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ORIGINAL ARTICLE

Polymeric three-layered particles for the delivery of prednisolone to the lower gastrointestinal tract in rats

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Abstract

Background: The aim of this study is to investigate the feasibility of three-layered particles as a drug delivery system to the lower part of small intestine. *Methods*: The particle surface and basement layers were made of enteric polymer, Eudragit® S100, and water-insoluble polymer, ethylcellulose. Prednisolone (PSL), as a model drug, was sealed with the surface and basement layers. After the administration of the test preparations to the duodenum of rats, blood samples were collected and plasma PSL levels were measured by high-performance liquid chromatography. The retention and transit characteristics of the three-layered particles in rat small intestine were studied by direct observation after abdominal incision up to 8 hours. *Results*: Three-layered PSL particles showed C_{max} of 0.32 ± 0.07 μg/mL and T_{max} at 6 hours, whereas the mean C_{max} and T_{max} of PSL powder, as a reference preparation, were 0.42 ± 0.03 μg/mL and 1 hour, respectively. With the direct observations, after administration of particles, about 77.5% of them were detected in duodenum at 1 hour, 45% in distal jejunum at 3 hours, and 50% in proximal ileum at 4 hours. Then, they were gradually transferred to the lower part of the small intestine at 5–8 hours time intervals. In comparison with PSL powder, three-layered particles delayed the intestinal transit and released PSL during their passage through the small intestine. *Conclusion*: These results suggested that three-layered particles adhered to the gastrointestinal mucosa and sustained the release of drug, resulting in drug delivery to the lower part of gastrointestinal tract.

Key words: Ethylcellulose, Eudragit® S100, gastrointestinal delivery, prednisolone, retention and transit, three-layered particles

Introduction

Controlled drug release technology has attracted increasing interest over recent years and devices incorporating this technology have been introduced into clinical use in several fields of medicinal therapy. Controlled release preparations can maintain the drug in the desired therapeutic range. The advantages of them are (1) localizing delivery of the drug to a particular body compartment, which lowers the systemic drug level; (2) reducing the need for follow-up care and preserving medications that are rapidly degraded by the body; and (3) increasing patient comfort and compliance. Controlled delivery systems are thought to be effective for the oral delivery of drugs used for the treatment of lower gastrointestinal (GI) diseases, such as Crohn's disease or ulcerative colitis[1].

For more than four decades, microcapsules and microspheres have been studied and then applied to pharmaceutical products. Among them, sustained-release subcutaneous injection preparation of leuprolide acetate gave a great contribution to the cancer therapy[2–4]. They are produced by a batch system. The conventional preparation methods of microcapsules and microspheres are classified into two categories: dispersion of the preformed polymers and polymerization of monomers[5,6]. In addition, dispersion method is classified into the three groups: (1) emulsion solvent extraction/evaporation method, (2) phase separation (coacervation) method, and (3) spray-drying method. Scientists have developed many modified methods to improve the disadvantages of the three methods, that is, low drug-loading efficiency and wide variation of the particle size.Many scientists have been struggling for more than three decades to

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solve the low drug-loading efficiency. In addition, conventional microparticles have only one function, controlled-release, because of their spherical shape. However, to date, there is no general solution to these problems of the batch production method.

Several methods of drug delivery to the lower small intestine have been developed: coating with biodegradable polymers; coating with pH-sensitive polymers; GI pressure-controlled release; and prodrug approach[7,8]. We developed a new oral delivery system composed of three-layered structures, where (1) surface layer, (2) drug carrying layer, and (3) basement layer are formed. Earlier, we developed the three-layered particles for the delivery of peptide/protein drug by adhering to the GI wall[9]. This result led to a hypothesis that with an adequate polymer for surface layer, the system is useful for the delivery of oral drugs to a target site of the GI tract such as the lower part of small intestine. Enteric polymers like hydroxypropylmethyl cellulose phthalate (HP®55), methacrylic acid and methacrylic acid methyl ester copolymer (Eudragit®L100), acrylic acid and methacrylic acid ester copolymer (Eudragit® S100) are highly used for preparing oral delivery systems. These polymers do not dissolve in the acidic condition, that is, stomach. However, they dissolve in the neutral and alkaline pH. The threshold pHs are 5.5 for HP55, 6.0 for Eudragit L100, and 6.8 for Eudragit S100. By selecting the proper polymer as an enteric layer, three-layered particles can deliver PSL to the lower part of the GI tract. Therefore, the delivery efficiency of the three-layered particles to the lower part of the GI tract has been studied using PSL as a model drug. Three-layered particles containing PSL with Eudragit S100 layer and ethylcellulose (EC) layer were prepared and administered to rats. Delivery efficiency, retention, and transit characteristics of the three-layered particles were studied from plasma PSL concentration and direct observation of the particles in the rat GI tract.

Materials and methods

Materials

Prednisolone (PSL) was obtained from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). EC (10 cP) and talc (average particle size, 7–12 μm) were obtained from Wako Pure Chemical Industries, Co. Ltd. (Osaka, Japan). Eudragit® S100 (Röhm Pharm, Damstadt, Germany) was obtained through Higuchi Inc. (Tokyo, Japan). Triethyl citrate (TEC), ethanol, polyethylene glycol (PEG) 6000, and PEG 20,000 were procured from Nacalai Tesque Inc. (Kyoto, Japan). All other materials were of reagent grade and were used as received.

Animals

Male Wistar Hannover rats were obtained from Nippon SLC Co. Ltd. (Hamamatsu, Japan). All animal experiments were performed in accordance with the guidelines for animal experimentation of Kyoto Pharmaceutical University. Rats had free access to food and water and were housed in temperature control facility (22 ± 2°C) with a 12 hours light/dark cycle for at least 1 week before use.

Preparation of three-layered particles

Enteric layer solution was prepared by dissolving 675 mg of Eudragit S100, 67.5 mg of PEG 6000, and 0.15 mL of triethyl citrate in 4.5 mL of ethanol. As water-insoluble layer solution, 500 mg of EC dissolved in 4 mL of ethanol was used. Drug-carrying layer solution was prepared by mixing PSL 75 mg with 125 mg of PEG 20,000 melted at 60°C and 300 μL of deionized water. All processes of preparation of three-layered particles were carried out manually. At first, by dropping Eudragit S100 enteric layer solution through conventional syringe with a 23-gauge needle, an array of spots (e.g., 20×20) was formed on Teflon® plate. The plate was kept under room temperature for complete drying of the solvent. The thickness of the dried spots of enteric layer was measured using a Sony μ-mate (Sony Manufacturing System Co., Saitama, Japan). The diameter and thickness of the enteric layer were approximately 2.0 mm and 70 μm, respectively. Second, using a microsyringe, a drop of the drug layer solution (0.5 μL) was placed in the center of enteric layer spots. After complete drying of drug layer, water-insoluble layer was formed by discharging EC solution over the drug layer and the plate was kept under room temperature to dry the water-insoluble layer. Thereafter, prepared hemispherical three-layered particles were carefully removed from the plate, mixed with talc powder to prevent from coagulations, and used in the following experiments.

Drug contents in three-layered particles

The mean PSL content in the prepared three-layered particles was assayed with 20 particles. A particle was put in a 1.5-mL microtube and dissolved with 1 mL of phosphate-buffered saline (PBS, pH 6.8) by vortexing for 15 minutes. After centrifugation, 100 μL of supernatant was used for high-performance liquid chromatography (HPLC) assay.

Dissolution study

Dissolution experiments of PSL from three-layered particles were performed using NTR-6100 apparatus (Toyama Sangyo Ltd., Osaka, Japan) according to the dissolution test method 1, basket method, outlined in the Japanese Pharmacopoeia Fifteenth Edition (JP 15) with some modifications as follows: at first each particle was put in 900 mL of 0.1 N HCl (pH 1.2) maintained at 37°C and dissolution study was started at a rotation paddle speed of 50 rpm. Samples, 5 mL, were collected at 0, 0.5, 1, 1.5, and 2 hours from each vessel and then filtered through a 0.45-μm membrane filter (Millex-HV, Millipore Co., Billerica, MA, USA) and the same volume of fresh medium was replaced. Subsequently, at 2 hours,

the dissolution medium was changed to PBS (pH 6.8) maintained at 37°C according to the United States Pharmacopeia 26 (USP 26). Likewise, 5 mL of the dissolution samples was withdrawn at predetermined time intervals and then filtered. The same volume of fresh medium was replaced. A 100 μL of the filtrate was assayed by LC/MS/MS analysis.

Absorption experiment in rats

Male Wistar Hannover rats of body weight 352–425 g were used after fasting them overnight for at least 12 hours. They were divided into two groups: Group 1 received three-layered PSL particles and Group 2 received PSL powder. After blank blood sampling, 0.25 mL blood, was obtained from the left jugular vein under light ether anesthesia, a small incision of 0.5 mm was made in the stomach near to pylorus. PSL powder or three-layered particles were loaded into a cannula (i.d. 3.5 mm) at PSL dose of 15 mg/kg under drying conditions. For example, in the case of the administration of three-layered particles to a rat weighing 400 g, 70 particles were loaded according to the mean PSL content of particles as described below, 85.94 ± 8.44 μg/particle. Subsequently, test preparations were administered into the duodenum through an incision in the stomach by flushing with 0.5 mL of water. After administration, abdominal incision was sutured and rats were placed back in individual cages. Blood samples, 0.25 mL, were obtained with heparinized syringes from the left jugular vein at 1, 2, 3, 4, 5, 6, 7, and 8 hours without constraint. Plasma fraction was obtained by centrifugation at 15, 780 × *g* for 10 minutes at 4°C (Kubota 1720 centrifuge, Tokyo, Japan) and then stored at −80°C until HPLC analysis.

Retention and transit experiment in the rat GI tract

To study the retention and/or transit of three-layered particles in the rat small intestine, abdominal incision was made under light ether anesthesia and 20 pieces of three-layered PSL particles were administered into the duodenum of rats through a cut on the stomach near to pylorus. After suturing, rats were placed back in individual cages. At 1, 2, 3, 4, 5, 6, 7, and 8 hours after the administration of particle preparation, rats were killed and abdominal incision was performed. The whole GI tract from the stomach to the cecum was removed and the small intestine from the pyloric sphincter to the ileocecal junction was equally marked into five fractions (section #1, duodenum; section #2, proximal jejunum; section #3, distal jejunum; section #4, proximal ileum; and section #5, distal ileum) of approximately 10 cm length and the number of retaining PSL particles in each fraction was visually counted. The image of the distribution characteristics of the test particles was recorded by a digital camera Nikon D200 (Nikon, Tokyo) at 3, 5, and 8 hours.

HPLC and LC/MS/MS assay methods

The rat plasma PSL concentrations were analyzed by modifying the HPLC assay method reported by AbuRuz et al.[10] and Zhang et al.[11] Namely, 100-μL aliquots of rat plasma sample were mixed with 0.1 mL of 0.5 M NaH_2PO_4 and 0.75 mL of distilled water in a glass tube. Thereafter, 4 mL of ethyl acetate was added to extract PSL from the plasma matrix. The resultant mixture was well mixed and was centrifuged at 1,958 × *g* for 10 minutes. Ethyl acetate fraction was decanted into a new glass tube and was evaporated to dryness at 60°C under the flow of nitrogen gas. After complete evaporation, 100 μL of the mobile phase was added and mixed well of which 20 μL was injected into an isocratic-HPLC system. The HPLC system for PSL analysis consisted of a pump, Shimadzu LC-10AS (Kyoto, Japan), and Shimadzu UV-VIS Detector (Kyoto, Japan). The column used was Cosmosil 5C18 AR-2 (4.6 × 250 mm, Nacalai Tesque, Kyoto, Japan). The mobile phase was the mixture of water and acetonitrile (70 : 30). The flow rate of the mobile phase was 1.0 mL/min and the column temperature was 55°C. PSL eluted from the analytical column was detected by a UV detector at a detection wavelength of 240 nm. The plasma PSL concentrations were determined from the calibration curve. The mean extraction efficiency of PSL from plasma was approximately 80%. The calibration curve of PSL in the spiked plasma samples was linear over the range of 0.05–2.0 μg/mL and passed through the origin.

The dissolution test samples were analyzed by modifying the LC/MS/MS assay method reported by Chen et al.[12] The extraction procedure was the same as HPLC method. However, 20 μL of the extracted sample was injected into the following LC/MS/MS system. The LC/MS/MS system consisted of an API 3200 triple quadrupole mass spectrometer equipped with turbo ion spray sample inlet as an interface for electrospray ionization (ESI), an analyst workstation (Applied Biosystems, Carlsbad, CA, USA), a micropump (LC-10AD, Shimadzu Co., Kyoto, Japan), and an automatic sample injector (AS8020; Tosoh Co., Tokyo, Japan). The mobile phase, 10 mM ammonium acetate : acetonitrile (30 : 70, v/v), was degassed and pumped through an ODS column (2.1 mm i.d. × 100 mm, 3 μm size, Quicksorb; Chemco Scientific Co. Ltd., Osaka, Japan) at a flow rate of 0.2 mL/min. The column temperature was maintained at 25°C. For PSL detection, the transitions of m/z 361.2→325.2 were optimized for the following conditions. Ionization occurred through the turbo ion spray inlet in the positive ion mode. The flow rates of curtain gas and collision gas were set at 10.0 and 5.0 L/min, respectively. The ion spray voltage and temperature were set, respectively, at 5.5 kV and 500°C. The declustering potential, the entrance potential, the collision energy, and the collision cell exit potential were set, respectively, at 27.0, 4.0, 15.0, and 28.0 V. The calibration curve of PSL in the spiked plasma samples was linear

over the range of 1.0–250 ng/mL and passed through the origin.

Pharmacokinetic analysis

The following pharmacokinetic parameters were determined from the plasma PSL concentration–time data. The maximum plasma drug concentration, C_{max}, and the time when plasma PSL concentration reaches C_{max}, T_{max}, were obtained as the measured values. The area under the plasma PSL concentration versus time curve from time 0 to the time of the last measurable plasma concentration (AUC_{0-t}) was calculated by linear trapezoidal rule, followed by an extrapolation to infinity ($AUC_{0-\infty}$) using the correction term, Cp_{last}/λ_z, where Cp_{last} and λ_z are the last measurable plasma concentration and the terminal elimination rate constant, respectively. The terminal elimination half-life ($t_{1/2}$) was determined by dividing ln 2 by λ_z. The area under the first-moment curve to the last measured plasma concentration (AUMC) was also calculated using the linear trapezoidal rule and the addition of the concentration term after the last measured point (t_{last}) to infinity, namely, $t_{last} \times Cp_{last}/\lambda_z + Cp_{last}/\lambda_z$. The mean residence time (MRT) was calculated by AUMC/AUC. The apparent total body clearance (CL_{tot}) was calculated by dose/$AUC_{0-\infty}$.

Statistical analysis

All values are expressed as their mean ± SE. Statistical differences were assumed to be reproducible when $P < 0.05$ (t-test).

Results

Drug contents and dissolution study of three-layered particles

Three-layered PSL particles were designed as a multiple unit system. Therefore, at first drug contents and dissolution characteristics were studied with each unit, a particle. The mean particle diameter obtained in this study was 1.96 ± 0.32 mm (n = 20, mean ± SD), where the relative standard deviation (RSD) was 16.1%. The PSL contents in the prepared three-layered particles were 85.94 ± 8.44 μg/particle (n = 20, mean ± SD), where the RSD was 9.82%. The release rate of PSL from three-layered particle was studied and the result is shown in Figure 1. After the start of the experiment, PSL was not released from the particles for 2 hours, where pH 1.2 HCl medium was used to simulate the gastric pH. By changing the dissolution medium from acidic one to pH 6.8 PBS, which simulated the intestinal pH, PSL was gradually released and was released within 3 hours after changing the medium to enteric one (totally 5 hours).

Absorption experiment in rats

In vivo pharmacokinetic evaluations of PSL powder and three-layered particles were performed using rats, and

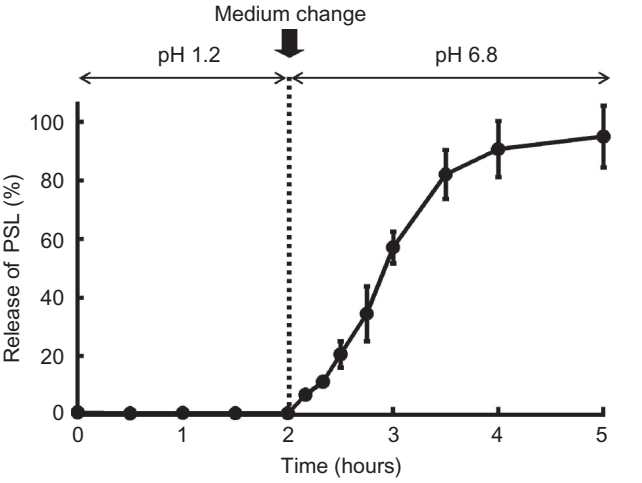

Figure 1. In vitro dissolution profile of PSL from three-layered particle. pH 1.2 HCl solution was used to simulate the gastric pH for 2 hours. Thereafter, dissolution medium was changed to pH 6.8 PBS to simulate the intestinal pH according to USP 26. Each point shows the mean ± SD (n = 6).

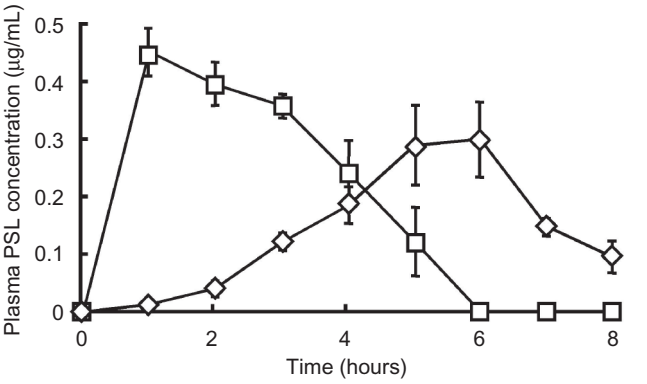

Figure 2. Plasma PSL concentration versus time curves after intraduodenal administration of test preparations, (◇) three-layered PSL particles, and (□ PSL powder to rats, 15 mg/kg. Three-layered particles were composed of three layers: Eudragit S100 surface layer, drug-carrying layer, and basement layer made of EC. Each point shows the mean ± SE (n = 6).

the plasma PSL concentration versus time curves after administration to rats are shown in Figure 2. The obtained pharmacokinetic parameters are summarized in Table 1. After administration of PSL powder into the duodenum of rats, the plasma PSL concentration started to increase immediately and reached its peak level, T_{max}, at 1.6 ± 0.3 hours, where the mean C_{max} was 0.42 ± 0.03 μg/mL. The mean AUC and MRT were 1.45 ± 0.16 μg·h/mL and 2.4 ± 0.1 hours, respectively. On the contrary, after administration of three-layered PSL particles, PSL started to appear in the systemic circulation at about 2 hours after administration and reached T_{max} at 5.5 ± 0.2 hours, where the mean C_{max} was 0.32 ± 0.07 μg/mL. The mean AUC and MRT of three-layered PSL particles were 1.31 ± 0.17 μg·h/mL and 6.3 ± 0.3 hours, respectively.

Table 1. Pharmacokinetic parameters of PSL after administration of three-layered particles to rats.

Formulation	$AUC_{0-\infty}$ (μg. h/mL)	$t_{1/2}$ (h)	MRT (h)	CL_{tot} (L/min/kg)	T_{max} (h)	C_{max} (μg/mL)
Powder	1.45 ± 0.16	0.3 ± 0.04	2.4 ± 0.1	11.05 ± 0.05	1.6 ± 0.3	0.42 ± 0.03
Three-layered particles	1.31 ± 0.17	1.9 ± 0.4*	6.3 ± 0.3*	12.53 ± 0.22	5.5 ± 0.2*	0.32 ± 0.07

$AUC_{0-\infty}$, area under the plasma PSL concentration versus time curve from time 0 to infinity; $t_{1/2}$, terminal elimination half-life; MRT, mean residence time; CL_{tot}, apparent total body clearance. Each value represents the mean ± SE of six rats. *$P < 0.05$, significantly different from reference preparation.

Retention and transit experiment in the rat GI tract

The direct inspection study was performed with abdominal incision at 1, 2, 3, 4, 5, 6, 7, and 8 hours after administration and the results are shown in Figure 3. The typical photographs of the small intestinal tract at 3, 5, and 8 hours after administration of 20 pieces of three-layered particles into the rat duodenum are shown in Figure 4. At 1 hour after administration, about 77.5% of the particles were detected in section #1, duodenum. Then, 47.5% of the particles were transferred to section #2 at 2 hours, proximal jejunum. At 3 and 4 hours, they were transferred to sections #3 and 4, distal jejunum 45% and proximal ileum 50%, respectively. Thereafter, they were gradually transferred to the lower part of the small intestine at 5–8 hours time intervals. All particles were detected in section #5 at 8 hours after administration (Figure 3).

Discussion

Site-specific delivery of oral drugs is useful for the treatment of a specific disease such as ulcerative colitis. We developed a method to prepare three-layered particles composed of surface layer, drug-loading layer, and basement layer one above the other[13,14]. Most conventional preparation methods of microcapsules and microspheres have the disadvantages that drug-loading efficacy is relatively low and the additional function of products might be limited to be only one due to their spherical shape. The advantages of the three-layered particles are the following: (1) drug-loading efficiency is very high, theoretically 100%; (2) size variation of the obtained particles is low; and (3) the obtained particles have not only sustained-release function but also other functions like site selectivity, targeting, and adhesiveness. In this study, the mean particle diameter obtained

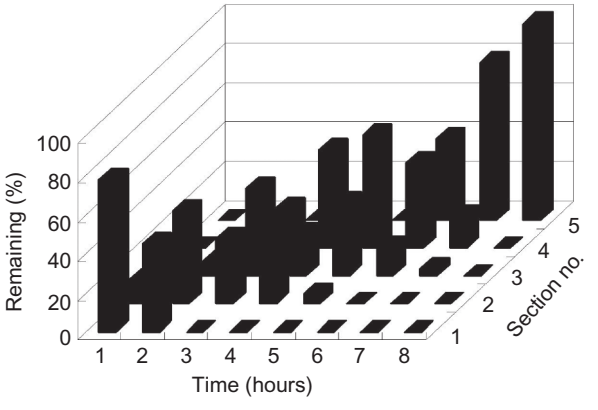

Figure 3. Transit and retention of three-layered PSL particles in the rat small intestine after administration of particles in the rat duodenum. The whole GI tract from the stomach to the cecum was removed and the small intestine from the pyloric sphincter to the ileocecal junction was equally marked into five fractions (section #1, duodenum; section #2, proximal jejunum; section #3, distal jejunum; section #4, proximal ileum; and section #5, distal ileum) of approximately 10 cm length and the number of retaining PSL particles in each fraction was visually counted. Each bar shows the mean of three to four rats.

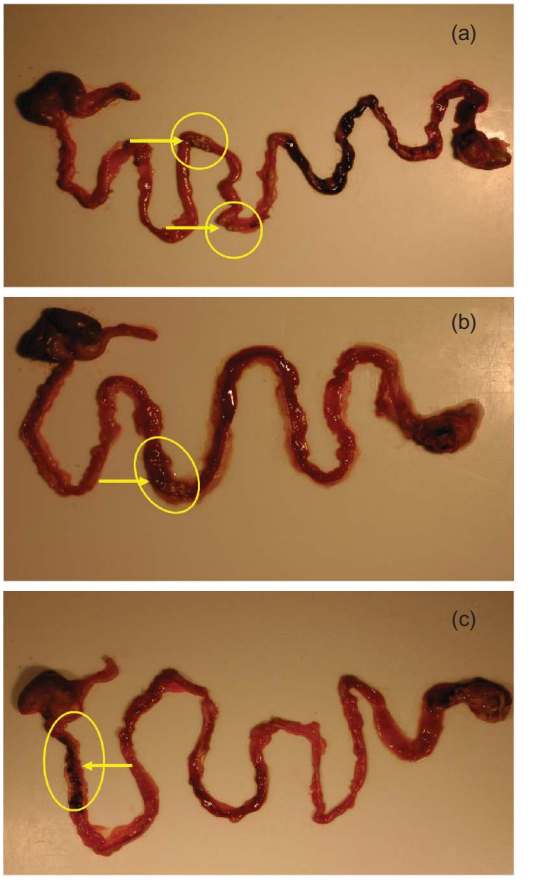

Figure 4. Photographs of the small intestinal tract at (a) 3 hours, (b) 5 hours, and (c) 8 hours after intraduodenal administration of three-layered PSL particles. After 20 pieces of three-layered PSL particles were administered into the rat duodenum, whole GI tract was removed at the predetermined time. Arrows and circles indicate the locations of three-layered particles.

was 1.96 mm, where the relative standard deviation was 16.1%. It was thought to be relatively large because of handmade preparation in this study. However, the aim of this study was to investigate the feasibility of three-layered particles as the delivery system of drugs to the lower part of the rat small intestine.

In the original protocol for in vivo experiments, three-layered PSL particles were administered to the rat stomach using a cannula. However, it was difficult for three-layered particles to pass through the rat pylorus because the size of prepared particles in this study, 1.96 mm, was relatively large for rats. Therefore, the test preparations were administered into the rat duodenum. After the administration of PSL powder into the duodenum of rats, T_{max} and C_{max} were 1.6 ± 0.3 hours and 0.42 ± 0.03 μg/mL; PSL powder rapidly showed high plasma PLS concentration and disappeared from the systemic circulation (Figure 2 and Table 1). On the contrary, after the administration of three-layered PSL particles, plasma PSL concentration was gradually increased and reached T_{max} at 5.5 ± 0.2 hours. In addition, the MRT of three-layered PSL particles was 2.63 times longer than that of PSL powder. From these results, PSL powder was thought to be well dispersed in the rat small intestine, dissolved and was absorbed during the passage through the whole small intestine. On the contrary, PSL was slowly released from the three-layered particles.

Examination of the GI transit profiles is essential to evaluate the delivering system of drugs used for the treatment of the lower GI tract like ulcerative colitis. In this study, the retention and transit experiment showed the long retention of three-layered particles in the small intestine. Especially, at 5 and 6 hours after administration of three-layered particles, the particles were mainly detected at sections #4 and #5, which correspond to the lower small intestine (Figures 3 and 4). Furthermore, by taking into account the plasma PSL concentration versus time curves after administration of three-layered particles, it was strongly suggested that PSL was slowly released and delivered to the lower part of the rat small intestine where PSL was absorbed.

Sugimoto et al. reported that polyvinyl alcohol (PVA) gel or chitosan (Ch)/PVA gel microspheres with similar size (a few micrometers to a few dozen micrometers) could move fairly fast to lower intestine[15], namely, they were distributed mainly in lower small intestine at 3 hours, more than 60% of them reached the large intestine within 4.5 hours, and almost all of them reached the large intestine at 6 hours. Therefore, Eudragit L100-coated chitosan/succinyl–PSL conjugate microspheres (Ch-SP-MS/EuL) or regenerated microparticles might show fairly fast GI transit. On the contrary, Eaimtrakarn et al. reported that the film system preparation consisting of HP55, Eudragit L100, and Eudragit S100 was found to deliver drugs to the upper, middle, and lower part of the small intestine at 1, 2, and 4 hours, respectively, after administration[9]. This study suggested that changing the pH-sensitive surface layer of the mucoadhesive film systems can control the retention/transit of the film preparation. The adhesion and retention of film system to the intestinal site is pH-dependent. The dissolution threshold pH of HP55, Eudragit L100, and Eudragit S100 is 5.5, 6.0, and 6.8, respectively. The pH of the distal ileum was reported to be 6.8–7.1[16]. The luminal pH of the colon is about 7.0. As the threshold pH of Eudragit S is 6.8, it was easily speculated that Eudragit S100 did not dissolve immediately after delivery to the colon. Therefore, release of PSL from the three-layered particles occurred slowly and it was considered that sustained-release characteristics were provided by Eudragit S100 layer. From the MRTs obtained in this study, Eudragit S100 layer was thought to have two functions (1) colon delivery and (2) sustained-release.

As colon delivery system, several drug delivery systems like pressure-controlled system, time-dependent system, pH controlled system, and biodegradable systems have been reported[17]. Most of these systems are prepared by a single unit system such as capsule or tablet. Asghar and Chandran prepared a matrix-based tablet comprising hydrophilic polymers, polycarbophil and Carbopol®, in combination with pH-sensitive polymers, Eudragit L100 or Eudragit S100, and successfully reported the colon-specific delivery of indomethacin as a model drug in healthy human subjects[18]. However, there is no guarantee that these systems work completely in the GI tract because the physiological condition of the GI has a wide variation due to the physiological change, disease state, and posture condition of the patients. In addition, digested food affects the performance of these delivery systems. To decrease the effect of these factors on the colon delivery efficiency of the oral drug delivery system, multiple unit system is thought to be better than single unit system.

Although handmade three-layered particles were used in this report, a machine to produce three-layered particles is under development, where each particle is prepared by discharging polymer and/or drug solutions through a micronozzle that has been used in printing technology. In the next stage of this study, we will prepare the three-layered particles with the machine and evaluate them based on the results obtained in this report.

Conclusion

Three-layered GI mucoadhesive particles composed of enteric layer (Eudragit S100 layer), drug layer, and basement layer were examined in rats for the delivery of PSL to the lower part of GI tract. In a direct observation experiment, three-layered particles were localized in the lower part of the small intestine, near to ileocecum, at 8 hours after administration. Pharmacokinetics study based on the plasma PSL levels versus time profile suggested that PSL was released gradually from three-layered

particles after delivery there. These results suggest that three-layered particles adhered to the GI mucosa and sustained the release of drug, resulting in drug delivery to the lower part of small intestine tract.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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