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Effects of absorption promoters on insulin absorption through colon-targeted delivery

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Abstract

The aim of this study were to investigate the effect of sodium glycocholate (GC-Na) as an absorption promoter and the effects of the co-administration of GC-Na and various absorption promoters on orally administered insulin absorption utilizing a colon-targeted delivery system. The system containing insulin and GC-Na (CDS) was administered to dogs, and plasma glucose and insulin levels were then measured at 24 h after administration. For CDS, the C_{max} in plasma glucose level was significantly higher than a reference formulation without GC-Na. The pharmacological availability for CDS was not significantly higher than the reference formulation. In contrast, CDS with poly(ethylene oxide) as a gelling agent (CDSP) showed prolonged hypoglycemia effects. The pharmacological availability was 5.5% and significantly different from the reference formulation. The absolute bioavailability for CDS was 0.25%, and for CDSP it was 0.42%. Consequently, the results of this study demonstrated that colon-specific delivery of insulin with GC-Na was more effective in increasing hypoglycemic effects after oral administration, and the combination of GC-Na and poly(ethylene oxide) tended to prolong the colonic absorption of insulin and might be more effective for improvement of orally administered insulin absorption utilizing the colon-targeted delivery system.

Keywords: Absorption promoters; Colon-targeted delivery; Sodium glycocholate; Insulin; Lactulose; Enterobacteria

1. Introduction

Recently, several peptides that have strong biological activities have been discovered and many researchers have tried to utilize them as medicines for the treatment of disease. However, administrations of these peptide drugs so far have been almost exclusively limited to the parenteral route. Oral administration of such drugs, like insulin, is considered to be the most suitable route, but the problem of low bioavailability comes into play. Many kinds of approaches have been investigated in order to overcome the problem (Takeuchi et al., 2003; Radwan, 2001). For example, the co-administration of peptide drugs and various absorption promoters has been tried (Hosny et al., 2002). Generally, the peptides were easily degraded by proteases, not only in the gastrointestinal lumen, but also in the intestinal mucosa (Yamamoto et al., 1994; Tozaki et al., 1997a). Therefore, some successes were achieved through the co-administration of peptide drugs and protease inhibitors in improving the problem of low absorption in the intestine (Bai, 1995). Their permeability into the intestinal mucosa is extremely low, though, because they are highly hydrophilic materials with a high molecular weight. It has therefore been also reported that it is necessary to add some absorption promoters that increase the permeability of the intestinal mucosa in order to remedy the low absorption from the intestine (Rao and Ritschel, 1995).

A lot of colon-specific drug delivery systems as another approaches have been tried to improve the low bioavailability (Saffran et al., 1986, 1991; Kraeling and Ritschel, 1992), but, generally, the colon is not as suitable a site for drug absorption as is the small intestine. This is because the water content in the colon is much lower and there is less colonic surface area for drug absorption available (Edwards, 1997; Kimura et al., 1994). However, the colon is the preferable site for the absorption of peptide drugs because proteolytic enzyme activity in the colon, such as

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digestive enzyme and metabolic enzyme activity, is lower than in the small intestine (Langguth et al., 1997; Rubinstein et al., 1997). Therefore, many researchers have focused on the colon as a potential delivery site for peptide and protein drugs. A novel colon-targeted delivery system (CODES^{TM}), which was developed by us, that releases drug by the generation of organic acids when the lactulose is degraded by enterobacteria in the colon, was used as the model of a colon-specific drug delivery system. It has already been reported in previous studies that CODES^{TM} achieved colon-specific drug delivery in both dogs and humans (Katsuma et al., 2002, 2004; Yang et al., 2003).

In this study, insulin-containing CODES^{TM} was prepared as a model drug (Insulin-CODES). Combinations of Insulin-CODES with various absorption promoters for insulin were tried in order to try and overcome the low absorption problem. Sodium glycocholate (GC-Na, as an absorption promoter), as well as citric acid (as a solubilizer for insulin) and meglumine (as a pH adjusting agent) were incorporated into the core of the Insulin-CODES. It has been reported that GC-Na is effective in preventing the aggregation of insulin in an aqueous solution (Ritschel, 1991), and reduces the degradation of insulin when incubated in homogenates of rat large intestine. It was found that the effect in the large intestine was more remarkable than that in the small intestine (Yamamoto et al., 1994). Camostat mesilate (CM) and sodium laurylsulfate (SLS), which are protease inhibitors, disodium ethylenediaminetetraacetate (EDTA), which is a permeation enhancer and poly(ethylene oxide) (PEO), which is a water-soluble polymer, were also used as absorption promoters in this study. CM is known to inhibit the degradation of insulin in both rat caecal contents and large intestine homogenate (Yamamoto et al., 1994; Tozaki et al., 1997a). SLS is an anionic surfactant, which exhibits inhibitory behavior against both insulin aggregation in water and the activities of proteases, such as trypsin, which is contained in pancreatic juice (Fuchs and Ingelfinger, 1954; Ritschel, 1991). EDTA is known to open the tight-junctions of the intestinal mucosa by chelation with calcium ions and enhance permeability of insulin across paracellular routes in the large intestine (Uchiyama et al., 1999; Yamashita et al., 1987). PEO can form a rigid gel in water and be used as an oral sustained-release material (Sako et al., 1996).

In this study, we evaluated the hypoglycemic effects and plasma insulin level profiles after oral administration of several kinds of Insulin-CODES loaded GC-Na and various absorption promoters to fasting dogs. This was done in order to investigate the effect of GC-Na on insulin absorption through colon-targeted delivery. Furthermore, the aim of this study was to investigate the effects of combining GC-Na with other absorption promoters to try to improve orally administered insulin absorption.

2. Material and methods

2.1. Materials

Bovine insulin (Sigma Chemical Co., St. Louis, USA) was used as the model drug. Lactulose was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Sodium glycocholate was obtained from Sigma Chemical Co., Citric acid, disodium ethylenediaminetetraacetate and sodium laurylsulfate were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Eudragit® E100, Eudragit® L100 and Eudragit® RL100 (Röhm Pharm, Darmstadt, Germany) were kindly provided by Higuchi Inc. (Tokyo, Japan). Hydroxypropylmethylcellulose 2910 (TC-5E®) was obtained from Shin-etsu Chemical Industry Co., Ltd. (Tokyo, Japan) and poly(ethylene oxide) (Polyox® WSR Coagulant) was obtained from Union Carbide Japan (Tokyo, Japan). Meglumine was obtained from Merck KGaA (Darmstadt, Germany) and camostat mecilate was obtained from Shiono Chemical Co., Ltd. (Tokyo, Japan). All other chemicals and solvents were of analytical grade.

2.2. Preparation of the colon-targeted delivery system containing insulin and various absorption promoters (Insulin-CODES)

A CODES^{TM} containing insulin and various absorption promoters (Insulin-CODES) was prepared in order to evaluate the effect of co-administration on orally administered insulin absorption. Insulin-CODES contained insulin (10 mg, 50 IU/kg), lactulose (100 mg) as a trigger for colon-specific drug release, citric acid (10 mg) as a solubilizer for insulin, meglumine (30 mg) as a pH adjusting agent, and sodium glycocholate (GC-Na, 100 mg) as an absorption promoter in the core. The 30 mg of meglumine was added to neutralize the 10 mg of citric acid since it dissolves the acid-soluble coating layer of Insulin-CODES. GC-Na (100 mg) was added to the core since about 70 mg of GC-Na was reported to enhance intestinal insulin absorption in dogs (Scott-Moncrieff et al., 1994). All ingredients were thoroughly mixed with a mortar and pestle. Tableting was performed under a compression force of 250 kg/cm^2 using an oil pressure jack (Sanki Industry, Tokyo, Japan). It was confirmed that the tablet core would dissolve in 1 mL of water.

First, the tablet cores were coated with the acid-soluble coating material, Eudragit® E100. A coating solution was prepared by dissolving 9% (w/w) Eudragit® E100 and 1% (w/w) Eudragit® RL100 in methanol. Coating was performed using a coating machine (Hi-coater HT-30; Freund Industrial Co., Ltd., Shizuoka, Japan) under the following conditions: spray air pressure, 1.4 kg/cm^2; spray solution feed, 10 g/min; inlet temperature, 60 °C; outlet temperature, 30–32 °C; pan rotation speed, 12 rpm. The amount of coating was 12 mg per tablet core. Second, the tablets were coated with water-soluble coating material, TC-5E® as an undercoating. A coating solution was prepared by dissolving 10% (w/w) TC-5E® in water. Coating was performed with the same coating machine under the following conditions: spray air pressure, 1.4 kg/cm^2; spray solution feed, 10 g/min; inlet temperature, 60 °C; outlet temperature, 35–40 °C; pan rotation speed, 12 rpm. The amount of coating was 3.5 mg per tablet core. Finally, the tablets were coated with enteric coating material, Eudragit® L100. A coating solution was prepared by dissolving 10% (w/w) Eudragit® L100 and 2% (w/w) castor oil in methanol. Coating was performed with the same coating machine under the following conditions: spray air pressure, 1.4 kg/cm^2; spray solution feed, 10 g/min; inlet temperature, 52 °C; outlet temperature, 30–35 °C; rotating speed of

pan, 12 rpm. The amount of coating was 15 mg per tablet core. In the following pages, CDS is used as an abbreviation for Insulin-CODES containing only GC-Na as absorption promoter.

Additionally, several kinds of Insulin-CODES tablets were prepared in order to evaluate the effects of co-administration of GC-Na and other absorption promoters for oral insulin absorption. CDSSC was loaded with sodium laurylsulfate (30 mg) and camostat mecilate (5 mg) in the tablet core of CDS as protease inhibitors. The amounts of these substances were selected because, based on previous reports (Tozaki et al., 1997a, 2001), they should be effective promoters at these levels. Disodium ethylenediaminetetraacetate (EDTA-Na_2, 50 mg) was loaded into the tablet core of CDS to make CDSE. This was done because the presence of 20 mM EDTA reportedly enhances the permeability of 0.5 mM insulin significantly in the colonic membrane (Uchiyama et al., 1999). CDSP tablets were made containing 10 mg of poly(ethylene oxide) for sustained-release in the tablet core. The dissolution time for the uncoated CDSP tablet core in pH 6.8 phosphate buffer solution was twice as long as that for the CDS core (data not shown). Insulin-CODES without absorption promoters (CD) was also prepared as a reference formulation.

2.3. In vivo oral administration of Insulin-CODES to dogs

Three male beagle dogs (weighing 10–15 kg) were fasted for 12 h prior to and during the experiment. Water was allowed ad libitum during the experiments. After oral administration of insulin bulk (50 IU/kg) and Insulin-CODES (50 IU/kg) with 30 mL of water, 5 mL blood samples were obtained using a heparinized syringe before and 1, 2, 4, 6, 8, 10, 12 and 24 h after drug administration. Plasma was immediately separated by centrifugation at 3000 rpm for 15 min. Insulin absorption was evaluated by its hypoglycemic effect and plasma insulin levels.

2.4. Intravenous administration of insulin to dogs

Three male beagle dogs (weighing 10–15 kg) were fasted for 12 h prior to and during the experiment. Water was allowed ad libitum during the experiments. After intravenous administration of aqueous insulin solution (10 IU/mL; 1 IU/kg) into a vein of a forepaw, 5 mL blood samples were obtained from the vein of another forepaw using a heparinized syringe before and 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h after drug administration. Plasma was immediately separated by centrifugation at 3000 rpm for 15 min. The plasma glucose and insulin levels were measured to evaluate its pharmacological availability and pharmacokinetic availability.

2.5. Measurement of plasma glucose level

The glucose levels in the plasma were determined by a glucose oxidase method using the Glucose CII test (Wako Pure Chemical Industries, Osaka, Japan). The percent plasma glucose levels (%) were expressed as percentages of the initial plasma glucose levels. Area under the curve (AUC)-Glu (% response-time) was calculated from the total AUC of the percentage plasma glucose-time profile. The pharmacological availability (PA%) was calculated using a method similar to that described by Ritschel et al. (1988) in the following equation:

$$\text{Pharmacologic availability (\%)} = \frac{\text{AUC-Glu (\% reponse time)}_{\text{p.o.}}}{\text{AUC-Glu (\% reponse time)}_{\text{i.v.}}} \times \frac{\text{Dose}_{\text{i.v.}}}{\text{Dose}_{\text{p.o.}}}$$

The maximum reduction in the percent glucose levels (C_{max}-Glu) and the mean residence time for the reduction of percent glucose level (MRT-Glu) were also calculated using a method similar to that described by Ritschel et al. (1988).

2.6. Measurement of plasma insulin level

The insulin levels in the plasma were determined using an enzyme immunoassay kit (Glazyme Insulin-EIA Test; Wako Pure Chemical Industries, Osaka, Japan). The area under the plasma insulin-time curve (AUC-Insulin), the maximum insulin levels (C_{max}-Inslin), the time when the maximum insulin level was observed (T_{max}-Insulin) and the mean residence time for the insulin level (MRT-Insulin) were calculated using a method similar to that described by Ritschel et al. (1988).

2.7. Statistical analyses

Results are expressed as the mean ± standard deviation (S.D.). Statistical analyses were performed using the Student's *t*-test.

3. Results

3.1. Effect of GC-Na on insulin absorption through colon-targeted delivery

In order to investigate the effect of GC-Na on insulin absorption through colon-targeted delivery, a colon-targeted delivery system containing insulin (50 IU/kg) and GC-Na as an absorption promoter in the tablet core (CDS) was prepared along with a system without absorption promoters (CD) as a reference formulation. Glucose level profiles and insulin level profiles in the plasma were evaluated after oral administration to fasting dogs. Levels after intravenous administration of insulin aqueous solution (1 IU/kg) were also evaluated. The initial value was considered the 100% level and all following level-time data recorded were expressed as a percent of the initial value. These values denote a percent pharmacological response. Fig. 1 shows these hypoglycemic effect-time curves and Table 1 shows pharmacodynamic parameters closely related to the pharmacological responses, AUC-Glu, C_{max}-Glu, T_{max}-Glu and MRT-Glu. Fig. 2 shows plasma insulin level profiles after the oral administration. Table 2 shows the pharmacokinetic parameters. All data were presented as the mean ± S.D. When insulin aqueous solution was intravenously administered, T_{max}-Glu was 1.1 h and C_{max}-Glu was 72.3%. The hypoglycemic effect appeared rapidly after the intravenous administration and almost disappeared 5 h later.

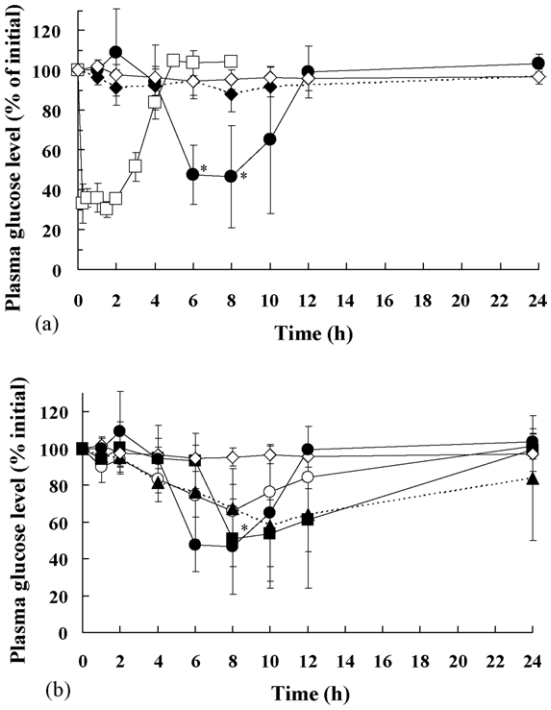

Fig. 1. Plasma glucose level profiles after i.v. and oral administration of insulin to beagle dogs under fasting conditions. Treatment: (a) (□) i.v. of insulin solution (insulin, 1 IU/kg), (♦) p.o. of insulin bulk (insulin, 50 IU/kg), (◊) p.o. of CD (insulin, 50 IU/kg + lactulose, 100 mg + citric acid, 10 mg + meglumine, 30 mg), (●) p.o. of CDS (CD + sodium glycocholate, 100 mg), (b) (○) p.o. of CDSSC (CDS + sodium laurylsulfate, 30 mg + camostat mecilate, 5 mg), (■) p.o. of CDSE (CDS + disodium ethylenediaminetetraacetate, 50 mg), (▲) p.o. of CDSP (CDS + poly(ethylene oxide), 10 mg). Each point represents the mean ± S.D. of three experiments. Asterisk (*) indicates $P < 0.05$, compared with the administration of CD.

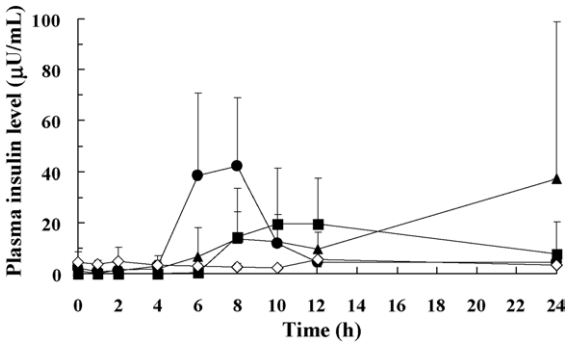

Fig. 2. Plasma insulin level profiles after oral administration of insulin to beagle dogs under fasting conditions. Formulation: (◊) CD (insulin, 50 IU/kg + lactulose, 100 mg + citric acid, 10 mg + meglumine, 30 mg), (●) CDS (CD + sodium glycocholate, 100 mg), (■) CDSE (CDS + disodium ethylenediaminetetraacetate, 50 mg), (▲) CDSP (CDS + poly(ethylene oxide), 10 mg). Each point represents the mean ± S.D. of three experiments.

When insulin bulk and CD were orally administered as reference formulations, the C_{max}-Glus were 12.1 ± 8.4 and 7.8 ± 5.1% and the AUC-Glus were 159.7 ± 111.8 and 90.3 ± 85.5% h, respectively, and few hypoglycemic effects were observed. The pharmacologic availabilities were 1.4 ± 0.1 and 0.8 ± 0.8%, and there was no significant difference between the PA of either formulation. In contrast, when Insulin-CODES with GC-Na as an absorption promoter (CDS) was orally administered, the hypoglycemic effects appeared drastically at 6 h after oral administration and were sustained for 12 h (Fig. 1(a)). The C_{max}-Glu was 59.9 ± 14.9%. This increase was statistically significant compared to the oral administration of CD. The AUC-Glu was also 352.0 ± 192.3% h, and the PA was 3.2 ± 1.7%. CDS induced an approximately four-fold increase in the hypoglycemic effect compared with that of CD. However, the increase was not significantly different.

Table 1
Pharmacodynamic parameters on the basis of plasma glucose level after i.v. and oral administration of insulin via the CODES^{TM} tablets (mean ± S.D., $n = 3$)

Treatment	Dose (U/kg)	C_{max}-Glu (% gluc. red.)	T_{max}-Glu (h)	AUC-Glu (% h)	MRT-Glu (h)	PA (%)
Control i.v.	1	72.3 ± 1.8	1.1 ± 0.7	221.1 ± 11.1	1.9 ± 0.1	–
Bulk p.o.	50	12.1 ± 8.4	13.3 ± 9.2	159.7 ± 111.8	11.3 ± 4.0	1.4 ± 1.0
CD (Insulin-CODES^{TM})	50	7.8 ± 5.1	12.7 ± 9.9	90.3 ± 85.5	15.0 ± 6.2	0.8 ± 0.8
CDS (CD + GC-Na)	50	59.9 ± 14.9**	6.7 ± 1.2	352.0 ± 192.3	7.6 ± 0.9	3.2 ± 1.7
CDSSC (CDS + SLS + Camostat)	50	34.5 ± 14.3*	7.3 ± 1.2	343.8 ± 103.2	9.2 ± 0.8	3.1 ± 0.9
CDSE (CDS + EDTA)	50	55.7 ± 14.8**	9.3 ± 2.3	547.5 ± 362.5*	10.3 ± 1.6	5.0 ± 3.3*
CDSP (CDS + PEO)	50	59.6 ± 4.3**	14.7 ± 8.1	608.2 ± 41.4*	12.5 ± 4.6	5.4 ± 0.4*

* $P < 0.05$, compared with the administration of CD.
** $P < 0.01$, compared with the administration of CD.

Table 2
Pharmacokinetic parameters on the basis of plasma insulin level after i.v. and oral administration of insulin via the CODES^{TM} tablets (mean ± S.D., $n = 3$)

Treatment	Dose (U/kg)	C_{max}-Insulin (μU/mL)	T_{max}-Insulin (h)	AUC-Insulin (μU h/mL)	MRT-Insulin (h)	BA (%)
Control i.v.	1	–	–	1465.1 ± 293.0	0.5 ± 0.1	–
CD (Insulin-CODES^{TM})	50	7.1 ± 4.0	5.0 ± 6.1	92.8 ± 39.6	12.2 ± 2.0	0.13 ± 0.05
CDS (CD + GC-Na)	50	49.0 ± 25.2	6.7 ± 1.2	249.9 ± 121.9	8.6 ± 2.7	0.34 ± 0.17
CDSE (CDS + EDTA)	50	27.2 ± 15.2	10.0 ± 2.0	254.1 ± 197.9	11.7 ± 3.7	0.35 ± 0.27
CDSP (CDS + PEO)	50	54.0 ± 48.2	14.7 ± 8.3	363.4 ± 304.9	14.3 ± 7.8	0.50 ± 0.42

Fig. 2 shows plasma insulin level profiles after oral administration of the formulations to fasting dogs. When CD was orally administered, plasma insulin levels did not change markedly. In contrast, when CDS was orally administered, plasma insulin level increased drastically at 6 h after the oral administration. C_{max}-insulin for CDS was 49.0 ± 25.2 μU/mL, AUC-insulin was 249.9 ± 121.9 μU h/mL and BA was 0.34 ± 0.17%. Although there were no significant differences between pharmacokinetic parameters for CD and CDS, the BA for CDS was two times higher than the one for CD. These BA results were as good as those for PA.

3.2. Effect of co-administration of GC-Na and various absorption promoters on orally administered insulin absorption utilizing a colon-targeted delivery system

Next, the effects of the co-administration of various absorption promoters on orally administered insulin absorption utilizing the colon-targeted delivery system were investigated. Fig. 1(b) shows plasma glucose level-time curves after oral administration of Insulin-CODES with GC-Na, and various absorption promoters. When camostat mesilate and sodium laurylsulfate were added to CDS as protease inhibitors (CDSSC), and the tablet was orally administered to fasted dogs, no drastic hypoglycemic effects were observed and the plasma glucose level decreased gradually after 4 h. The hypoglycemic effect was sustained for 12 h. Although the C_{max}-Glu after oral administration of CDSSC was 34.5 ± 14.3% and the increase was statistically significant compared with CD, it was smaller than that for CDS (Table 1). The AUC-Glu (343.8 ± 103.2% h) and PA value (3.1 ± 0.9%) were almost similar to those for CDS.

EDTA-Na_2 was then loaded into the tablet core of CDS (CDSE) in order to enhance the permeability of insulin, and the CDSE tablets were orally administered to fasted dogs. Dramatic hypoglycemic effects appeared at 8 h after oral administration and were sustained for 12 h (Fig. 1(b)). As shown in Table 1, the C_{max}-Glu (55.7 ± 14.8%), the AUC-Glu (547.5 ± 362.5% h) and the PA (5.0 ± 3.3%) after oral administration of CDSE were significantly larger than those for CD. The C_{max}-Glu for CDSE was not higher than that for CDS, but the AUC-Glu and the PA for CDSE were higher than those for CDS. However, there differences between them were not significant. Table 2 shows pharmacokinetic parameters based on the plasma insulin level profile after the oral administration of CDSE. The C_{max}-insulin for CDSE was 27.2 ± 15.2 μU/mL, the AUC-insulin was 254.1 ± 197.9 μU h/mL and the BA was 0.35 ± 0.27%. The BA as well as the PA for CDSE was two times higher than the one for CD. However, they were not significantly different from those for CD and CDS.

Additionally, CDS was prepared with a water-soluble polymer, poly(ethylene oxide) (CDSP) for sustained-release and orally administered to fasted dogs. As shown in (Fig. 1(b)), the plasma glucose level profile for CDSP was different from that for CDS. Although the C_{max}-Glu for CDSP (59.6 ± 4.3%) was almost similar to those of CDS and CDSE, the AUC-Glu and the PA for CDSP were 608.2 ± 41.4% h and 5.4 ± 0.4%, respectively, and the greatest hyperglycemic effect was observed (Table 1). The increase in the C_{max}-Glu, the AUC-Glu and the PA were statistically significant compared with CD. However, they were not statistically significant when compared with the other formulations. As shown in Table 2 and Fig. 2, the plasma insulin level profile and PK parameters after the oral administration of CDSP correlated well with the plasma glucose level profile and pharmacodynamic parameter. The AUC-Insulin for CDSP was 363.4 ± 304.9 μU h/mL and the absolute BA (0.50 ± 0.42%) was three times higher than that for the CD. These PK parameters, as well as the pharmacodynamic parameters calculated on the basis of the observed hyperglycemic effects were not significantly different from those for the other formulations. CDSP decreased the plasma glucose levels between 6 and 24 h after oral administration and prolonged the pharmacological response compared to CDS (Fig. 1(b)). The MRT-Glu [12.5 ± 4.6 h (range 9.2–17.7)] for CDSP was longer than that for CDS [7.6 ± 0.9 h (range 6.7–8.5)] in all of dogs. However, the parameter was not statistically significant when compared with CDS.

4. Discussion

We found that the hypoglycemic effects appeared drastically after oral administration of a colon-targeted delivery system containing insulin (50 IU/kg) with GC-Na as an absorption promoter (CDS), although few hypoglycemic effects were observed after oral administration of the system without absorption promoters (CD). GC-Na is known to inhibit the degradation of insulin in large intestine homogenate (Yamamoto et al., 1994), and the aggregation of insulin in water (Fuchs and Ingelfinger, 1954; Ritschel, 1991). Therefore, we consider that the hypoglycemic effects for CDS could be increased by absorption-enhancing action of GC-Na. Tozaki et al. (1997b) reported that when insulin solution was administered orally to rats, the PA was less than 1%. When chitosan capsules, which were developed to deliver drugs colon-specifically, containing only insulin or containing insulin and GC-Na were administered orally, the PAs were 1.6 and 3.5%, respectively. Their results were almost consistent with our results. Thus, our findings suggested that although delivery of only insulin to the colon did not improve insulin absorption when orally administered, colon-specific delivery of insulin with GC-Na was more effective in increasing hypoglycemic effects after oral administration.

It was reported that a colon-specific drug delivery system coated with an azo-polymer, which contains insulin with camostat mesilate, could improve low absorption in the colon (Tozaki et al., 2001). However, when CM and sodium laurylsulfate were added to CDS as protease inhibitors (CDSSC), the increase in the hypoglycemic effect was not observable with the addition of CM and SLS to CDS in this study. It was also reported that CM could inhibit the degradation of insulin in large intestine homogenate, just like GC-Na (Yamamoto et al., 1994), and SLS could inhibit both insulin aggregation in water and the activities of proteases as well as GC-Na (Fuchs and Ingelfinger, 1954; Ritschel, 1991). Because of this, it might be perceived that CDSSC does not have a synergistic effect on the improvement of orally administered insulin absorption compared with CDS.

It is known that peptides have a very low permeability with respect to colonic mucosal membranes because of their hydrophilic nature and large molecular weight. The addition of a permeation enhancer, such as EDTA improved colonic absorption (Uchiyama et al., 1999; Yamashita et al., 1987). We found that the PA for CDSE added EDTA to CDS was higher than that for CDS. Yamashita et al. (1987) reported that EDTA could open the tight-junctions of the intestinal mucosa by chelation with calcium ions, and enhance permeability of paracellular routes in the intestine. Based on these findings, we consider that co-administration of EDTA might have the potential to exhibit a synergistic effect on the improvement of orally administered insulin absorption in this study because EDTA had a different mechanism of action from other absorption promoters, such as GC-Na. However, the difference between the hypoglycemic effects of CDS and CDSE was not significant in this study. The decrement of plasma glucose levels started 8 h after oral administration of CDSE and were relatively slower than the rates of the others (Fig. 1(b)). The reason is not fully understood, but it may be due to fluctuation in gastrointestinal transit time to the colon. We reported in previous papers that the onset of colonic absorption in fasting dogs was 5.5 ± 1.9 h (range 4–8 h, $n = 6$) (Yang et al., 2003). The delayed onset of the effect of the CODES^{TM} tablet in this study may be within a predictable range. Although one of three dogs showed a prolonged hypoglycemic effect between 8 and 24 h post-dose, the PAs of the other dogs could not be precisely evaluated without points between 12 and 24 h. Thus, the results in this study could not obviously demonstrate that the addition of EDTA might lead to improve the colonic absorption of insulin.

To prolong the colonic absorption, CDS was prepared with a water-soluble polymer, poly(ethylene oxide) (CDSP). CDSP decreased the plasma glucose levels between 6 and 24 h after oral administration and prolonged the pharmacological response compared to CDS (Fig. 1(b)). As a result, the greatest hyperglycemic effect was observed (Table 1). It was observed that the T_{max}-Glu and the MRT-Glu of all dogs after dosing with CDSP were larger than those for CDS. We consider that the hypoglycemic effects for CDSP could be prolonged by sustained-release action of PEO. One of three dogs that was administered CDSP exhibited an outlying high hypoglycemic effect 24 h post-dose. Insulin first appeared in the plasma 9 h post-dose, and a prolonged pharmacological effect was observed up to 24 h post-dose. This may have been due to the arrival to the colon of the CODES^{TM} tablet being delayed. We reported in previous papers that the onset of colonic absorption in fed dogs was about 9 h post-dose (Katsuma et al., 2002; Yang et al., 2003). The delayed drug absorption profile in this study agreed with these findings. Therefore, the stomach in the dog may have changed from being in a fasting condition to a fed condition because of some factor, possibly coprophagy, and the gastric emptying of the CODES^{TM} tablet may have been delayed. These results demonstrated that addition of PEO to the CODES^{TM} tablet core tended to prolong the colonic absorption of insulin delivered to the colon, and combination of GC-Na and PEO might be effective for improving the PA after oral administration of insulin. Kimura et al. (1999) reported that 5-aminosalicylic acid, which was used to directly treat inflammatory bowel disease, was microencapsulated with a water-insoluble polymer, ethylcellulose and encased in a pressure-controlled colon delivery capsule. Their findings suggest that it is possible to achieve sustained release in the large intestine. Few studies on sustained-release in the colon from a colon-targeted delivery system, however, have been investigated so far. Therefore, more formulation studies on sustained-release by CODES^{TM} need to be done in order to obtain sufficient insulin absorption when orally administered.

In this study we tried to improve orally administered insulin absorption by delivery of insulin with GC-Na to the colon using CODES^{TM}. Additionally, we investigated the effects of the co-administration of GC-Na and various absorption promoters on improvement of orally administered insulin absorption. The BAs observed in this study were not satisfactory, but they agreed with the data Cheng et al. (1994) reported. Bendayan et al. (1994) reported that when insulin was co-administered with sodium cholate and aprotinin to normal and diabetic rats, the plasma insulin level and hyperglycemic effects in diabetic rats were significantly higher than those in normal rats. Saffran et al. (1991) conducted the same studies in diabetic dogs. When insulin was loaded into a colon-targeted delivery system with 5-methoxy salicylate as an absorption promoter and administered orally to diabetic dogs, the BA was significantly higher compared with normal dogs. Therefore, when administering CODES^{TM} containing both insulin and absorption promoters to diabetic dogs, the effects of the combination of GC-Na and various absorption promoters might have been more evident. It has been also reported that it is necessary to add some absorption promoters that increase the permeability of the intestinal mucosa in order to remedy the low absorption of insulin from the intestine (Rao and Ritschel, 1995). But, a major limiting factor in the application of the permeability enhancer approach is the potential toxicity of the enhancers themselves (Swenson et al., 1994). Although no histological studies that evaluate the mucosal toxicity of the absorption promoters have been carried out, no clinical signs that were suggestive of gastrointestinal disturbance, such as vomiting or diarrhea were observed in this study. Therefore, it might be possible to further improve insulin absorption by increasing the amount of absorption promoter. A chelating agent, EDTA, is known to enhance absorption to the intestinal wall, but is highly toxic (Uchiyama et al., 1999). In contrast, GC-Na has a reportedly high absorption enhancing effect with low intestinal toxicity. Increasing amounts of GC-Na in the CODES^{TM} tablet could lead to further enhancement of the insulin absorption. However, more formulation work needs to be done in order to obtain sufficient orally administered absorption of peptides, such as insulin. In conclusion, the results of this study demonstrated that the absorption of insulin, when orally administered, could be improved by a combination of the colon-targeted delivery system, CODES^{TM} and GC-Na, which inhibited not only insulin aggregation in the colonic lumen, but also insulin degradation in the colonic mucosa. They also demonstrated that the combination of GC-Na and PEO as an oral sustained-release material lead to prolong the colonic absorption of insulin through colon-targeted delivery and might be more effective for improvement of the pharmacological effect.

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