Enhancing Effect of Cyclodextrins on Nasal Absorption of Insulin and Its Duration in Rabbits[1)]

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The absorption of insulin (from porcine pancreas) in rabbits after the nasal administration of aqueous preparations containing insulin and five kinds of cyclodextrins (CyDs) in phosphate buffer solution at pH 7.0 was investigated. Without CyD, the insulin and glucose levels in plasma were unchanged, whereas a marked increase in the plasma levels of insulin and a decrease in glucose concentrations were observed following the simultaneous administration of insulin and CyD such as α- and heptakis (2,6-di-*O*-methyl)-β-CyD (DM-β-CyD). The largest enhancing effect on the nasal absorption of insulin was obtained by DM-β-CyD. To evaluate the duration of the absorption-enhancing effect of CyDs, preadministration (administration of CyD 0.5, 6, 12 and 24 h before insulin administration) was performed. The area under plasma concentration–time curve (*AUC*) and C_{max} of insulin significantly decreased with the preadministration of DM-β-CyD 6, 12 and 24 h before nasal administration. The absorption-enhancing effect disappeared 24 h after the preadministration. These findings demonstrate that CyDs enhance the nasal absorption of insulin, and the recovery of the membrane transport barrier function in nasal mucosa is achieved, at the latest, 24 h after the administration of CyDs.

Keywords cyclodextrin; absorption-enhancing effect duration; insulin nasal absorption enhancement; rabbit

Introduction

In a previous paper,[1)] we demonstrated that the co-administration of insulin and cyclodextrin (CyD), particularly heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD), results in a considerable increase in the rectal absorption of insulin as a model drug of polypeptides in rabbits. These findings led us to inquire whether insulin absorption from another delivery route might be caused by CyDs. The nasal route is an attractive alternative to parenteral injection because of rich vascularization, ease of administration, and so on.[2)] Attempts to achieve nasal absorption of insulin promoted by absorption-enhancing agents (absorption enhancers) such as surfactants,[3,4)] bile salts,[5)] medium-chain fatty acids,[6)] and sodium tauro-24,25-dihydrofusidate[7,8)] in animals and humans suggest that the nasal administration of insulin shows promise as a means of performing insulin therapy.

Recently, chemically modified CyDs such as methylated CyDs have been successfully employed in a formulation for nasal administration containing 17-β-estradiol in rabbits and rats,[9)] since they appear to have only minor effects on ciliary beat frequency.[10)] Furthermore, Arima *et al.*[11)] and Merkus *et al.*[12)] reported improvement in the nasal delivery of insulin with chemically modified CyDs in rats.

In this study, to evaluate the enhancing effect of CyDs on nasal absorption of insulin in rabbits, aqueous preparations containing insulin and CyDs were instilled intranasally. The absorption-enhancing effect of CyD administered nasally was compared with that obtained by the rectal administration.

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 Co., Tokyo, Japan. γ-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. All other reagents used were of analytical grade.

Preparation for Nasal Administration The aqueous preparations (freshly prepared) for nasal administration were made by suspending appropriate amounts of insulin and each CyD in an isotonic phosphate buffer solution[13)] (PBS) at pH 7.0. The placebo formulations contained the same amount of CyDs in PBS without insulin. The insulin and CyD contents are listed in Table I.

Animal Experiments and Determination of Insulin and Glucose in Plasma Male Japan White rabbits weighing 3.0 to 4.0 kg were used in this investigation. They had free access to water and food and were housed individually in cages under environmentally controlled conditions (23±1 °C, 55% relative humidity, 12-h on/off light/dark cycle). The method of the nasal absorption study in rabbits reported by Maitani *et al.*[14)] was applied with a slight modification. Briefly, rabbits were fasted overnight with freely available tap water and they were held with their heads in a vertical position. For simultaneous administration, after mixing the suspension for 30 s using a Vortex Mixer, a 200 μl aliquot was administered into one nostril with a micropipette (Eppendorf®). For the pretreatment method, a solution containing CyD without insulin was instilled intranasally, then the insulin suspension was administered 0.5, 6, 12 and 24 h after CyD administration. Immediately after the nasal administration of drugs, rabbits were placed in a supine position for 2 min,[14)] then they were secured in a crouching posture during the experimental period. Two-milliliter aliquots of blood samples were taken from the auricular vein by a syringe containing ethylenediaminetetraacetic 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 and glucose.

The plasma insulin and glucose concentrations were determined by the enzyme immunoassay method (EIA) employing an EIA Insulin test-S kit (Medical & Biological Laboratories, Nagoya, Japan) and by the Glucose-test kit (Wako Pure Chemicals, Tokyo, Japan) as described in our previous paper.[15)]

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 nasal administration

TABLE I. Amounts of Insulin and CyD Added in Aqueous Preparations For Nasal Administration

Insulin dose[a)] (IU)	CyD (mg)	Volume (μl)
5.2	0	200
5.2	30	200
5.2	50	200
5.2	100	200
26	0	200
26	30	200

Aqueous preparations containing insulin and each CyD in an isotonic phosphate buffer solution (pH 7.0) were used. *a*) Insulin doses of 5.2 and 26 IU correspond to amounts of 0.2 and 1 mg, respectively.

(AUC_{0-6}) was calculated using the trapezoidal rule.[16] Plasma glucose concentrations after insulin administration were expressed as a mean value of observed concentration.

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 and Discussion

Plasma Insulin Concentration after Simultaneous Nasal Administration of Insulin and CyDs To investigate the enhancing effects on natural CyDs (α-, β-, and γ-CyD) and chemically modified CyDs (HP-β- and DM-β-CyD) on the nasal absorption of insulin after the simultaneous administration of insulin and CyD, solutions of porcine insulin with each CyD (30 mg) in 200 μl of PBS were administered intranasally. To determine the dose of insulin, 5.2 IU (0.2 mg, one-fifth of the amount used in the rectal administration),[1] we referred to the reports of Hirai *et al.*[3] and Aungst *et al.*[17]

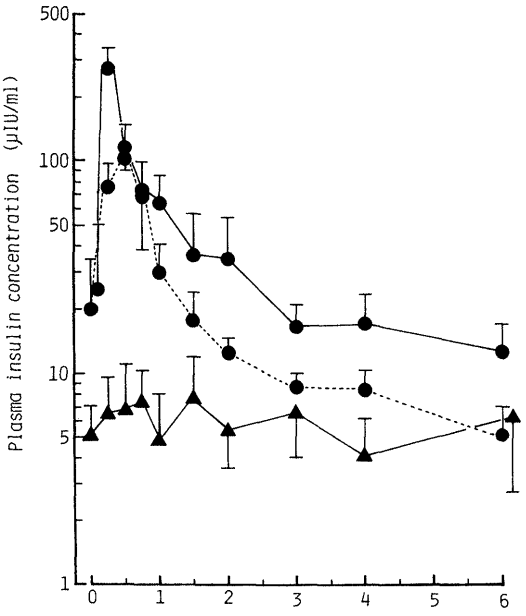

Fig. 1. Mean Plasma Concentrations of Insulin Following the Nasal Administration of Aqueous Preparations Containing Insulin and CyDs in Phosphate Buffer Solution at pH 7.0 (Simultaneous Administration) in Rabbits

Each point represents the mean±S.E. (vertical bar) of three or four rabbits. Amounts of insulin and CyD in the preparations are 5.2 IU and 30 mg, respectively. Key: —▲—, insulin without CyD; ---●---, α-CyD; —●—, DM-β-CyD.

TABLE II. Pharmacokinetic Parameters of Insulin Following Nasal Administration of Aqueous Preparations Containing Insulin and CyD in Rabbits

CyD	AUC_{0-6} (h·μIU/ml)	C_{max} (μIU/ml)	t_{max} (min)
None	35±14	11± 4	—
α-CyD	120±28	129±51	15± 6
β-CyD	45±10	17± 6	41± 9
γ-CyD	76±45	28±15	38±10
HP-β-CyD	69±17	21± 4	270±57
DM-β-CyD	241±56[a,b]	276±68[c,d]	15± 0

Insulin: 5.2 IU, CyD: 30 mg. Each value represents the mean±S.E. of three or four experiments. Statistically significant differences: *a*) $p<0.01$ in DM-β-CyD *vs.* none, β- and HP-β-CyD, *b*) $p<0.05$ in DM-β-CyD *vs.* γ-CyD, *c*) $p<0.01$ in DM-β-CyD *vs.* none, β-, γ- and HP-β-CyD, *d*) $p<0.05$ in DM-β-CyD *vs.* α-CyD.

Figure 1 illustrates the mean semilog plasma insulin concentration–time curves following nasal administration of the aqueous suspensions containing insulin and CyD. The mean values of pharmacokinetic parameters of insulin are summarized in Table II. The use of a placebo formulation, which is a solution containing CyD without insulin, did not change plasma insulin or glucose concentrations from the physiological levels (endogenous levels). Plasma insulin concentrations after the administration of an aqueous suspension containing insulin without CyD were not increased in the control experiments. These results indicate that insulin absorption following nasal administration without CyD was negligible.

On the other hand, the extent of nasal insulin absorption was indeed significantly enhanced by CyDs. High plasma levels of insulin were obtained after the simultaneous administration of insulin (5.2 IU) and 30 mg of α- or DM-β-CyD. A marked decrease in plasma glucose levels was also recognized. However, the increases in plasma insulin concentrations were not significant when insulin was administered with β-, γ- or HP-β-CyD. The highest values ($p<0.01$) of mean AUC_{0-6} (241±56 h·μIU/ml) and C_{max} (276±68 μIU/ml) were obtained with DM-β-CyD (Table II). DM-β-CyD appears to be the most potent enhancing agent of nasal insulin absorption in rabbits. It has been demonstrated that the enhancement of nasal insulin absorption was obtained after nasal instillation of insulin (10 IU/kg[11] and 0.4 IU[12]) with α- or DM-β-CyD in rats. The effects of α-CyD were not as strong as those observed with DM-β-CyD. Our results (insulin, 5.2 IU (approximately 1.5 IU/kg)) obtained with rabbits are in general agreement with those observed in rats.[11,12] However, the extent of bioavailability (*EBA*) of insulin was approximately 11 and 5% for DM-β- and α-CyD, at an amount of 30 mg, respectively. These *EBA* values are somewhat lower in rabbits than those obtained in rats.[12] This difference in *EBA* is possibly related to the fact that the influence of CyDs on nasal absorption differs largely between animal species.[18]

With reference to the enhancing mechanism of insulin absorption by CyD, since minimal interaction between insulin and CyD was observed by the UV spectroscopic method in our preliminary studies, the modification of biophysical determinants for nasal absorption, *i.e.*, some interaction between CyDs and mucosal membrane, would be expected. CyDs may extract lipids from the gastrointestinal mucosa[19] and solubilize various components from the membranes of human erythrocytes, with various CyDs possessing different solubilizing potencies.[20] Similar mechanisms are probably relevant to the effects of CyDs on the permeability of the nasal epithelium, such as nasal absorption enhancement of insulin. Other absorption enhancing mechanisms of CyDs may also be involved. The half-life (29 min) of insulin caused by enzymatic degradation was shorter in tissue homogenates of nasal mucosa from rabbits than in those of rectal (72 min), vaginal (106 min) and buccal (318 min) mucosa.[18] It is presumed that CyDs inhibit proteolytic activity of enzymes. CyDs significantly inhibited the activity of leucine aminopeptidase, which is present in both the plasma membrane and cytosol of nasal mucosal cells, and cleaves the B-chain of insulin from the N-terminal.[11] CyDs, particularly DM-β- and α-CyD, may

enhance nasal absorption of insulin by decreasing the metabolic polypeptide degradation in the nasal mucosa.[12]

As shown in Fig. 2, the mean value of AUC_{0-6} should have increased with increases in the coadministered amount of CyD, for instance DM-β-CyD. A similar result was obtained with α-CyD. However, the plasma insulin concentrations and the value of AUC_{0-6} tended to decrease when a larger amount (100 mg) of DM-β-CyD was employed. In some cases after instillation of insulin (5.2 IU) with 100 mg of DM-β-CyD, a remarkable secretion from the nasal meatus was induced in rabbits. In all probability, tissues in the nasal cavity were excessively irritated by DM-β-CyD when a larger amount was used. Consequently, it seems that this phenomenon of secretion caused by excess CyD detrimentally affects the absorption enhancement of insulin.

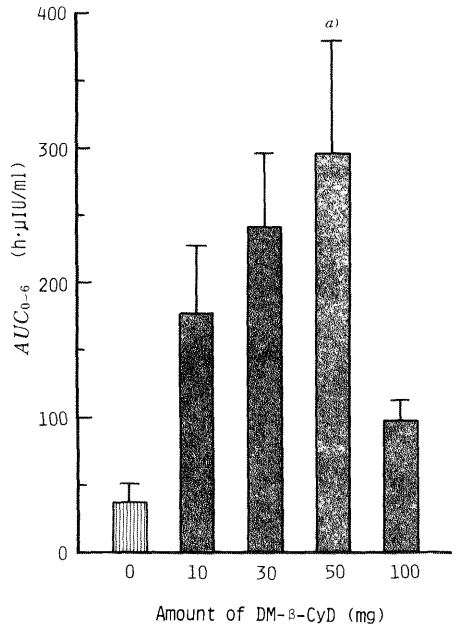

Fig. 2. The *AUC* of Insulin Following Nasal Administration of Aqueous Preparations Containing Insulin (5.2 IU) and Various Amounts of DM-β-CyD in Phosphate Buffer Solution (pH 7.0) in Rabbits

Each value represents the mean ± S.E. of three or four experiments. Statistically significant differences: *a*) $p<0.05$ in 50 mg *vs.* 0 mg.

To observe the plasma insulin levels after the nasal administration of insulin at larger amounts with a lesser amount of CyDs, a formulation comprising an aqueous suspension containing 26 IU of insulin with 30 mg of DM-β-CyD was instilled intranasally. The plasma insulin concentration was not increased when a higher dose (26 IU) of insulin without CyDs was administered, whereas a very high level (C_{max}, 1452 ± 282 μIU/ml) of insulin in plasma (Fig. 3A) and marked decreases in plasma glucose concentrations (Fig. 3B) were obtained by the simultaneous administration of insulin with DM-β-CyD. In studies of nasal delivery using various doses of insulin, 10 IU/kg in rats,[3,6] 5 IU/kg in dogs,[21] 1.4—2 IU/kg in sheep[22,23] and 0.5 IU/kg in human volunteers[5] were administered with various excipients. From our observations in rabbits, it is possible to regulate plasma insulin concentrations using aqueous preparations containing insulin at various doses (5.2—26 IU (approximately 1.5—8 IU/kg)) combined with at least 30 mg of DM-β-CyD. Even small amounts of insulin in the nasal delivery formulations, for instance 0.2 mg (approximately, 5 IU), were effective.

With respect to the preparations for the nasal delivery of insulin in this investigation, insulin was suspended and CyDs, except for β-CyD having low solubility, were dissolved in pH 7.0 PBS. Although the effect of formulation factors, such as suspension form, on the nasal absorption of insulin has not been elucidated, the sustained absorption of insulin in the form of a suspension would be expected both by the continuous dissolution of crystalline insulin and its larger residence in the nasal cavity.[11] However, such phenomena caused by the insulin suspensions were not clear in our observations in rabbits.

Nasal Insulin Absorption by the Preadministration of CyD CyDs, particularly DM-β-CyD, significantly enhanced insulin absorption following simultaneous nasal

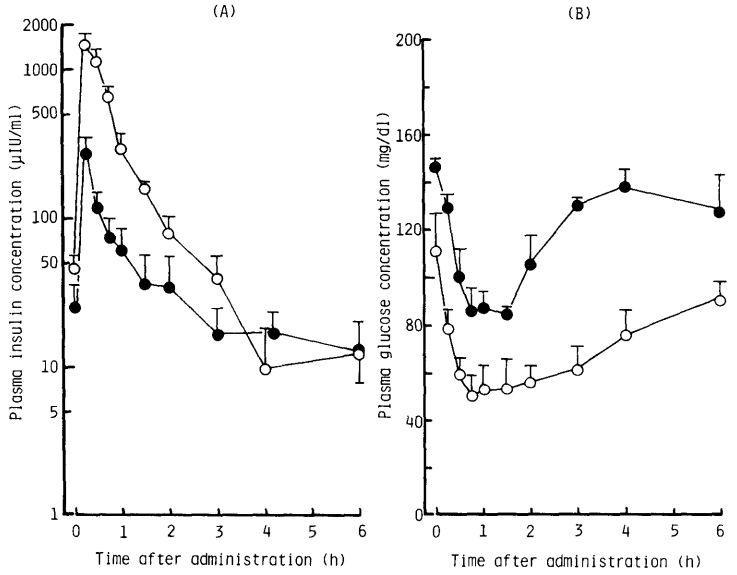

Fig. 3. Mean Plasma Concentrations of Insulin (A) and Glucose (B) Following Nasal Administration of Aqueous Preparations Containing Various Amounts of Insulin with 30 mg of DM-β-CyD in Phosphate Buffer Solution in Rabbits

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.

TABLE III. Pharmacokinetic Parameters of Insulin Following Nasal 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]	240±56	276±68
0.5	190±25	208±50
6	177±52	111±43
12	91±33[c]	63±28[c]
24	41±12[c,d]	17± 3[c,d]

Insulin: 5.2 IU, DM-β-CyD: 30 mg. Each value represents the mean±S.E. of four experiments. *a*) Preadministration time 0.5—24 h is the time interval between CyD and insulin administration. *b*) Preadministration time 0 h represents the simultaneous administration of insulin and CyD. Statistically significant differences: *c*) $p < 0.01$ in 12 and 24 h *vs.* 0 h, *d*) $p < 0.01$ in 24 h *vs.* 0.5 h.

administration in rabbits. To evaluate the applicability of CyDs, studies concerning the duration of the enhancing effect by CyDs in the nasal absorption of insulin are important. The duration of the absorption-enhancing effect by DM-β-CyD, the most effective CyD, was investigated using the preadministration method (administration with only CyD before insulin administration).

Table III shows the mean values of C_{max} and AUC_{0-6} after nasal administration with DM-β-CyD preadministration. Plasma insulin concentrations were significantly decreased when the time interval between the preadministration of DM-β-CyD and the insulin administration was prolonged. The values of C_{max} and AUC_{0-6} observed as a result of the preadministration of DM-β-CyD at 12 h and longer time intervals were significantly lower ($p < 0.01, 0.05$) than those obtained by the simultaneous administration of insulin and DM-β-CyD. These findings are in general agreement with those obtained after the rectal administration reported in our previous paper.[1] The mean values of C_{max} (17±3 μIU/ml) and AUC_{0-6} (41±12 h·μIU/ml) observed 24 h after the preadministration of DM-β-CyD were nearly equal to those (C_{max}, 11±4 μIU/ml: AUC_{0-6}, 35±14 h·μIU/ml) obtained by administration without CyD. These results suggest that the absorption-enhancing effect of CyD after nasal instillation is decreased with increasing time intervals between DM-β-CyD preadministration and insulin administration. The absorption-enhancing effect may be reduced by decreasing the amount of CyD in the absorption sites of insulin. Although the absorption of CyDs from the nasal cavity has not been confirmed, it is conceivable that CyDs could be absorbed in nasal mucosa if they act as an enhancing agent for the nasal absorption of polypeptides. On the ohter hand, the duration of the absorption-enhancing effect by CyDs is probably related to the recovery of mucosal irritation caused by these materials. The enhancing effect observed in this study is quite reversible. Consequently, the recovery of the membrane barrier function in nasal mucosa is achieved, at the latest, 24 h after the treatment with at least 30 mg of DM-β-CyD.

Comparison of Plasma Insulin Levels between Nasal and Rectal Administration of Insulin and CyD The mean values of AUC_{0-6} following the simultaneous administration of insulin and CyD in the nasal cavity were compared with those in the rectum[1] and the results are expressed in Fig. 4. In the case of a lower dose (5.2 IU) of insulin with 30 mg of DM-β-CyD, the difference in the mean AUC_{0-6} obtained by nasal and rectal administrations was not significant. However, despite the fact that the same amount of insulin was employed, the AUC_{0-6} (1123±116 h·μIU/ml) observed after nasal administration was significantly higher than that (290±42 h·μIU/ml) observed following rectal administration when a higher dose (26 IU) of insulin with 30 mg of DM-β-CyD was administered. The AUC_{0-6} increased in proportion to the dose of insulin for nasal administration, whereas this relationship was not found following rectal administration. The difference in AUC_{0-6} between nasal and rectal administration may be caused by the difference in the effectiveness of CyD on insulin absorption in the nasal mucosa or the rectal lumen. Even though a lower dose (5.2 IU) of insulin was employed, the *AUC* values obtained by nasal administration are nearly equal to those obtained after rectal administration using a higher dose (26 IU) of insulin. It seems that DM-β-CyD shows more effective absorption enhancement of insulin after nasal instillation than after rectal administration, whereas, the durations of the absorption-enhancing effects of DM-β-CyD in nasal and rectal administration are similar.

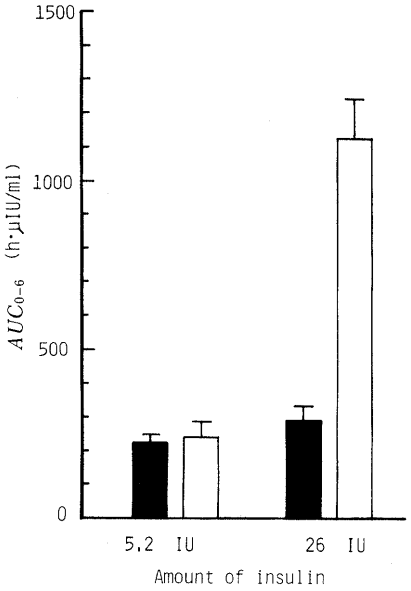

Fig. 4. Comparison of Mean Values of *AUC* between Nasal and Rectal Administration of Insulin and DM-β-CyD

Column: □, nasal administration (with 30 mg of DM-β-CyD); ■, rectal administration (with 30 mg of DM-β-CyD). Each value represents the mean±S.E. of four experiments.

In conclusion, this study demonstrates that insulin is efficiently absorbed through the nasal cavity of rabbits when aqueous preparations containing insulin and CyDs such as α- and DM-β-CyD are administered. However, the attenuation of the membrane transport barrier function is recovered, at the latest, 24 h after nasal administration of CyD. CyD is useful as a practical absorption enhancer for the nasal delivery system of polypeptide drugs.

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