration of CyD. CyD is useful as a practical absorption enhancer for the rectal dosage form of polypeptide drugs.

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References and Notes

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