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New Mucosal Dosage Form of Insulin[1,2]

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The present study was intended to develop a new oral mucosal dosage form with a view to solving the problems of the administration of insulin by injections. We prepared a potentially suitable dosage form and tested it in beagle dogs.

The new oral mucosal dosage form of insulin consists of the core-base, which contains cacao butter, insulin and additive, and the peripheral-base, which contains a mixture of hydroxypropyl cellulose-H (HPC) and Carbopol-934 (CP). The suitable mixing ratio of HPC and CP in the peripheral-base was chosen as 1 : 2 on the basis of experimental results concerning the stickiness, dissolution properties, viscosity and fracture resistance. This dosage form could stick tightly to the oral mucosa of beagle dogs for 6 hr.

The change of blood sugar and plasma insulin levels in beagle dogs was investigated by the three-way crossover method with group A as a reference, group B given insulin alone and group C given insulin and sodium glycocholate. Absorption of insulin was recognized only in group C, indicating that sodium glycocholate effectively promoted the absorption of insulin from the oral mucosa. The percentage absorbed of insulin in this dosage form was about 0.5% compared with the amount absorbed upon intramuscular injection of insulin.

Keywords——insulin; mucosal dosage form; hydroxypropyl cellulose; Carbopol-934; mucosal absorption; beagle dogs; blood sugar level; plasma insulin level; enzyme immunoassay

Although the need for insulin for the therapy of diabetes is increasing, it is still administered in the form of an injection. However, the use of injections throughout the life of a patient presents various problems, such as physical and psychic pain, hypertrophy or atrophy of the subcutaneous fat at an injection site and occasionally allergy over the whole body.

Attempts have been made to prepare dosage forms of insulin other than the injection,[3–10] but satisfactory results have never been obtained. In the present study, we attempted to prepare a new oral mucosal dosage form of insulin with a view to solving the above problems of the injection.

The oral mucosal administration has the advantage that there is no "first-pass effect" for the drug, as in the case of rectal absorption. Further, the application is easy and convenient compared with the suppository.

Following a previous study concerning the topical dosage form for *carcinoma colli* consisting of hydroxypropyl cellulose and Carbopol-934,[11] the present study was carried out to investigate both the basic problems in preparing the mucosal dosage form and the application in beagle dogs.

Experimental

Materials——Hydroxypropyl cellulose-H (HPC), Carbopol-934 (CP) and cacao butter J.P.IX used were commercial products. Sodium glycocholate was supplied by Tokyo Kasei Chemical Ind., Ltd. Insulin (26.8 I.U./mg) was from Sigma Chemical Co., Ltd., "Glucose B-Test Wako" from Wako Pure Chemical Co., Ltd., and "Insulotec Mochida" from Mochida Pharmaceutical Co., Ltd.

Preparation of the New Mucosal Dosage Form of Insulin——A schematic illustration of the new oral mucosal dosage form is shown in Fig. 1(a); it was prepared as follows. Cacao butter of the core-base was melted at 37° in a beaker, then insulin and sodium glycocholate were added and mixed sufficiently, and the whole was allowed to solidify at room temperature overnight. Fifty milligrams of the solidified mass was

compressed into a cylindrical form of 5 mm diameter with 1 mm thickness to make the core. The core was covered with peripheral-base consisting of 150 mg of a mixture of HPC and CP, and compressed directly to make a surface with a radius of 8 mm curvature under 200 kg/cm² for 30 sec using a Shimadzu evacuable die and hydraulic press designed to produce KBr tablets for infrared spectroscopy.

Basic Examination of the Dosage Form——1) Stickiness Test: The tablet prepared by compressing 200 mg of the mixture of HPC and CP in a mixing ratio of 3: 1, 2: 1, 1: 1 or 1: 2 was stuck to the mouse peritoneal membrane,[12] and the sticking force (g) was measured after 10 minutes by means of the stickiness test apparatus shown in Fig. 2.

2) Dissolution Test of Peripheral-base: Tablets consisting of 100 mg of HPC and CP in a mixing ratio of 100: 0 to 0: 100 containing 5.00 mg of insulin,[13] which had been compressed directly under 200 kg/cm² for 30 sec, were tested in 1/15 M phosphate buffer (pH 7.38) at 37±0.2° at 100 rpm in the apparatus shown in Fig. 3. The concentration of insulin was determined according to the Lowry–Folin method using a Hitachi 124 spectrophotometer. The 63.2% dissolution time was calculated according to the Weibull distribution.[14]

3) Viscosity of the Solution of Peripheral-base: A 1% solution in 1/15 M phosphate buffer (pH 7.38) of HPC and CP in the same mixing ratio as in the dissolution test was tested in a B-type viscometer (Tokyo Keiki) at 37±0.5°.

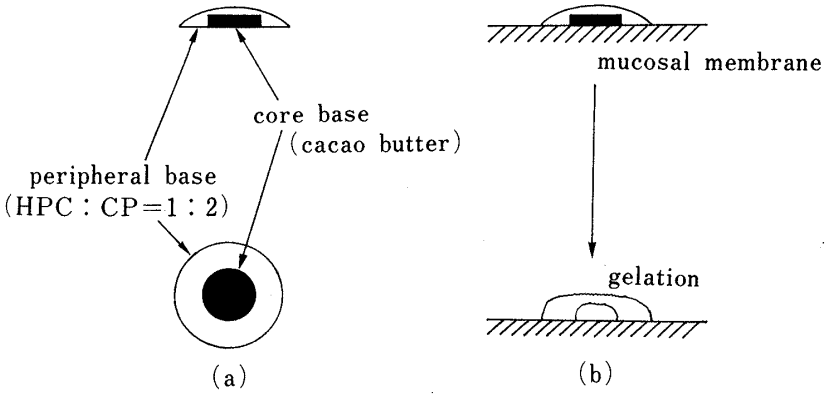

Fig. 1. Schematic Illustration of the New Mucosal Dosage Form of Insulin

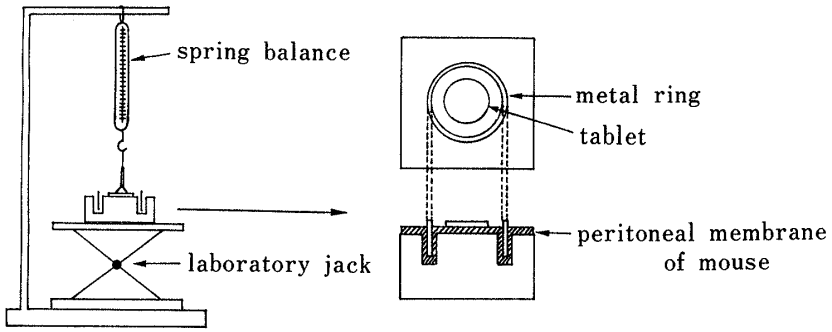

Fig. 2. Schematic Illustration of Stickiness Test Apparatus

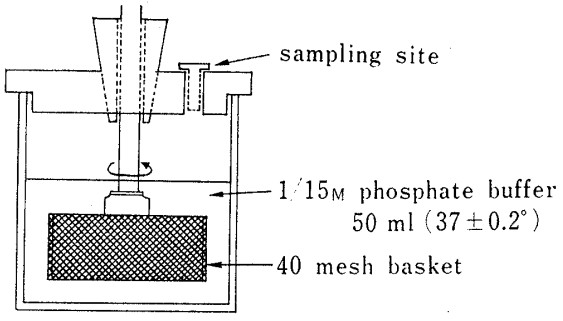

Fig. 3. Schematic Illustration of Dissolution Test Apparatus

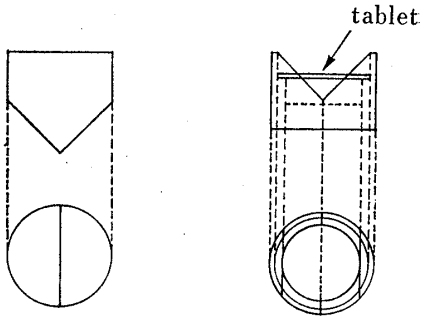

Fig. 4. The Hardness Tester Accessory used in the Fracture Resistance Test

4) Fracture Resistance Test of the Peripheral-base:[15] Tablets of HPC and CP in the same mixing ratio as in the dissolution test were tested in a Kiya hardness tester using the specially[16] designed accessory shown in Fig. 4.

Application of the Dosage Form to the Mucosal Membrane of Beagle Dogs——The new mucosal dosage form of insulin (Table I) was examined in nine beagle dogs (two male and seven female beagle dogs weighing 9 to 12 kg, fasted previously for 24 hr) by the three-way crossover method, with group A as a reference, group B given insulin alone and group C given insulin and sodium glycocholate. The mixing ratio of 1:2 of HPC and CP was selected on the basis of the results obtained by the basic pharmaceutical examination of the dosage form mentioned above, as will be described later in detail. Before this *in vivo* experiment, in order to exclude the effect of stress to the dog by the application, a tablet containing no insulin (group A) was stuck to the oral mucosa for 6 hr every day for 4 days. Blood samples were obtained from a forefoot vein at 0, 0.5, 1, 2, 3, 4 and 6 hr after the application.

Table I. Mucosal Dosage Forms of Insulin tested in the Present Experiment

Group	Core-base			Peripheral-base
	Cacao butter (mg)	Insulin[b] (mg)	Na-glycocholate (mg)	HPC: CP (1: 2) (mg)
A[a]	50	—	—	150
B	40	10	—	150
C	35	10	5	150

a) Reference.
b) 26.8 I.U./mg, bovine crystalline insulin, Sigma Chem. Co.

Determination of Blood Sugar Level and Plasma Insulin Level——Blood sugar level was determined by an enzymatic method using glucose-oxidase and peroxidase.[17] Plasma insulin level was determined by enzyme immunoassay using insolubilized anti-insulin antibody and enzyme-labeled anti-insulin antibody.[18]

Determination of Plasma Concentration Curve after Intramuscular Administration of Insulin——To determine the relative bioavailability of insulin from the new oral mucosal dosage form in comparison with that from intramuscular administration, the area under the curve (AUC) of plasma insulin levels in beagle dogs for 6 hr after the injection was obtained. The injection was prepared according to the preparative method of insulin injection in J.P.IX, with a dose of 2.68 I.U./kg, which is one-tenth of the dose used for mucosal administration.

Results and Discussion

Basic Pharmaceutical Examination of the Dosage Form

In a preliminary experiment, a compressed disk similar to the topical dosage form reported in the previous paper,[11] which consisted of HPC, CP and insulin, was applied to the oral mucosa in beagle dogs. No absorption of insulin was found, possibly for the following two reasons: (1) Only a very small amount of insulin reached the membrane because insulin was released in all directions and the part released into saliva was swallowed and inactivated. (2) HPC and CP seemed to be unsuitable as the base materials for mucosal absorption of insulin. On the other hand, it has been reported that insulin is absorbed from cacao butter suppositories through the rectal membrane.[19] Thus, the dosage form shown in Fig. 1 was designed in this study.

In order to find a suitable mixing ratio of HPC and CP for use as the peripheral-base, the stickiness to the mucosal membrane, dissolution properties, viscosity of the solution and fracture resistance were investigated. No clear relationship was found between the mixing ratio of HPC and CP and the stickiness to the mucosal membrane, suggesting that the stickiness was related to the content of moisture on the membrane. The tablet did not stick to a very moist membrane, but stuck tightly to one with little moisture. If the moisture level was satisfactory, however, a tablet containing more CP usually stuck more tightly to the mucosal membrane for a longer time period. Thus, in order to stick this tablet to the oral mucosa, it was necessary to wipe away the saliva with absorbent cotton.

In the dissolution test, as shown in Fig. 5, it was recognized that the dissolution was delayed as the concentration of CP was increased. It was recognized that the viscosity increased with increase in the concentration of CP, as shown in Fig. 6, in the same way as in the dissolution test. Thus, it was assumed that as the concentration of CP was increased, the tablet would stick more tightly and for a longer time.

In the fracture resistance test, as shown in Fig. 7, it was found that the greater the concentration of CP, the greater the fracture resistance. The desired characteristics of this dosage form are: 1) ability to stick to the oral mucosa; 2) ability to protect the cacao butter in the core; and 3) susceptibility to gelation of the peripheral-base, followed by gradual dissolution and elimination of the tablet by the saliva. Thus, the most suitable mixing ratio of HPC and CP was chosen as 1: 2. Tablets of this mixing ratio in peripheral-base were subjected to *in vivo* examination in beagle dogs. However, the mixing ratio of HPC and CP can be adjusted according to the application and the kind of drug.

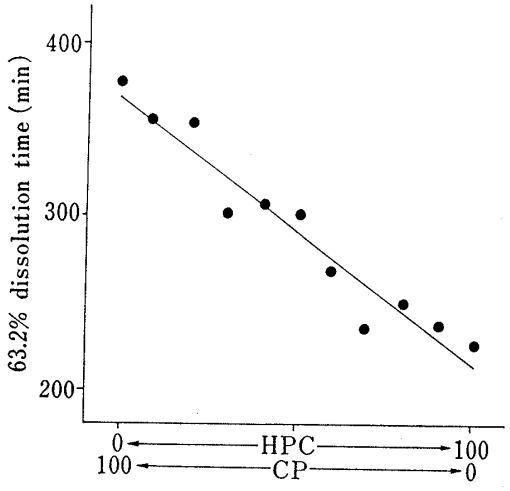

Fig. 5. Relationship between HPC–CP Mixing Ratio and 63.2% Dissolution Time according to the Weibull Distribution

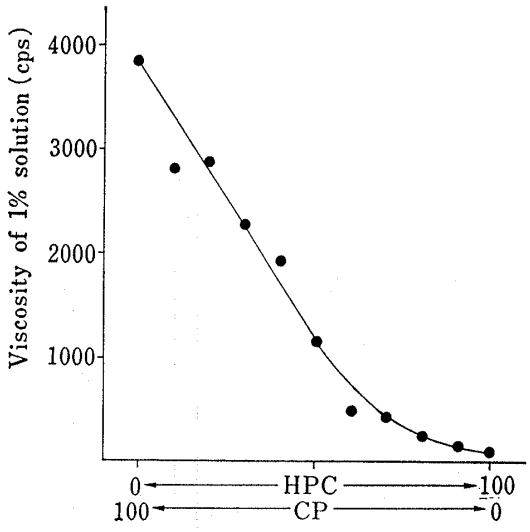

Fig. 6. Relationship between HPC–CP Mixing Ratio and the Viscosity of 1% Solution in 1/15 M Phosphate Buffer at $37 \pm 0.5°$

Figures 8 and 9 show the tablet with the peripheral-base of HPC and CP (1: 2) in the initial state and at 6 hr respectively, after mucosal application in a beagle dog. The tablet remained stuck in place in a swollen state for 6 hr, but had disappeared completely after 12 hr due to gelation by the saliva.

Change of Blood Sugar Levels

Figure 10 shows the change of blood sugar levels after mucosal administration of the new mucosal dosage form of insulin in beagle dogs. The fact that the blood level did not change for 6 hr in group A (the reference) indicated that there was no effect of the stress accompanying the application of the dosage form. There was also no change of blood sugar level in group B, which contains insulin alone, and this indicated that insulin could not permeate through the oral mucosa. This result is consistent with other reports.[20,21] On the other hand, the blood level in group C was significantly different from those in the other two groups according to the *t*-test ($p<0.05$) at 0.5, 1 and 2 hr. The decrease of the blood level in group C was about 40% after 1 hr. This result shows that insulin could permeate through the oral mucosa in the presence of sodium glycocholate. However, the amount of insulin absorbed was not satisfactory compared with the dose of insulin (26.8 I.U./kg). In the nasal absorption of insulin reported by Hirai *et al.*,[22] the decrease of blood sugar level

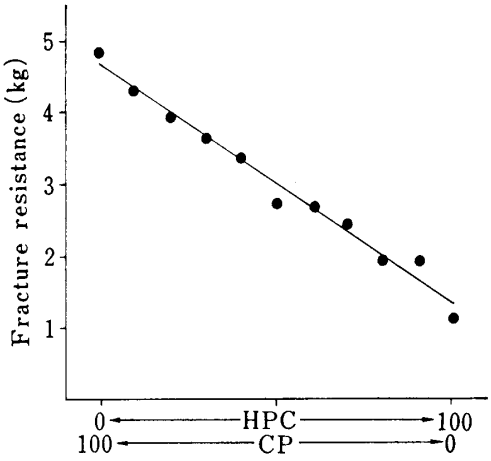

Fig. 7. Relationship between HPC-CP Mixing Ratio and Fracture Resistance against Vertical Pressure

Fig. 8. The New Mucosal Dosage Form stuck to the Oral Mucosa in a Beagle Dog

Fig. 9. The Swollen Tablet stuck tightly at 6 hr after Mucosal Administration

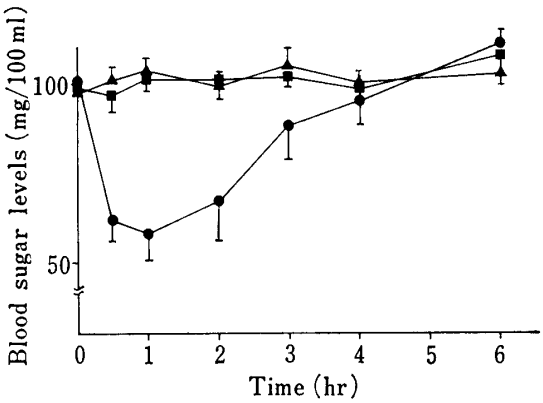

Fig. 10. Change of Blood Sugar Levels after Mucosal Administration of the New Mucosal Dosage Form of Insulin in Beagle Dogs

Each point represents the mean ± S.E. of 9 determinations.
-▲-: group A, -■-: group B, -●-: group C.

was about 75% at 1.5 hr and about 25% at 6 hr with 50 I.U./dog. In the intestinal absorption of W/O/W type insulin micelle reported by Shichiri *et al.*,[9] the decrease of blood sugar level was 60% at 1 hr, and was maintained at over 70% for 3 hr. In the present dosage form, however, the decrease of blood sugar levels was not well maintained. The reason for this result may be that continuous release of the drug did not take place from the core-base, and thus the absorption ratio became small. To overcome this problem, it is necessary to make a dosage form with better sustained-release properties by suitable pharmaceutical treatment.

Change of Plasma Insulin Levels

Figure 11 shows the change of plasma insulin levels after mucosal administration of the new mucosal dosage form of insulin in beagle dogs. The results corresponded well to those of the blood sugar level study in Fig. 10; the plasma insulin level was about 50 μU/ml after 30 min in group C. The difference of level in group C compared with the other two groups was significant according to the *t*-test ($p<0.05$) at 0.5, 1, 2 and 3 hr. There was no difference between groups A and B. Therefore, it was concluded that insulin was absorbed from the oral mucosa in the presence of sodium glycocholate. Thus, other more effective additives able to increase further the absorption of insulin from the oral mucosa may exist.

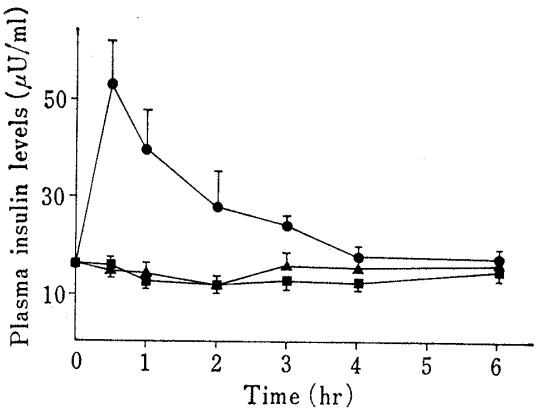

Fig. 11. Change of Plasma Insulin Levels after Mucosal Administration of the New Mucosal Dosage Forms of Insulin in Beagle Dogs

Each point represents the mean ± S.E. of 9 determinations.
-▲-: group A, -■-: group B, -●-: group C.

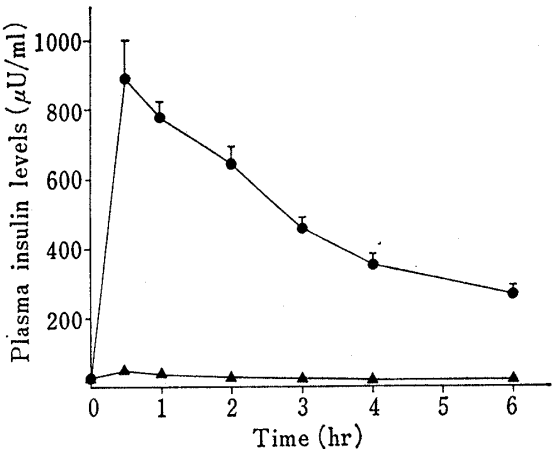

Fig. 12. Bioavailability of Insulin Following I.M. Administration and Mucosal Administration in Beagle Dogs

Each point represents the mean ± S.E. of 9 determinations.
-●-: I.M., -▲-: mucosal.

Bioavailability of Insulin upon Mucosal Administration in Comparison with That upon Intramuscular Administration

Figure 12 shows the change of plasma insulin levels after intramuscular insulin injection. The area under the curve (AUC) of the absorption of insulin given by intramuscular administration was 2916.5 μU/ml·hr, while it was 150.7 μU/ml·hr for the present dosage form, so that the amount absorbed of insulin from this dosage form was about 0.5% compared with that upon intramuscular administration. The following factors may be involved.

(1) Insulin crystals, which were under 200 mesh in size and were dispersed in cacao butter of the core-base in the present dosage form, might be barely soluble in saliva, because the pH of the saliva is about 6.2—7.6, while the pK_a of insulin is 5.3—5.8.

(2) The area of the core-base in contact with the oral mucosa is very small, and consequently the absolute quantities of insulin in contact with the oral mucosa may be insufficient.

(3) Aoki *et al.*[23] reported that drugs of molecular size under 20—50 Å were absorbed from the rat oral mucosa, and Walton[24] indicated that drug absorption from the oral mucosa was related to the partition coefficient. Thus, insulin, a hydrophilic polymer, may be poorly absorbable. Consequently, in order to increase the absorption of a hydrophilic polymer such as insulin or heparin, it will be necessary to choose a suitable additive, *i.e.* a so-called absorption-accelerating agent.

Finally, although the amount absorbed of insulin was low in the present dosage form, we did confirm that insulin was absorbed at the oral mucosa. Further investigations may improve the bioavailability of insulin in the present dosage form, as regards both the extent of absorption and the duration of enhanced blood level.

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