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Retention and transit of intestinal mucoadhesive films in rat small intestine

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Abstract

The retention and transit characteristics of intestinal mucoadhesive film systems have been studied after intraduodenal administration in rats. Small size four layered film preparations, 0.5×0.5 mm, were prepared, where the backing layer (45.1 ± 2.9 μm thick) was made of a water-insoluble polymer, ethylcellulose (EC), the surface layer was made of enteric pH-sensitive polymers, Eudragit® L100, S100 or HP-55® and the middle layer was made of cellulose membrane. The surface layer was attached to the middle layer with an adhesive layer composed of carboxyvinyl polymer (Hiviswako® 103). After administration of ten films to the duodenum, the rats were sacrificed hourly and the distribution of the films in the whole small intestine was directly observed after abdominal incision. The HP-55, Eudragit L100 and S100 film systems were found to adhere to the upper, middle and lower part of the small intestine after 1, 2 and 4 h, respectively, for 2–3 h. Direct inspection study suggests that intestinal mucoadhesive film system has functions of: (1) pH-dependent intestinal adhesion site specificity; (2) adhesion to the intestinal wall; and (3) retention in the small intestinal adhesion site for at least 2 h. Intestinal mucoadhesive film system has been suggested to be a targeting system for drugs to the gastrointestinal tract.

Keywords: Mucoadhesive; Film system; Retention; Small intestine; Rat

1. Introduction

As an oral delivery system for macromolecular drugs such as proteins and carbohydrates, many drug delivery systems (DDS) have been investigated (Szkrybalo, 1987; Bienz-Tadmor, 1993; Wilding et al., 1994; Drews, 1995; Vermeij and Blok, 1996; Crommelin and Sindelar, 1997). However, there are several barriers for the oral delivery of macromolecular drugs, i.e. (1) degradation in the acidic pH of the stomach; (2) hydrolytic degradation by proteolytic enzymes; (3) metabolism by luminal, brush border and cytosolic enzymes; and (4) poor membrane permeability across the intestinal epithelium (Zhou, 1994). Among these, luminal enzymatic hydrolysis and low membrane permeability are the major causes of low oral bioavailability (BA) of macromolecu-

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lar drugs. To overcome these barriers, many oral DDSs have been developed. Mention may be made of lipid systems such as Macrulin® and Macritonin® that contain insulin and calcitonin, respectively, are now under clinical phase II–III trials (Fletcher, 1998; Gomez-Orellana and Paton, 1999). Protein unfolding was another technology applied to increase the membrane permeability of calcitonin (Leone-Bay et al., 1996; Bergeron et al., 1997). However, even in these systems, degradation by proteolytic enzymes in the gastrointestinal (GI) tract was not fully prevented.

We have developed a new oral delivery system for macromolecular drugs, i.e. gastrointestinal mucoadhesive patch system (GI-MAPS) (Eaimtrakarn et al., 2001). This system consists of four layers, namely (1) the backing layer made of a water-insoluble polymer, ethylcellulose (EC); (2) surface layer made of an enteric pH-sensitive polymer; (3) the middle cellulose membrane layer containing drug; and (4) an adhesive layer between middle layer and surface layer. Soon after the enteric surface layer is dissolved in the small intestine, the adhesive layer is exposed and the system gets adhered to the intestinal wall. This results in high drug concentration gradient between the system and the enterocytes thereby accelerating the permeation of macromolecules. The water insoluble backing layer protects the drug from the action of the proteolytic enzymes of the GI lumen and as a result, the hydrolytic loss of the protein drugs is extremely less than the above mentioned oral protein delivery systems. With the use of these GI-MAPS, the pharmacological availability of a model protein drug, recombinant human granulocyte-stimulating factor (G-CSF), was found to be about 23% as compared with i.v. injection in beagle dogs. As it can be easily understood from the concept of GI-MAPS, the most important factor of GI-MAPS to work as a new oral drug delivery system is the adhesive efficiency of the system to the intestinal wall.

In this report, intestinal mucoadhesive films having different pH-sensitive surface layers were prepared and their retention and/or transit characteristics in the rat small intestine have been studied precisely.

2. Materials and methods

2.1. Materials

Hydroxypropyl methylcellulose phthalate (HP-55®) was obtained from Shin-etsu Chemical Industry Co. Ltd. (Tokyo, Japan) and Eudragit® S100 and L100 (Röhm Pharm, Darmstadt, Germany) were obtained through Higuchi Inc. (Tokyo, Japan). Carboxyvinyl polymer (Hiviswako® 103), ethylcellulose (EC, 10 cp) and triethyl citrate were obtained from Wako Pure Chemical Industries, Co. Ltd. (Osaka, Japan). Polyethylene glycol (PEG) 400, sudan black and citric acid were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Magnesium silicate was obtained from Kyowa Chemical Industry Co. Ltd. (Takamatsu, Japan). Rats were obtained from SLC (Hamamatsu, Japan). All other materials were of reagent grade and were used as received.

2.2. Preparation of intestinal mucoadhesive films

The backing layer and the pH-sensitive surface layer were prepared by casting/solvent evaporation technique. EC solution was prepared by dissolving 550 mg of polymer in 5 ml of a mixture of methylene chloride and methanol (4:1) and 150 mg of triethyl citrate and 3.5 mg of sudan black were added to it. Triethyl citrate was used as plasticizer and sudan black as staining agent for backing membrane. The EC solution was casted on a 10×10 cm size Teflon plate and the solvent was evaporated at 10°C for 12 h. Then, the EC layer was attached to the middle layer of similar size, made of cellulose membrane, by thermal bonding at 80°C. Thereafter, this bilayer was cut in to 3.0×10.0 cm size pieces. The thickness of the EC film itself was 45.1 ± 2.9 μm and the total thickness of bilayer was 108.4 ± 12.1 μm.

Three kinds of enteric polymers, HP-55, Eudragit L100 and S100, were used to prepare pH-sensitive surface layers. HP-55 solution was prepared by dissolving 550 mg of polymer and 50 μl of triethyl citrate in 10 ml of a 4:1 mixture of methylene chloride and methanol. Similarly, Eudragit L100 and S100 solutions were prepared by dissolving 550 mg of respective polymer and 300

μl of triethyl citrate in 10 ml of 1:1 mixture of methylene chloride and methanol. Each solution was casted on the same teflon plate and the solvent was evaporated at 10°C for 12 h. Then, the pH-sensitive film was cut in to 3.0 × 10.0 cm size pieces. The thickness of the enteric film was 37.9 ± 3.1 μm for HP-55, 35.7 ± 2.4 μm for Eudragit L100 and 38.1 ± 2.5 μm for Eudragit S100. The mucoadhesive glue was prepared by mixing 0.8 g of Hiviswako 103, 250 μl of PEG 400 and 2 ml of water. After mixing well, the glue was uniformly spread on the surface of the pH-sensitive surface layer, and the resulting surface layer was attached to the other cellulose membrane layer of the bilayer prepared above.

The four-layered film was cut into small pieces of 0.5 × 0.5 mm size and they were treated with micro-pulverized stearic acid and magnesium silicate to cover the edges of the films in order to prevent sticking of the films to each other.

2.3. Determination of retention and/or transit of films in the small intestine

Male Wistar rats weighing 350 ± 10 g were fasted for 12 h before the onset of experiments. Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation in Kyoto Pharmaceutical University. Water was allowed ad libitum. An abdominal incision was made under light ether anesthesia, and ten pieces of each test film were administered into the duodenum through a cut on the stomach near the pylorus. To detect the small films in the GI tract, the water-insoluble backing layer was stained with sudan black dye. After abdominal suturing, rats were returned to their cages. After 1, 2, 3, 4, 5 and 6 h of administration, the rats were sacrificed. The GI tract containing stomach, small intestine and cecum was isolated and spread out on a sheet. The whole small intestine from the pyloric sphincter to the ileo–cecal junction was equally marked into 5 portions (sections # 1–5) of approximately 13 cm length and the number of remaining test film preparations in each portion were visually counted after cut opening each section.

2.4. pH measurement of rat small intestine

Male Wistar rats were anesthetized with an i.p. injection of sodium pentobarbital, 50 mg/kg, and an abdominal incision was made. The whole small intestine was divided into five sections as in the retention/transit experiment. A microelectrode (model 6069MP-10C, Horiba, Kyoto, Japan) was directly inserted into both the stomach and the small intestine through a small abdominal incision and the pH was directly measured.

3. Results and discussion

After intraduodenal administration of three kinds of film preparations, i.e. HP-55, Eudragit L100 and S100 systems, to rats, the distribution of the administered films was directly monitored by an abdominal incision. Fig. 1 shows the results of HP-55 system. At 1 h after administration, all the films were detected in section # 1, i.e. duodenum. The films were retained in section # 1 for more than 2 h as the percent of films remained at 3 h was 80% in this section. All the films were found transferred to section # 2, i.e. jejunum at 4 h. Thereafter, they were gradually transferred to the lower part of the small intestine. The dissolution threshold pH of HP-55 is 5.5 (Wade and Weller, 1994). As the pH of the duodenum was reported to be 7.1 (Ward and Coates, 1987), the surface

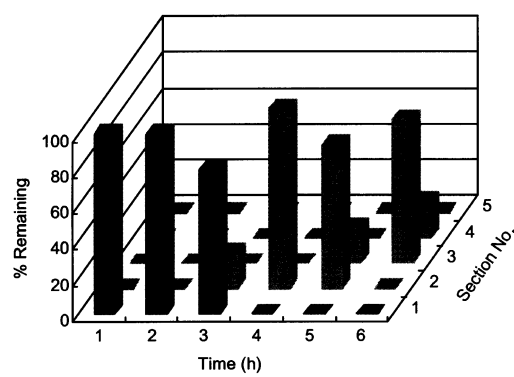

Fig. 1. Distribution of HP-55 mucoadhesive film preparations in the rat small intestinal tract after intraduodenal administration of ten preparations. Each bar shows the mean of three or four rats.

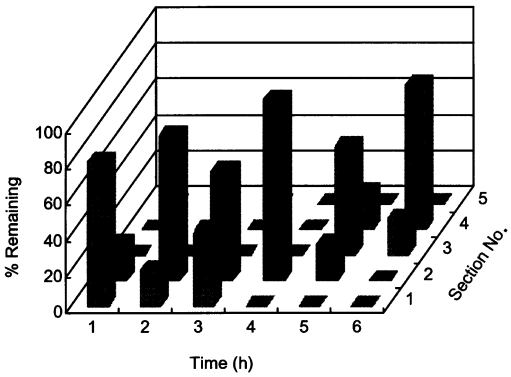

Fig. 2. Distribution of Eudragit L100 mucoadhesive film preparations in the rat small intestinal tract after intraduodenal administration of ten preparations. Each bar shows the mean of three or four rats.

enteric layer of the film was thought to be quickly dissolved after they were administered into the duodenum thereby resulting in the attachment of films to the duodenum wall. Therefore, a retention time of more than 2 h was observed in the duodenum.

Fig. 2 shows the results of Eudragit L100 system. Though the dissolution threshold pH of Eudragit L100 is 6.0 (Dittgen et al., 1997), the surface layer of the system did not dissolve in the section # 1 (pH 7.1). As a result, 80% of the films were found in section # 1 (duodenum) at 1 h and the remaining 20% were in section # 2 and after 2 h of administration, 80% of the films transferred to section # 2 and 20% remained in section # 1. The films retained in section # 2 for 2 h as evident from the retention of all films in this section even after 4 h of administration. Thereafter, the films transferred gradually to sections # 3 and 4 at 5 and 6 h, respectively. From these results it is clear that there was a difference in the adhesive site between HP-55 and Eudragit L100 systems. Though the dissolution threshold pH of Eudragit L100 is 6.0, it does not mean that the polymer spontaneously and completely dissolves at pH 6.0. It starts to dissolve at pH 6.0 and the dissolution progresses as the pH increases to above 6.0. To confirm the difference in the dissolution site of HP-55 and Eudragit L100 layers, the pH of the rat stomach and small intestine was measured and the results are shown in Fig. 3. The pH in stomach was found to be 3.77 ± 0.26. It gradually increased from the duodenum to the ileum, though a small decrease was observed in the jejunum. The mean pH in the section # 1 was 6.67 ± 0.13 and that in sections # 2 and 3 (jejunum) were 6.63 ± 0.03 and 7.13 ± 0.09, respectively, which were almost the same as reported earlier (Ward and Coates, 1987). Also, soaking time in the small intestinal fluid affects the dissolution rate of enteric polymer films. In our experiment, the test films were administered as a dried state into the rat duodenum. We think that it took about 0.5 h for the administered films to be soaked with the intestinal fluid. Therefore, Eudragit L100 layer of films was thought to dissolve in the jejunum, i.e. section # 2. Consequently, they retained there for approximately 2 h.

When Eudragit S100 system was administered to rats, they gradually transferred in the small intestine and most of the films reached to section # 5 after 4 h of administration as shown in Fig. 4. Section # 5 was the lowest part of the small intestine, distal ileum, and was approximately 10 cm away from the ileo–cecal junction. The pH of the distal ileum was reported to be 6.8–7.1 (Ward and Coates, 1987). On the other hand, the pH was found to be 7.50 ± 0.02 in this study. As the dissolution threshold pH of Eudragit S100 is 6.8 (Dittgen et al., 1997), the surface layer of Eu-

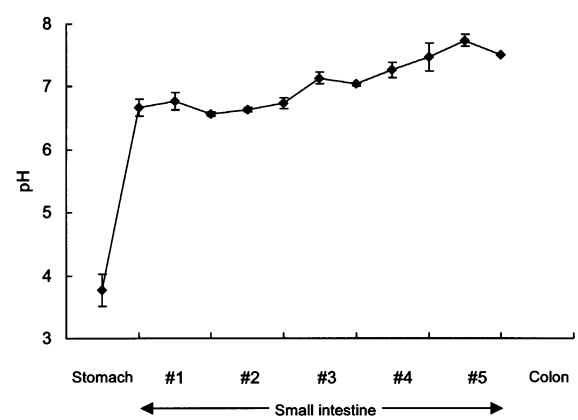

Fig. 3. Luminal pH in the rat small intestinal tract. The whole small intestine was divided into five sections and the pH of the middle and the end part of each section was measured. Each bar shows the mean of three rats.

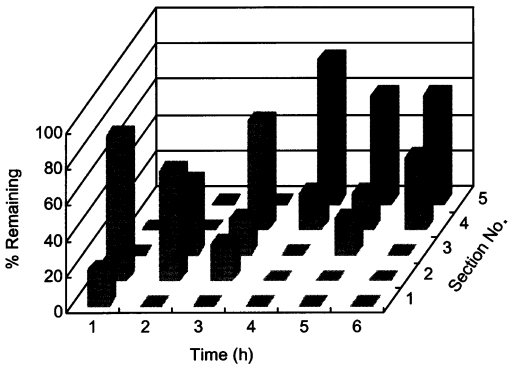

Fig. 4. Distribution of Eudragit S100 mucoadhesive film preparations in the rat small intestinal tract after intraduodenal administration of ten preparations. Each bar shows the mean of three or four rats.

dragit S100 system dissolved. Therefore, the retention of the films in that section was approximately 2 h. Of course, the delayed movement of the gastrointestinal content in the ileum was thought to be a critical factor on the retention of the film preparations in the ileum.

Film preparations have been usually used in transdermal therapeutic systems (TTS) (Ridout et al., 1988). Some pharmaceutical scientists have applied film preparations for oral mucosal delivery of oxytocin, thyrotropin-releasing hormone and chlorhexidine gluconate as a buccal patch system (Li et al., 1996, 1997; Senel et al., 2000). Our attempt is the first study on the film preparations as a gastrointestinal mucoadhesive delivery system. From this rat study, it has been demonstrated that changing the pH-sensitive surface layer of the mucoadhesive film systems can control the retention and/or transit of the film preparations. In our earlier study, G-CSF was formulated in GI-MAPS, in which the same pH-sensitive surface layers as used in this study were used (Eaimtrakarn et al., 2001). Among them, Eudragit L100 system showed the highest pharmacological availability of G-CSF in dogs, i.e. 23% as compared with the i.v. injection. Smith compared the pH values of the contents of different parts of the alimentary tract in animals of 15 different species, where the pHs in the anterior portion of stomach were 5.5 (dog) and 5.0 (rat), that in the posterior portion were 3.4 (dog) and 3.3 (rat). The pHs of the upper small intestine were 6.2 (dog) and 6.5 (rat), that of middle part of the small intestine were 6.0 (dog) and 6.8 (rat), and that of lower small intestine were 7.5 (dog) and 7.1 (rat). The pHs of the colon were 6.5 (dog) and 6.6 (rat), respectively. Based on these results, Eudragit L100 system was thought to dissolve in the dog jejunum thereby resulting in the adherence of the film system to the jejunum wall, where the hydrolytic enzyme activity is lower than that of duodenum and the effect of intestinal content is less than that of ileum.

According to a recent report (Zheng et al., 1999), the hydrolytic enzyme activity is still high in the jejunum and the ileum has the lowest hydrolytic enzyme activity. By considering the hydrolytic enzyme activity alone, it can be predicted that the system which dissolves and adheres in the ileum is the best for oral protein delivery. However, besides the hydrolytic enzyme activity, one must consider the other barrier, i.e. membrane permeability. Morishita et al. (1993) studied the pharmacological activity of insulin delivered by enteric microspheres (MS). The MSs were prepared with Eudragit L100, S100 and a 1:1 mixture of these enteric polymers. The study indicated that Eudragit L100 MS showed the highest insulin activity after oral administration in rats. They explained the results by assuming that insulin release from the Eudragit L-MS occurred at higher concentrations in the jejunum to upper ileum as compared with that of Eudragit S-MS. In addition, other reports have shown that the enhanced absorption of octreotide occurred after administration into the rat jejunum (Fricker et al., 1992). However, more elaborate studies are required to understand the reason for better absorption of protein/peptide drugs from jejunum.

With respect to the adhesiveness of oral preparation, Akiyama et al. (1993, 1994, 1995) developed an adhesive micro matrix system (AdMMS). In their study, carboxyvinyl polymer particles were formulated as an adhesive clue. After oral administration, the carboxyvinyl polymer particles form gel structure and adhere to the gut wall, especially stomach. Targeting function in the GI tract is not involved in their system. In this study, film preparations were administered to rats in the

fasted condition. Especially, the rats were fasted over 12 h and consequently the small intestine did not contain any food debris. Therefore, the administered films were completely adhesive to the intestinal wall. However, when films were administered in the fed condition, the adhesion of the films would be affected. With respect to this point, precise study must be performed.

In conclusion, four-layered film preparations had a dissolution-site specific mucoadhesion in the rat small intestine and the adhesive layer has a function of mucoadhesiveness to the small intestinal wall. Though depend on the dissolution site, the duration of the adhesion was approximately 2 h in rats. These results support the usefulness of gastrointestinal mucoadhesive film preparations for the oral delivery of macromolecules.

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