Evaluation of Poly(vinyl alcohol)-Gel Spheres Containing Chitosan as Dosage Form to Control Gastrointestinal Transit Time of Drugs

Katsuyoshi Sugimoto, Minoru Yoshida, Takashi Yata, Kazutaka Higaki and Toshikiro Kimura *

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1, Tsushima-naka, Okayama 700-8530, Japan. Received June 24, 1998; accepted August 4, 1998

Two types of poly(vinyl alcohol)-gel spheres were prepared with chitosan (CS/PVA-GS) and without chitosan (PVA-GS), and comparative studies were performed using these gel spheres (GSs). No change in particle size was observed by the addition of chitosan: nearly 45% of both particles were in the 5—10 µm range. In an in vivo gastrointestinal transit test, CS/PVA-GS prolonged the small-intestinal transit time more than PVA-GS. In an in vitro intestinal perfusion study, the mean transit time of these GSs was markedly reduced by pretreatment of the intestinal surface with a mucolytic agent, N-acetyl-L-cysteine, suggesting that the mucous layer on the intestinal surface plays an important role in controlling the transit rate of these GSs. The oral administration of aminoephiline (theophylline) and ampicillin as model drugs incorporated in PVA-GS and CS/PVA-GS was examined in rats. While theophylline absorption from PVA-GS was not affected by the addition of chitosan, the improvement of ampicillin absorption by PVA-GS was enhanced by the chitosan combination.

Key words  poly(vinyl alcohol); gel microsphere; chitosan; gastrointestinal transit time; drug delivery system

The permeability of the gastrointestinal (GI) mucosa to a drug and the residence time of the dosage form in the absorptive site of the GI tract are the two major factors determining the absorption of the orally administered drug. The absorption of orally administered peptide drugs, for example, is very poor. One reason is the poor mucostral permeability to large molecular and highly hydrophilic peptide drugs in addition to their instability in the GI tract. Despite extensive studies on pharmaceutical modifications using absorption enhancers to overcome the poor membrane permeability to some drugs,3 these approaches are not always successful because of mucostral damage caused by the enhancers. Prolongation of the residence time in the absorption site of the GI tract would be effective in enhancing the absorption of poorly permeable drugs, if their stability were maintained. To accomplish this purpose, dosage forms using various polymers have been investigated as a new drug delivery system.

We have developed poly(vinyl alcohol)-gel spheres (PVA-GS) as an oral drug delivery system with characteristics of prolonging GI-transit time and controlling drug release. Oral PVA-GS enabled a prolongation of the residence time in the small intestine, resulting in a prolonged plasma concentration-time profile for cephalaxin. PVA-GS is an attractive dosage form for oral administration, since it can provide a drug a longer residence in the small intestine, a major site of drug absorption. We also have examined insulin orally administered as PVA-GS with the protease inhibitors, aprotinin and bacitracin, in streptozotocin-induced diabetic rats, and observed its significant intestinal absorption. The prolonged GI-transit characteristics of PVA-GS and the synchronous controlled release of insulin and aprotinin from these gel spheres are two major explanations for the improved oral bioavailability of insulin administered as PVA-GS containing aprotinin.

The use of bioadhesive polymers has been found to be essential in dosage forms developed to control the GI-transit rate. 5-7 We modified PVA-GS using chitosan (CS) as one such polymer. CS is a natural polyaminosaccharide prepared from chitin by N-deacetylation with alkali. It has been used in biomedical and pharmaceutical fields because of its favorable properties of low toxicity and good biocompatibility.8 These advantages are compatible with our concept that PVA-GS must be non-toxic and non-carcinogenic. PVA-GS was therefore prepared by the freezing and thawing method without using any crosslinking agents.

In this study, we formulated CS/PVA-GS, that is, PVA-GS containing CS, and compared it with PVA-GS as a dosage form to control GI-transit rate.

MATERIALS AND METHODS

Materials  PVA (average polymerization number=1700) was generously supplied by Kuraray Co. (Osaka, Japan). Chitosan (degree of deacetylation=100%) was obtained from Funakoshi Co. (Tokyo, Japan). Sorbitan sesquioleate (SO-15) and polyoxyethylene hydrogenated castor oil (HCO-60) were supplied by Nikko Chemicals Co. (Tokyo). Aminophylline and theophylline were obtained from Sigma Chemical Co. (St. Louis, MO) and Tokyo Kasei Kogyo Co. (Tokyo), respectively. Ampicillin was obtained from Wako Pure Chemical Industries (Osaka). Other reagents used in this study were reagent grade commercial products and were used without further purification.

Animals  Male Wistar rats weighing 220—330 g were used.

Preparation of GSs  GSs were formulated as described previously. 9 Briefly, viscous PVA saline solution (15%) 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. CS was dissolved in 1% (v/v) acetic acid solution. The two solutions and the drug solution were then mixed well. Two milliliter of the mixture was added to 10 mL of sesame oil with 0.8 mL of SO-15 and 0.4 mL of HCO-60, 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 20 h) and thawed (at 4°C for 4 h) to form GSs. n-Hexane washings to remove the oil phase, including the surfactants, were followed by vacuum filtration to purify the GSs.

Measurement of Particle Size  Statistical measurement

© 1998 Pharmaceutical Society of Japan
was made of data observed with a stereoscopic microscope (CK2, Olympus, Tokyo).

**Measurement of GI-Transit in Vivo** The GI-transit rate after oral administration was examined as described. Briefly, rats were fasted overnight with free access to water prior to the experiment. Under ethyl ether anesthesia, 0.5 ml of GSs containing phenol red (7.5 mg/g), followed immediately by 0.3 ml of isotonic phosphate buffer (pH 6.5), were administered by a gastric sonde to each rat. After a fixed time period, the rat was sacrificed and the entire GI tract was excised. The small intestine was further divided into 4 equal segments (about 20 cm each); we call these segments SI1, SI2, SI3 and SI4 from the upper part of the small intestine, respectively. The contents of each segment were washed out with 50 ml of saline. Phenol red concentration in the supernatant was determined to evaluate the GI-transit of the GSs. The value of GI-transit clearance in each segment i (CIgi) was calculated by 100/AUCgi, where AUCgi (%–h) is the area under the curve of observed recovery of the phenol red dose (%) against time of data in Fig. 2.

**In Vitro Perfusion Method** The intestinal transit properties were examined by the in vitro perfusion method as described. Briefly, the small intestine was excised from rats anesthetized with pentobarbital. A seventeen-cm length of jejunum or ileum was placed on absorbent cotton inclined at a 25° angle. The luminal inside was perfused with isotonic phosphate buffer with a constant flow rate, 0.2 ml/min, using a peristaltic pump. GSs containing phenol red were mixed beforehand with isotonic phosphate buffer, and 0.05 ml was injected at t=0. The perfusate was collected at fixed intervals. The small-intestinal transit characteristics of GSs were evaluated with phenol red concentration in the collected perfusate.

**Release of Aminophylline and Ampicillin from GSs** GSs (0.5 ml) containing aminophylline or ampicillin in a seamless cellulose tubing (UC8-32-25, molecular weight cutoff 12 kDa, Sanko Junyaku Co., Tokyo) was put into 40 ml of the second fluid for a disintegration test of JP XIII (pH 6.8), tightly closed, and was incubated at 37 °C. The appearance of theophylline or ampicillin, respectively, in the medium from GSs was measured periodically. These drugs were determined by HPLC.

**Oral Administration Study** Rats, whose jugular vein was cannulated to collect blood samples 2 d prior, were fasted overnight before the experiments. They were allowed free access to water except for the first 2 h after the administration of GSs. Under ethyl ether anesthesia, GSs, followed immediately by 0.3 ml of isotonic phosphate buffer (pH 6.5), were administered intragastrically using a gastric sonde. The rats moved freely and without restriction during the experiment. Blood samples were taken from the cannula of the jugular vein periodically and the plasma was separated immediately by centrifugation. These plasma samples were frozen and stored until assay, and the drug concentrations were determined by HPLC as described below.

**Analytical Methods** 1) Theophylline: The plasma concentration of theophylline was determined according to the method of Shiu et al. The plasma (0.1 ml) was deproteinized with the same volume of 10% trichloroacetic acid (TCA). After centrifugation, an aliquot of the clear supernatant was injected into a reverse-phase HPLC system with a Shimadzu (Kyoto, Japan) LC-6A chromatograph fitted with a 150 mm×4.6 mm i.d. Inertsil ODS column (GL Sciences, Tokyo) and equipped with a Shimadzu SPD-6A UV detector. The mobile phase, flow rate and operating wavelength of the detector were 5 mm acetate buffer (pH 4.8) and acetonitrile (87:13 by volume), 1.0 ml/min and 272 nm, respectively. A Shimadzu C-R4A data module was used for quantitative analysis.

2) Ampicillin: The plasma concentration of ampicillin was determined by HPLC after the pre-column derivatization according to the method of Lal et al. with slight modification. The plasma (0.1 ml) was deproteinized with the same volume of 10% TCA. To 0.1 ml of the clear supernatant was added 0.05 ml of 0.4 M citric acid solution containing 7% (w/v) formaldehyde and the mixture was heated at 90 °C for 2 h. The resultant solution was cooled to room temperature and stored in a refrigerator until HPLC analysis; the HPLC system used was the same as described for theophylline analysis, except for the mobile phase and the detector. The mobile phase was 0.1 M potassium phosphate buffer (pH 5.6) and acetonitrile (7:3 by volume). A fluorescence detector (Shimadzu RF-535) was operated at excitation and emission wavelengths of 346 and 422 nm, respectively.

**Pharmacokinetic Analysis** The moments, the area under the plasma concentration–time curve (AUC) and the mean residence time (MRT), were calculated by a trapezoidal method. The absolute bioavailability values were calculated by using the AUC values after intravenous administration. Student's t-test was utilized to determine the significance of differences.

**RESULTS AND DISCUSSION**

The GI-transit time of a dosage form is changed by many factors. When two dosage forms are compared, the particle size is a very important factor. Thus, the particle size distribution of PVA-GS and CS/PVA-GS was examined, and the results are shown in Fig. 1. The particle size was less than 40 μm in both GSs and nearly 45% of both were in the 5—10 μm range. The average diameter showed no significant difference between PVA-GS and CS/PVA-GS: 7.36 and 7.38 μm, respectively. The GSs made by our method without crosslinking agent are very small. Fieck and Peppas also developed microparticles without crosslinking agent, but theirs were not as small as ours. It seems that they could not get a stable w/o emulsion since they used sodium lauryl sulfate as an emulsifier, which has a hydrophilic lipophilic balance (HLB) value too high to obtain this type of emulsion.

Next, we measured the GI-transit time of the GSs after oral administration (Fig. 2). The small-intestinal transit of CS/PVA-GS was slower than PVA-GS, especially in the lower small intestine (phenol red recovery at 4.5 h in SI4 was 10.8% for PVA-GS and 27.3% for CS/PVA-GS), resulting in later arrival of CS/PVA-GS to the large intestine (phenol red recovery at 4.5 h in the large intestine was 88.8% for PVA-GS and 65.7% for CS/PVA-GS). While the values of Clgi in the stomach for GSs were much smaller than that for solution (2.03 h⁻¹), the values for PVA-GS and CS/PVA-GS were essentially the same (0.50 and 0.48 h⁻¹, respectively). The Clgi values in the small intestine, on the other hand, were different; those for CS/PVA-GS (10.39, 6.18, 1.27 and
0.85 h⁻¹ in SI1, SI2, SI3 and SI4, respectively) were smaller than those for PVA-GS (13.32, 6.78, 1.89 and 1.03 h⁻¹, respectively). This longer small-intestinal transit time of CS/PVA-GS would be due to the change in interaction between the mucosal surface of the small intestine and GSs because of the presence of CS.

An in vitro perfusion study was performed to determine the interaction between the small-intestinal mucosa and GSs. Table 1 shows the mean transit time (MTT) values for each formulation in the jejunum and ileum. For PVA-GS, the MTT value in the ileum was significantly larger than that in the jejunum, while the values for CS/PVA-GS in the two regions were similar. The great difference in transit rate in vivo would be due to the difference in the propulsive movement in the two regions. Harris et al. reported that the formulation using an acrylic acid derivative, Carbopol-934, delayed the GI-transit time after oral administration in the rat, and stated that this was due to the interaction between this polymer and the mucous layer of the intestinal tract. To learn the contribution of the mucous layer to retardation of the transit rate, the effect of pretreatment of the intestinal mucosal surface with N-acetyl-l-cysteine, a mucolytic agent, on this rate was examined. Table 1 shows that the transit rates of the two formulations were accelerated by the pretreatment in the ileum; while the acceleration in the jejunum was not statistically significant, a similar tendency was observed. This suggests that the mucous layer on the intestinal surface plays an important role in controlling the transit rate of the formulation. Since the difference in MTT values for PVA-GS in the jejunum and ileum disappeared by the treatment with N-acetyl-l-cysteine, it is suggested that the interaction of PVA-GS with the mucous layer in the ileum is stronger than that in the jejunum. Takeuchi et al. evaluated the mucoadhesive function of the polymer-coated liposomes in vitro using rat intestine, and CS-coated liposomes showed higher adhesive property than PVA-coated liposomes.

Since it was determined that CS/PVA-GS delayed the GI transit time more than PVA-GS (Fig. 2), comparative studies were made on the characteristics of drug absorption from the two types of gel spheres. Theophylline and ampicillin were used as model drugs. Both are absorbed from the entire intestinal tract without the first-pass metabolism, but the extent of absorption differs: the former is absorbed very well after oral administration but the latter is poorly absorbed due to its low mucosal permeability. Figure 3A shows the in vitro release profiles of theophylline from PVA-GS and CS/PVA-GS containing aminophylline. As is evident, the two GSs showed similar release profiles and linear relations in Higuchi's plot were observed in both GSs up to 80% release, suggesting that diffusion in the gel-matrix is the rate-determining step for the drug release. Approximately 80% of theophylline was released in 4 h from both GSs. The values of mean releasing time (MRelT) of theophylline for PVA-GS and CS/PVA-GS estimated from the curves were 2.11 and 2.02 h, respectively.

Figure 4 shows the plasma concentration profiles of theo-
Fig. 3. In Vitro % Released versus (Time)**2 Plots for Theophylline and Ampicillin from PVA-GS and CS/PVA-GS Containing Aminophylline (A) and Ampicillin (B), Respectively
(A) ○, PVA-GS (n=6); ●, CS/PVA-GS (n=4); (B) ○, PVA-GS (n=3); ●, CS/PVA-GS (n=2). Results are expressed as the mean±S.E.

Table 2. Pharmacokinetic Parameters of Theophylline after Oral Administration of PVA-GS and CS/PVA-GS Containing Aminophylline

<table>
<thead>
<tr>
<th>Dosage form (n)</th>
<th>AUC (µg·h/ml)</th>
<th>MRT (h)</th>
<th>MAT (h)</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution (5)</td>
<td>33.0±1.8</td>
<td>2.81±0.17</td>
<td>0.30±0.17</td>
<td>1.018±0.056</td>
</tr>
<tr>
<td>PVA-GS (7)</td>
<td>32.2±2.1</td>
<td>6.16±0.10{a}</td>
<td>3.65±0.10{a}</td>
<td>0.993±0.065</td>
</tr>
<tr>
<td>CS/PVA-GS (7)</td>
<td>32.0±1.8</td>
<td>6.02±0.49{a}</td>
<td>3.51±0.49{a}</td>
<td>0.987±0.056</td>
</tr>
</tbody>
</table>

Dose of aminophylline was 5 mg/kg. Results are expressed as the mean±S.E.  

<sup>a</sup> p<0.01, compared with solution.

Fig. 4. Plasma Concentration of Theophylline after Oral Administration of PVA-GS and CS/PVA-GS Containing Aminophylline

Dose of aminophylline was 5 mg/kg. Key: ●, solution (n=5); ○, PVA-GS (n=7); ●, CS/PVA-GS (n=7). Results are expressed as the mean±S.E.

Fig. 5. Plasma Concentration of Ampicillin after Oral Administration of PVA-GS and CS/PVA-GS

Dose of ampicillin was 50 mg/kg. ●, solution (n=3); ○, PVA-GS (n=5); ●, CS/PVA-GS (n=5). Results are expressed as the mean±S.E.

Theophylline following oral administration of PVA-GS and CS/PVA-GS containing aminophylline, GSS delayed the rise in the plasma concentration in comparison with the solution (T<sub>max</sub>, from 0.40 to 3.57 h; C<sub>max</sub>, from 10.1 to 4.4 µg/ml), and the plasma levels lasted for a longer period. However, the addition of CS did not affect the plasma concentration profile for PVA-GS. The pharmacokinetic parameters are listed in Table 2. The extent of bioavailability was almost 1.0 in all three dosage forms. The values of mean absorption time (MAT) for the solution, PVA-GS and CS/PVA-GS were 0.30, 3.65 and 3.51 h, respectively. MRelT values calculated from these MAT values are 3.35 and 3.21 h for PVA-GS and CS/PVA-GS, respectively, and are larger than the MRelT values estimated in vitro (Fig. 3A). The difference in MRelT values between in vitro and in vivo would be caused by the delayed gastric emptying rate by GSS: MRT values in the stomach for the solution, PVA-GS and CS/PVA-GS were 0.38{a}, 1.10 and 1.15 h, respectively. Theophylline can be rapidly absorbed in all regions along the intestine. Thus, the rate-determining steps of theophylline are the gastric emptying rate and the release rate from GSS. This would be the reason for the bioequivalence between PVA-GS and CS/PVA-GS. That is, the different intestinal transit rates of the two GSs affected neither the rate nor the extent of bioavailability of rapidly absorbable theophylline, since not only the gastric emptying rates but also the drug releasing rates are similar in the two dosage forms.

Contrary to theophylline, the bioavailability of poorly absorbable ampicillin was affected by the administration as the PVA-GS dosage form, which was further enhanced by the addition of CS. Figure 3B shows the in vitro release profiles of ampicillin from PVA-GS and CS/PVA-GS. As in the case of theophylline, both GSs showed closely similar release profiles and linear relations in Higuchi’s plot were observed in both GSs up to 80% release, again suggesting that diffusion in the gel-matrix is the rate-determining step for the drug release. Approximately 80% of ampicillin was released in 4 h from both GSs. MRelT values of ampicillin for PVA-GS and CS/PVA-GS estimated from the curves were exactly the same: 1.95 h. In spite of the larger molecular weight, this is slightly shorter than those for theophylline. The reason remains to be determined, but the hydrophilic nature of ampicillin would be one factor causing the faster release.

Figure 5 shows the plasma ampicillin levels following oral administration of PVA-GS and CS/PVA-GS, and the pharmacokinetic parameters are listed in Table 3. When ampicillin was administered as the solution, approximately 70% of the dose was unabsorbed and entered the large intestine. The
Table 3. Pharmacokinetic Parameters of Ampicillin after Oral Administration of PVA-GS and CS/PVA-GS

<table>
<thead>
<tr>
<th>Dosage form (n)</th>
<th>AUC (µg·h/ml)</th>
<th>MRT (h)</th>
<th>MAT (h)</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution (3)</td>
<td>6.91±0.45</td>
<td>1.77±0.07</td>
<td>1.26±0.07</td>
<td>0.316±0.021</td>
</tr>
<tr>
<td>PVA-GS (5)</td>
<td>8.60±0.46</td>
<td>4.08±0.11(a)</td>
<td>3.57±0.11(b)</td>
<td>0.393±0.021</td>
</tr>
<tr>
<td>CS/PVA-GS (5)</td>
<td>10.63±0.63(c)</td>
<td>4.79±0.05(d)</td>
<td>4.28±0.05(e)</td>
<td>0.485±0.029(f)</td>
</tr>
</tbody>
</table>

Dose of ampicillin was 50 mg/kg. Results are expressed as the mean±S.E. a) p<0.01, compared with solution; b) p<0.05, compared with PVA-GS; c) p<0.001, compared with solution; d) p<0.01, compared with PVA-GS.

GSs increased the residence time in the small intestine, the absorption site, resulting in increased bioavailability. The addition of CS further increased the bioavailability. MAT of ampicillin in CS/PVA-GS (4.28 h) was longer than that in PVA-GS (3.57 h), indicating that the antibiotic in GS containing CS could be absorbed for a longer period than GS without CS. Since the absorption of ampicillin is permeation-rate limited, the large amount of the released antibiotic from PVA-GS in the small intestine passes through the absorption site to the large intestine. The addition of CS prolonged the residence time of the GS in the small intestine and thus increased the period of absorption of the poorly absorbable drug. This would be the reason for the enhanced bioavailability by CS/PVA-GS.

In conclusion, it is suggested that CS/PVA-GS is useful not only for prolonged absorption but also to improve the bioavailability of poorly absorbable drugs.

REFERENCES