Oral Administration of Insulin as Poly(Vinyl Alcohol)-Gel Spheres in Diabetic Rats

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The oral administration of insulin in poly(vinyl alcohol)-gel spheres (PVA-GS), an oral dosage form with prolonged residence time in the ileum, was examined in streptozotocin-induced diabetic rats. Intragastric administration of PVA-GS containing insulin and a protease inhibitor, aprotinin or bacitracin, caused a significant and prolonged reduction of blood glucose levels, suggesting insulin absorption. The bioavailability of insulin estimated from the hypoglycemic effect was about 2% in the presence of either protease inhibitor. The release profiles of insulin and the protease inhibitors from the PVA-GS could be explained by Higuchi’s plot, and the rates were similar to each other. The site dependency of insulin absorption in the intestinal tract was examined by an in situ loop method. Insulin absorption estimated by plasma insulin levels was larger in the ileum and the large intestine than in the jejunum. The prolonged residence time of PVA-GS in the absorption site, the lower intestine, and the synchronous release of insulin and the protease inhibitors from the PVA-GS are the two major explanations for the improved bioavailability of insulin administered as PVA-GS containing a protease inhibitor.

Key words insulin; oral administration; poly(vinyl alcohol)-gel sphere; protease inhibitor; prolonged gastrointestinal residence time; diabetic rat

The permeability of the gastrointestinal (GI) mucosa to a drug and the residence time of the dosage form in the absorptive GI tract are two major factors determining the absorption of an orally administered drug. In order to develop an oral drug delivery system (DDS) for peptide drugs, two major problems, i.e., 1) proteolytic degradation in the GI tract and 2) poor mucosal permeability to large molecular- and highly hydrophilic-drugs, remain to be overcome.1) Pharmaceutical modifications using absorption enhancers to overcome the poor membrane permeability to some drugs have been studied extensively.2) However, the enhancer approaches are not always successful because the enhancers have little selectivity regarding the actions of the permeants. On the other hand, prolongation of the residence time in the absorption site would be effective in enhancing the absorption of poorly permeable drugs if they can be protected from the degradation.

We have prepared poly(vinyl alcohol)-gel spheres (PVA-GS) as a GI-transit time controlling oral DDS. Oral PVA-GS enables a prolongation in ileal residence time as well as a prolonged plasma concentration-time profile for cephalixin.3) While more than 90% of orally administered aqueous solution was transferred to the large intestine within 5 h, about 40% of orally administered PVA-GS still remained in the ileum.3) Since PVA-GS can provide drugs prolonged residence in the small intestine, a major site of drug absorption, PVA-GS is an attractive dosage form for drugs with poor mucosal permeability.

In this study, we examined the absorption of insulin orally administered as PVA-GS with or without protease inhibitors in streptozotocin-induced diabetic rats, and further assessed the absorption of insulin through the intestinal mucosa of diabetic rats by an in situ loop method, to clarify regional differences in permeability of the epithelium to insulin, as well as the usefulness of the incorporation of the protease inhibitor in PVA-GS.

MATERIALS AND METHODS

Materials PVA (average polymerization number = 1700) was kindly supplied from Kuraray Co. (Osaka, Japan). Sorbitan sesquioleate (SO-15) and polyoxyethylene hydrogenated castor oil (HCO-60) were supplied by Nikko Chemicals Co. (Tokyo, Japan). Insulin (from bovine pancreas) and streptozotocin were obtained from Sigma Chemical Co. (St. Louis, MO). Aprotinin and bacitracin were obtained from Okura Pharmaceuticals Co. (Kyoto, Japan) and Wako Pure Chemical Industries (Osaka), respectively. Other reagents used in this study were reagent grade commercial products and were used without further purification.

Preparation of PVA-GS Viscous PVA saline solution (15%) containing insulin with or without aprotinin (or bacitracin) was prepared as follows. PVA was dissolved in saline by heating at 105°C for 1 min using an autoclave (HA300M, Hirayama Co., Tokyo), and the solution was cooled to room temperature with stirring. Insulin was dissolved in dilute HCl solution, and the pH was adjusted to 6.8–7.4 by the addition of dilute NaOH solution. Then, both solutions were mixed well. Two milliliter of the solution was added to 10 mL of sesame oil with 0.8 mL of SO-15 and 0.4 mL of HCO-60. The mixture was vortexed sufficiently and emulsified by ultrasonic agitation (UT-104, Sharp, Osaka) for 1 min. The resulting w/o emulsion was frozen (at −20°C for 24 h) and thawed (at 4°C for 12 h) to form the PVA-GS. PVA-GS was purified by n-hexane washings to remove the oil phase, including the surfactants, followed by vacuum filtration. The diameters of the spheres measured by photomicroscopy were 5–25 μm. Loading efficacy of insulin to the PVA-GS was 40.4±3.4%.

Release of Insulin and Protease Inhibitors from PVA-GS PVA-GS (0.5 g) containing insulin, aprotinin or bacitracin in a seamless cellulose tubing (UC8-32-25, M.W. (molecular weight) cutoff 12 kDa, Sanko Junyaku

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Co., Tokyo) was put into 10 ml of the second fluid for a disintegration test of JP XII (pH 6.8) using a sinker, and was incubated at 37 °C. The appearance of insulin or protease inhibitors in the medium from the PVA-GS was measured periodically. Insulin was determined using an enzyme immunoassay kit (Insulin-EIA, Dainabot Co., Tokyo). The calibration curve was constructed with the insulin used in this study. Aprotinin was determined spectrophotometrically at 280 nm. Bacitracin was determined by HPLC after a derivatization with fluorescamine at pH 8. The HPLC apparatus was a Shimadzu LC-5A (Kyoto) equipped with a fluorescence detector (RF-353), a column oven (CTO-2A), a pump control unit (GTF-3A) and an integrator (Chromatopac C-R3A). The chromatographic conditions were as follows: column, YMC-Pack ODS-AM (4.6 × 150 mm, Yamamura Chemical Laboratories Co., Kyoto); column temperature, 40 °C; flow rate, 1.0 ml/min; fluorescent detection, excitation and emission wavelengths were 390 nm and 475 nm, respectively. Mobile phases were (A) 25 mm phosphate buffer (pH 7.4) and (B) 25 mm phosphate buffer (pH 7.4); acetonitrile (1:1); A/B (1/1) for the first 3 min which was then gradually changed to 100% B for 10 min.

Preparation of Diabetic Rats Streptozotocin (STZ) (60 mg/kg) was administered intravenously to male Wistar rats (250—300 g). The rats were used for the following experiments 8 d after the STZ-treatment. Blood glucose level was determined by a glucose-oxidase method (Iatron-Chrome GLU-LQ, Iatron Laboratories Co., Tokyo). Urine glucose was assayed by reagent strips for urine analysis (Diastrict, Miles-Sankyo Co., Tokyo). After 12 h fasting, rats with a urine glucose level higher than 1.0% and blood glucose level higher than 120 mg/dl were used as diabetic state animals.

Oral Administration of PVA-GS Under ether anesthesia, the femoral vein of the diabetic rats was cannulated with vinyl tubing. PVA-GS containing insulin (3 mg/2.5 g gel) with or without aprotinin (4 mg/2.5 g gel) or bacitracin (6 mg/2.5 g gel) was prepared, and the PVA-GS (2.5 g/kg), followed by 0.5 ml of saline, was administered intragastrically to the diabetic rats using a gastric sonde. The dosed rats were kept in restraining cages, with free access to water. Blood samples were periodically taken from the cannulated femoral vein. Blood glucose levels were determined by the glucose-oxidase method, and changes were expressed as the % of each initial level.

Absorption of Insulin from Intestinal Loop The diabetic rats were fasted for 12 h before use. Under urethane anesthesia (1.0 g/kg, i.p.), a jejunal (20 cm), ileal (20 cm) or large-intestinal (colon to anus) loop was prepared, and the femoral vein was cannulated with vinyl tubing. PVA-GS (2.5 g/kg) containing insulin or an aqueous solution of insulin was administered into the intestinal loop. Blood samples were periodically taken from the cannulated femoral vein. Plasma insulin levels were determined periodically using an enzyme immunoassay kit (EIA Insulin TEST-S, MBL, Nagoya, Japan). The calibration curve was constructed with the insulin used in this study. The bioavailability of insulin after intraintestinal loop administration was calculated from the AUC values of plasma insulin. Insulin absorption from PVA-GS containing both insulin and aprotinin was similarly examined.

Data Analysis Pharmacokinetic evaluations were carried out by non-compartmental analysis of the plasma concentration—time data based on the statistical moment theory. The moments, the area under the plasma concentration—time curve (AUC) and the mean residence time (MRT), were calculated by a trapezoidal method. Student's t-test was utilized to determine the significance of differences.

RESULTS AND DISCUSSION

The oral delivery of insulin is limited by at least two absorption barriers; one is a transport barrier, the poor permeability of mucosal membrane to the peptide drug, and the other is an enzymatic barrier, the enzymatic degradation of the peptide in the gastrointestinal tract. In this study, we examined PVA-GS as an oral dosage form of insulin which could overcome both barriers against insulin absorption.

As to poor mucosal membrane permeability, absorption can be expected to be increased by increasing the residence time in the small intestine and prolonging the contact time with the mucosal membrane. PVA-GS can increase the residence time in the ileum, resulting in keeping the plasma level of cephalixin, whose absorption site is limited in the small intestine, constant up to at least 12 h. Figure 1 shows the result of blood glucose levels in STZ-induced diabetic rats after oral administration of PVA-GS. However, no hypoglycemic effect was observed in the diabetic rats following administration of PVA-GS containing only insulin, as in the case of the empty preparation. This indicates that prolongation of only the ileal residence time of the formulation was not sufficient to increase the absorption of insulin. On the other hand, a significant continuous hypoglycemic effect was observed when insulin was orally administered as PVA-GS.

![Fig. 1. Changes in Blood Glucose Levels after Oral Administration of PVA-GS Containing Insulin with or without Aprotinin in Diabetic Rats](image-url)

Doses of insulin and aprotinin were 75 U/kg and 4 mg/kg, respectively. Key: ○, empty PVA-GS; ●, PVA-GS containing insulin; △, aqueous solution of insulin and aprotinin; ▲, PVA-GS containing insulin and aprotinin. Results are expressed as the mean ± S.E. of 3–4 experiments. a) p < 0.05; b) p < 0.01, compared with the values of empty PVA-GS.
containing protease inhibitors, aprotinin (Fig. 1) and bacitracin (Fig. 2). Aprotinin is an inhibitor of proteasome, while bacitracin is an inhibitor of an insulin-degrading enzyme located in the cytosol of enterocytes. These results suggest that it is primarily the second barrier, an enzymatic barrier, which makes insulin absorption difficult. The bioavailabilities of insulin estimated from the hypoglycemic effect up to 12 h were 2.0 ± 0.4 and 1.8 ± 0.8% for PVA-GS containing aprotinin and bacitracin, respectively. By macroscopic observation, PVA-GS in the intestinal lumen maintained its original shape at 3 h after the administration. Some aggregation was observed at 6 h after the administration, but it still seemed to keep the gel state.

Although oral PVA-GS enables prolongation in ileal residence time of the formulation, synchronous release of insulin and the protease inhibitor from the PVA-GS must be guaranteed to ensure its anti-proteolytic effect. As shown in Fig. 3, insulin and protease inhibitors showed a similar release profile from the PVA-GS, and linear relations in Higuchi’s plot were observed in both peptides, suggesting that diffusion in the gel-matrix is the

Fig. 2. Changes in Blood Glucose Levels after Oral Administration of PVA-GS Containing Insulin with or without Bacitracin in Diabetic Rats

Doses of insulin and bacitracin were 75 U/kg and 6 mg/kg, respectively. Key: O, empty PVA-GS; ●, PVA-GS containing insulin; □, aqueous solution of insulin and bacitracin; ■, PVA-GS containing insulin and bacitracin. Results are expressed as the mean ± S.E. of 3–4 experiments. a) p < 0.05, compared with the values of empty PVA-GS.

Fig. 3. Release Profiles of Insulin and Protease Inhibitors from PVA-GS

Profiles are expressed as (A) % released vs. time, and (B) % released vs. square root of time. Key: ●, insulin; ▲, aprotinin; ■, bacitracin. Points are the mean of duplicate experiments.

Fig. 4. Plasma Insulin Levels after Administration of PVA-GS Containing Insulin with or without Aprotinin into Jejunal (A), Ileal (B) and Large-intestinal (C) Loops in Diabetic Rats

Doses of insulin and aprotinin were 75 U/kg and 4 mg/kg, respectively. Key: ×, empty PVA-GS; O, aqueous solution of insulin; ●, aqueous solution of insulin and aprotinin; △, PVA-GS containing insulin; ■, PVA-GS containing insulin and aprotinin. Results are expressed as the mean ± S.E. of 3–4 experiments. a) p < 0.05; b) p < 0.01, compared with the values of insulin aqueous solution.
Table 1. Pharmacokinetic Parameters of Insulin after Administration of Aqueous Solution or PVA-GS with or without Aprotinin into Different Intestinal Loops in Diabetic Rats

<table>
<thead>
<tr>
<th>Site</th>
<th>Formulation</th>
<th>Aprotinin</th>
<th>MRT (h)</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>Aq. solution</td>
<td>+</td>
<td>2.1 ± 0.5</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Aq. solution</td>
<td>+</td>
<td>1.4 ± 0.4</td>
<td>0.74 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>PVA-GS</td>
<td></td>
<td>4.0 ± 2.0</td>
<td>0.20 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>PVA-GS</td>
<td>+</td>
<td>3.2 ± 0.8</td>
<td>0.74 ± 0.07</td>
</tr>
<tr>
<td>Ileum</td>
<td>Aq. solution</td>
<td>+</td>
<td>1.5 ± 0.5</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Aq. solution</td>
<td>+</td>
<td>1.9 ± 0.1</td>
<td>1.72 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>PVA-GS</td>
<td></td>
<td>3.5 ± 0.6</td>
<td>0.53 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>PVA-GS</td>
<td>+</td>
<td>2.2 ± 0.7</td>
<td>2.12 ± 0.51</td>
</tr>
<tr>
<td>Large-intestine</td>
<td>Aq. solution</td>
<td>+</td>
<td>1.2 ± 0.2</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Aq. solution</td>
<td>+</td>
<td>1.0 ± 0.2</td>
<td>0.29 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>PVA-GS</td>
<td></td>
<td>2.6 ± 0.8</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>PVA-GS</td>
<td>+</td>
<td>2.8 ± 0.1</td>
<td>2.19 ± 0.96</td>
</tr>
</tbody>
</table>

Doses of insulin and aprotinin were 75 U/kg and 4 mg/kg, respectively. Results are expressed as the mean ± S.E. of 3–4 experiments. a) p < 0.05; b) p < 0.01, compared with the values of aqueous solution of insulin alone.

rate-determining step for peptide release. Approximately 50% and 80% of both peptides were released in 6 h and 12 h, respectively. No marked change was observed in the state of PVA-GS in the dialysis bag during the 12 h release experiment.

Figure 4 shows the plasma insulin levels after intra-intestinal loop administration of insulin as the aqueous solution or the PVA-GS in diabetic rats. In this experiment, the intestinal contents were washed out before insulin administration. Thus, the effect of an enzymatic barrier in the lumen should be greatly reduced. Synchronous application of aprotinin with insulin was effective in increasing the plasma insulin level in all sites of the intestinal tract. In the case of insulin-PVA-GS containing aprotinin, prolongation of elevated plasma insulin levels was observed up to 6 h after administration into the ileal and large-intestinal loops. Absolute bioavailabilities of insulin calculated from the AUC values for 0–6 h by the dose–AUC relationship obtained in the i.v. administration study are summarized in Table 1. These absorption profiles of insulin administered as PVA-GS containing aprotinin are consistent with the prolonged hypoglycemic activity of oral PVA-GS incorporating insulin and aprotinin.

In the jejunum, plasma insulin levels after the administration of an aqueous solution containing insulin together with aprotinin were much higher than those by PVA-GS containing both insulin and the protease inhibitor. Insulin and aprotinin in the form of an aqueous solution can disperse along the jejunal mucosal surface. However, PVA-GS releases both components slowly, resulting in an incomplete protective effect in the jejunum with high degrading activity. On the other hand, in spite of its slowly releasing property, the PVA-GS form was effective in the lower intestine where the proteases are less active.

Studies on the mechanism of the slow transit of PVA-GS in the ileum are in progress. Our preliminary study found a significant role of mucous layer on the mucosa in the slow transit in the ileum, while the detailed mechanism of such remains to be clarified.

In conclusion, intestinal absorption of insulin from the PVA-GS is not inferior to that from an aqueous solution. The prolonged GI-transit characteristics of PVA-GS in the lower intestine and the synchronous controlled release of insulin and aprotinin from the PVA-GS are two major explanations of the improved oral bioavailability of insulin administered as PVA-GS containing aprotinin. The technique provides a novel oral DDS for peptides.

REFERENCES