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Integrated Drug Delivery for Ulcerative Colitis Treatment: Colonic Drug Targeting and Controlled Release

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Published Date: February 26, 2016

ABSTRACT

The author has been developed specific delivery systems of an anti-inflammatory drug for the treatment of ulcerative colitis. Many other researchers have also been challenging the same subject. The author designed and developed delivery systems using macromolecular prodrug approach for controlled release and enteric coating for specific delivery to the lower intestine. Namely, Eudragit-coated chitosan-succinyl-prednisone (Ch-SP-MP/Eu) were produced, and examined *in vitro* and *in vivo*. The microparticles had small size and acceptable drug content, suppressed release at gastric conditions and exhibited very slow and gradual release at intestinal pH. They promoted anti-inflammatory effects and improved toxic side effects *in vivo* using rats with TNBS-induced colitis. These excellent effects were consistent with good lower intestine-specific delivery, prolonged drug release and suppressed systemic absorption. Ch-SP-MP/Eu are suggested to be a superior integrated delivery system which allows prolonged drug supply specifically to the diseased colonic sites.

Keywords: Eudragit-coated chitosan-succinyl-prednisolone conjugate microparticles; Efficacy, Toxic side effect; Lower intestine-specific delivery; Prolonged drug release; Suppressed systemic absorption

INTRODUCTION

Infammatory bowel disease (IBD) is a serious problem in various developed countries [1,2]. Ulcerative colitis (UC) and Crohn's disease (CD) are major forms of IBD, and crucial diseases because they are often severe, refractory and chronic [3,4]. These diseases are regarded as autoimmune ones, and characterized by inflammation based on uncontrolled response of gastrointestinal immune system. IBD is considered to be induced by individual genetic factors and environments such as foods affecting enteric microflora. It can cause abdominal pain, chronic diarrhea and bleeding. Its etiology is not clear, and considered to be due to complex interplay among genetic, environmental, microbial and immune factors [5-7]. Therefore, a symptomatic therapy is mainly conducted in the pharmacotherapy of IBD [8]; recently, antibody drugs have been found to highly effective to suppress the inflammation [9,10]

As to UC, diseased area is limited to large intestinal sites. The chronic inflammation is caused on the intestinal mucosa [11]. Its treatment is performed mainly by pharmacotherapy using anti-inflammatory drugs or surgical removal of diseased regions. Although antibody drugs are highly effective, its high cost and difficulties in use, related to toxic side effects, are big problems, resulting in the fact that conventional anti-inflammatory drugs are still important and commonly used for the treatment of UC. Since UC is limited in the large intestine, the drug delivery to the lower intstine has been challenged in order to enhance the efficacy and reduce the toxic side effects. Namely, various chemical modifications [12-16] or drug delivery systems [17-22] have been developed in order to obtain a highly effective and low-toxic therapeutic system against UC. 5-Aminosalicyric acid (5-ASA), and steroidal and non-steroidal anti-inflammatory drugs are frequently chosen [23]. However, when these drugs are administered in simple conventional oral dosage forms, they are absorbed systemically to a large extent and not delivered efficiently to the disease sites, resulting in less effectiveness and greater toxic side effects. Therefore, in order to achieve of high effectiveness and low toxicity, it is necessary to deliver a drug specifically to the target site and supply the drug efficiently for a sufficient period. Salazosulfapyridine is a prodrug of 5-ASA, and releases 5-ASA in the colon based on reductive action by colonic bacteria [24,25]. Although salazosulfapyridine is clinically available, sulfapyridine, regenerated from salazosulfapyridine, can cause toxic side effects due to the intestinal absorption of sulfapyridine. A commercial tablet, Pentasa, is a delayed release system of 5-ASA around the colonic region, and is considered to be less toxic than salazosulfapyridine because it releases only 5-ASA [26]. Asacol has also been available as delayed release system of 5-ASA with enteric coating polymer [27]. Chitosan capsules containing 5-ASA have also been found to display a good effect as a result of their efficient biodegradation followed by the effective release of 5-ASA in the lower intestine [17].

As compared with 5-ASA, prednisolone (PD) is generally chosen for the treatment of more severe IBD. Although PD displays high efficacy at a low dose, it can induce severe toxic side effects such as immunosuppression [18]; therefore, site-specific delivery of PD is considered to be important to obtain high efficacy and suppress toxicity. Recently, micro- or nano-particulate dosage forms have been reported to be effective to deliver drugs to the intestine, Peyer's patches or colon [28-31]. Microparticles with a diameter larger than 200 μm are not appropriate for long residence at the intestinal disease site due to their elimination by diarrhea, and nanoparticles and microparticles with a smaller size are superior for long residence at the site of colitis because they are trapped by a thick mucous layer [32,33]. Further, microparticles of several hundred nanometers to several micrometers are effectively taken up by macrophages appearing in large numbers [28,31]. Biocompatible and biodegradable polymers are expected to be useful to deliver drugs to inflamed areas, and may accelerate drug release due to enzymes of bacteria and macrophages [17,34,35]. Furthermore, the prolonged supply of an anti-inflammatory drug is shown to be useful for effective treatment [30]. It is considered to be important that the drug is supplied continuously to the diseased area after the delivery of a drug has been achieved.

From those considerations, efficient delivery and prolonged supply of a drug are suggested as key points of the delivery systems for the treatment of UC. The author has been developing the delivery systems by preparing a macromolecular prodrug of PD, subsequently making its microparticles and finally enteric coating of the microparticles [36-39]. Chitosan, being biocompatible and biodegradable natural polymer [40], is employed as a macromolecular support [37,41]. PD was loaded by ester linkage, potentially acting as a prodrug. Eudragits L100 and S100 were applied as enteric coating polymers. Thus, chitosan-succinyl-prednisolone conjugate microparticles (Ch-SP-MP) coated with Eudragit (Eu), named Ch-SP-MP/Eu, were developed. The delivery system was designed to complete efficient delivery to the colonic diseased area and prolonged supply of the drug to the diseased site. The characteristics and functionalities of Ch-SP-MP/Eu were evaluated *in vitro* and *in vivo* by comparing PD itself, simple PD/Eu microparticles, Ch-SP-MP and Ch-SP-MP/Eu [38,39,42,43].

METHODS

Preparation of Microparticles

Simple chitosan microparticles loaded with PD (PD/Ch)

Prednisolone (PD) was added to chitosan (Ch) solution in acetic acid solution, and the suspension was put into liquid paraffin containing Span 80. The resultant mixture was stirred vigorously, and the aqueous phase was evaporated from that emulsion, resulting in simple Ch microparticles containing PD, named PD/Ch [41]. n-Hexan was used to wash PD/Ch.

Chitosan-succcinyl-prednisolone microparticles (Ch-SP-MP)

Ch was dissoloved in HCl aqueous solution, and succinyl-prednisolone (SP) sodium salt was

mixed to the Ch solution. To the solution, water-soluble carbodiimide (WSC) was added as an amide coupling agent. The resultant Ch-SP conjugate was precipitated by the addition of acetone. After Ch-SP was washed with aqueous acetone, it was obtained as powder after lyophilization (Figure 1) [41].

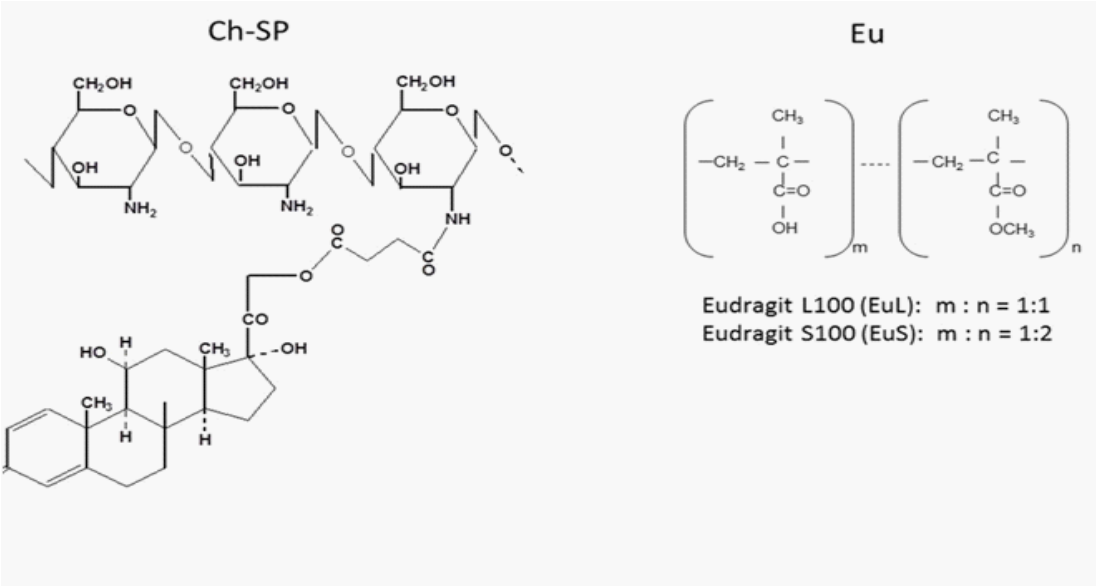

Figure 1: Chemical structures of chitosan-succinyl-prednisolone conjugate (Ch-SP) and Eudragit (Eu).

Ch-SP was dissolved in aqueous acetic acid solution, and the solution was put into liquid paraffin solution containing SO-15. The mixture was stirred vigorously, and the aqueous phase was evaporated under reduced pressure. After Ch-SP microparticles (Ch-SP-MP) were formed in liquid paraffin, they were collected by centrifugation **(Figure 2).** n-Hexane was used for washing the microparticles [36, 38, 42]. Ch-SP1-MP and Ch-SP2-MP were prepared using different lots of CH-SP (Ch-SP1 and CH-SP2, respectively).

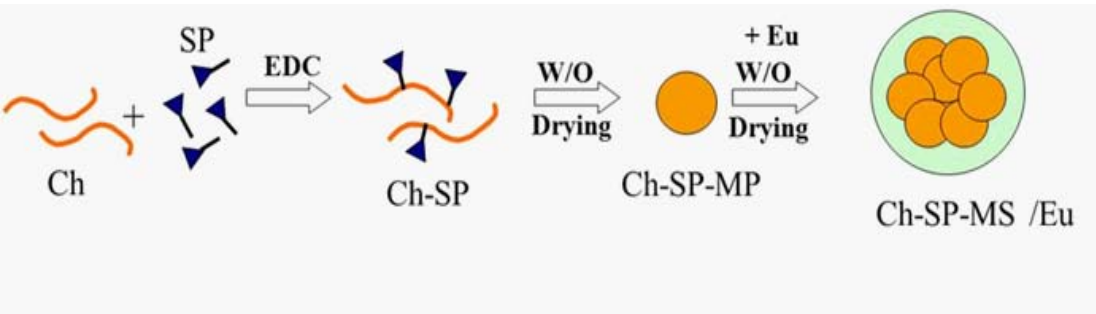

Figure 2: Preparative scheme of Ch-SP microparticles (Ch-SP-MP) and Eu coated Ch-SP microparticles (Ch-SP-MP/Eu).

Eudragit-coated Ch-SP-MP (Ch-SP-MP/Eu)

Eudragit L100 and S100, named EuL and EuS, respectively, were chosen as enteric coating polymers (Figure 1). Ch-SP-MP were added in Eudragit (Eu) solution in methanol, and the suspension was put in liquid paraffin containing SO-15. Methanol was evaporated from the emulsion, resulting in the formation of Eu-coated Ch-SP-MP (Ch-SP-MP/Eu) (Figure 2), in which n-hexane was used to wash the microparticles [36,38,42].

Simple Eudragit microparticles loaded with PD (PD/Eu)

Methanol solution containing EuL or EuS was put into liquid paraffin with SO-15. Methanol was evaporated from the resultant emulsion, and small volume of aqueous acetic acid solution was added and drying was continued under reduced pressure. The resultant simple Eu microparticles loaded with PD (PD/Eu) were washed with n-hexane [42].

The composition of each formulation shown in Table 1, and the detailed preparative methods were written in the references indicated in Table 1.

Table 1: PD-loaded chitosan microparticles (PD/Ch), PD-loaded EuL microparticles (PD/EuL), PD loaded EuS microparticles (PD/EuS), Ch-SP1 microparticles (Ch-SP1-MP), Ch-SP2 microparticles (Ch-SP2-MP), EuL-coated Ch-SP1-MP (Ch-SP1-MP/EuL), EuS-coated Ch-SP1-MP (Ch-SP1-MP/EuS) and EuS coated Ch-SP2-MP (Ch-SP2-MP/EuS)* [38,41,42].

	Ch	PD	Ch-SP	Ch-SP-MP	EuL	EuS	Particle size (μm)	PD content (% w/w)
PD/Ch	80	20					225±8.4	13.2±3.2
PD/EuL		10			90		4.5±3.2	4.1±0.7
PD/EuS		10				90	1.2±0.3	3.7±0.7
Ch-SP1-MP			100				1.3±0.4	4.6±0.7
Ch-SP2-MP			100				2.8±2.2	5.4±0.2
Ch-SP1-MP/EuL				50	50		31.8±11.1	3.2±0.7
Ch-SP1-MP/EuS				50		50	28.4±9.9	3.1±0.6
Ch-SP2-MP/EuS				33		67	18.7±12.3	2.3±0.3

Characteristics of microparticles

The obtained microparticles were investigated for their size, morphology and drug content. They were observed using a scanning electron microscope by JEOL after thin coating with platinum. The Green diameter (Feret diameter) was measured for 100 - 200 microparticles chosen at random from their scanning electron micrographs [38,41,42].

For PD/Ch, the drug content was measured spectrophotometrically at 246 nm after dissolved in 0.1 M HCl aqueous solution [41]. The PD content of Ch-SP-MP and Ch-SP-MP/Eu were investigated by hydrolysis of the ester bond in 0.1 M NaOH aqueous solution and subsequent spectrophotometric measurement (246 nm) of the supernatant [38,41,42]. As to PD/Eu, they

were dissolved in phosphate buffered saline at pH 7.4, and analyzed for the PD concentration by HPLC using an ODS column [38,41,42].

In Vitro Release Experiments

The drug release studies were performed by gently shaking the microparticles in JP16 first fluid (pH 1.2) and second fluid (pH 6.8). At appropriate time points, the supernatant was analyzed spectrophotometrically or HPLC using an ODS column [41,42].

In vivo examination of efficacy and toxic side effect

The *in vivo* experimental protocol was approved by the Committee on Animal Research of Hoshi University, Japan. Every animal experiment was performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, Japan.

After male Wistar rats (ca. 200 g) had been fasted for 48 h, TNBS (20 mg) dissolved in 0.25 ml of 50% (v/v) ethanol was instilled into the colonic section of each ,rat 7cm from the anus with a catheter, thereby inducing UC [17,18,39,44]. Rats were housed for 3 days without any treatment in order to allow the development of a full inflammatory bowel disease model, and used as rats with TNBS-induced colitis. Rats that developed colitis were divided into each group: Control (non-treated), PD(5) (5 mg PD /kg × 3 times (every 24 h)), PD(10) (5 mg PD/kg ×6 times (every 12 h)), Ch-SP1-MP(5) (5 mg PD eq./kg ×3 times (every 24 h)), Ch-SP1-MP(10) (5 mg PD eq./kg ×6 times (every 12 h)), Ch-SP1-MP/EuL(5) (5 mg PD eq./kg ×3 times (every 24 h)), Ch-SP1-MP/EuL(10) (5 mg PD eq./kg ×6 times (every 12 h)), Ch-SP2-MP/EuS(5) (5 mg PD eq./kg ×3 times (every 24 h)), Ch-SP2-MP/EuS(10) (5 mg PD eq./kg ×6 times (every 12 h)), PD/EuS(5) (5 mg PD eq./kg ×3 times (every 24 h)), and PD/EuS(10) (5 mg PD eq./kg ×6 times (every 12 h)). Ch/EuL group was EuL-coated Ch microparticles with no drug. In these groups, the dosing substance corresponding to 5 mg PD eq./kg was suspended in 1.5 ml of saline, and the whole suspension was administered via gastric intubation. Healthy rats were ones with no TNBS treatment.

The evaluation of each substance was performed 10 days after the TNBS treatment. Body weight (B) was measured after the sacrifice of animals with excessive ether anesthesia on that day, and the colon and thymus were excised. The colon was cut into the proximal colon (4-cm colonic segment from the end of the cecum) and distal colon (8-cm colonic segment next to the proximal colon) [18,35]. These segments were rinsed with saline to remove colonic contents, and the resultant proximal colon weight (Cp) and distal colon weight (Cd) were measured. The ratios, Cp/B and Cd/B, were calculated. Furthermore, the MPO activity in the distal colon, which has been used as an index of inflammation caused by the infiltration of activated neutrophils, was measured as an index of inflammation extent [18,35,45,46]. Colonic damage score (CDS) and colitis activity score (CAS) were also observed visually as colitis severity [39,43]. Furthermore, after the thymus had been removed and rinsed with saline, its weight (T) was measured, and the T/B ratio was calculated to evaluate the toxic side effects.

Gastrointestinal Drug Distribution Studies

Gastrointestinal distribution was investigated for PD itself, Ch-SP1-MP/EuL, Ch-SP2-MP/EuS and PD/EuS. They were administered at 5 mg PD eq./kg to rats with TNBS-induced colitis, which was fasted for 24 h or more. The distribution of free PD was examined for the distribution in each gastrointestinal region at early (3 – 4 h post administration) and later period (24 h post administration). The stomach (S), upper half of the small intestine (PI), lower half of the small intestine (DI), cecum (Ce), upper one third of the colon (CP), and lower two-thirds of colon (CD) were excised. For each part, after the whole (tissue + content) or only content was homogenized, the homogenate was diluted appropriately and PD was extracted with the mixture of *t*-butylmethyl ether and pentane (3:2, v/v). The extracted sample was measured for the amount of PD by HPLC. From the PD concentration, the distributed amount of free PD in each part was calculated. It was treated as the index of the drug distribution in the gastrointestinal tract [38,43, 47].

Gastrointestinal Absorption Studies Based On Plasma Concentration

The rats with TNBS-induced colitis were fasted for 24 h or more, PD, ES-MP, and Ch-MP/ES were administered using gastric intubation at 5 mg PD eq./kg with a 1.5-mL saline suspension per rat. Blood samples were withdrawn at appropriate time points. After the plasma was treated in a manner similar to the above, the concentration of PD was determined by HPLC [38,43, 47].

HPLC Assay

HPLC analysis was performed at room temperature using the following apparatuses and conditions. A Shimadzu LC-6AD pump was used with a Shimadzu SPD-10AV VP UV-VIS detector, which was set at a wavelength of 246 nm and connected with a Shimadzu C-R7A Chromatopac. A YMC Pack ODS-AM column (6 mm inner diameter × 150 mm length; YMC Co., Ltd., Kyoto, Japan) was used as the analytical column. A 26 % (v/v) 2-propanol aqueous solution containing 0.1 % (v/v) trifluoroacetic acid was used as the mobile phase, in which the flow rate was set at 1 ml/min and the injection volume was 20 μl. The PD concentration was determined by the absolute calibration curve method.

RESULTS AND DISCUSSION

Particle Characteristics

The chemical structures of Ch-SP and Eu (EuL and EuS) are shown in Figure 1. The preparative scheme of conjugate microparticles (Ch-SP-MP) and enteric-coated microparticles (Ch-SP-MP/Eu) is summarized in Figure 2. The obtained microparticles were obtained as spherical or ellipsoidal shape. PD/Ch, Ch-SP-MS and PD/Eu had fairly smooth surface, but Ch-SP-MS/Eu possessed rough surface. Their mean particle size and the PD content are shown in Table 1 [38,41,42]. PD/Eu and Ch-SP-MP were obtained as fully small microparticles, suggesting that they might be advantageous for the retention in the colonic mucosa.

In Vitro Release Characteristics

The obtained microparticles were investigated for release characteristics at pH 1.2 (gastric condition) and pH 6.8 (intestinal condition). The mean release profile profiles were obtained as shown in Figure 3 [38,42]. As PD/Ch exhibited high initial burst and subsequently very slow release at pH 7.4 [41], they were removed from the tests. All the tested microparticles showed suppressed release at pH 1.2, and the release of PD was observed at pH 6.8. As PD/EuL exhibited fairly fast release of PD at pH 6.8, they were presumed to be dissolved in the small, leading to high systemic drug absorption. PD/EuS showed release of PD gradually and efficiently. Ch-SP1-MP exhibited slow release. Ch-SP1-MP/EuL and Ch-SP1-MP/EuS showed fairly similar release profiles, in which PD was released a little more slowly than in Ch-SP1-MP. As to Ch-SP1-MP/EuL and Ch-SP1-MP/EuS, enteric coating was conducted to a small extent, resulting in the fact that the difference between EuL and EuS appeared to be not observed markedly. Ch-SP2-MP/EuS showed most slowly release rate at pH 6.8. This was considered to be because Ch-SP2-MP/EuS were obtained by enteric coating of Ch-SP2-MP using double amount of EuS as compared with Ch-SP1-MP/EuS. These release profiles were importantly associated with intestinal absorption, drug localization and drug retention. Furthermore, it will be made clear from their relationships with efficacy and toxicity how the release mode should be set in the delivery system.

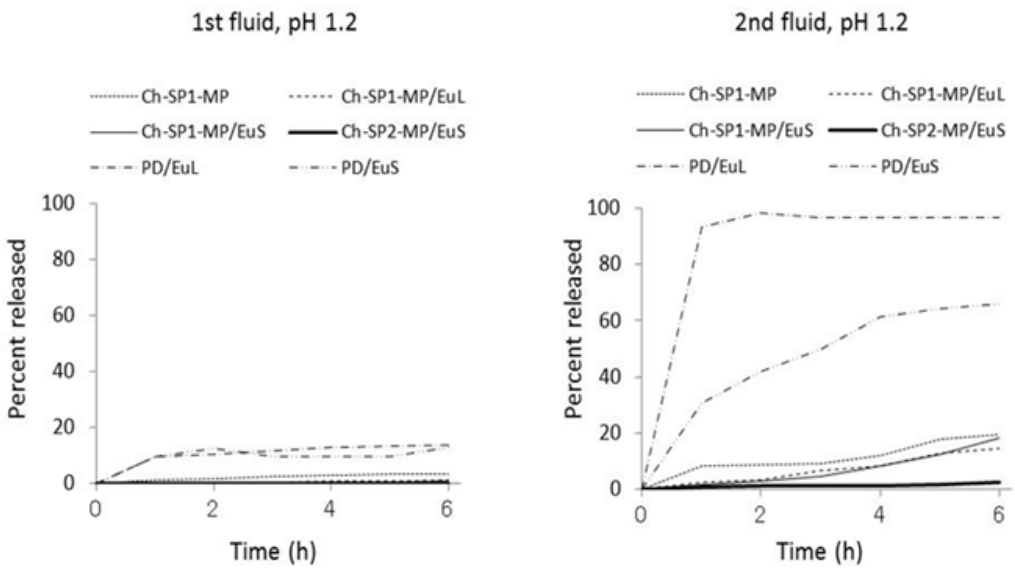

Figure 3: *In vitro* release profiles of PD from Ch-SP1-MP, Ch-SP1-MP/EuL, Ch-SP1-MP/EuS, Ch-SP2-MP/EuS, PD/EuL and PD/EuS in JP 16 1st fluid (pH 1.2)and 2nd fluid (pH 6.8) at 37°c* [38,42].

Efficacy and Toxic Side Effect

The degree of inflammation of the diseased site was investigated using several parameters or indices (Cp/B, Cd/B, MPO activity in CD, CDS, CAS) [39,43]. They can indicate efficacy of each dosage form. Their mean values are summarized in Table 2. PD/EuS hardly enhance the anti-inflammatory effect of PD. Ch-SP1-MP slightly promoted the effectiveness of PD from the decrease in MPO activity and colitis severities such as CDS and CAS. Ch-SP1-MP/EuL and Ch-SP2-MP/EuS showed much higher effectiveness than PD itself. These suggested that enteric coated Ch-SP-MP should be superior in the promotion of PD efficacy in ulcerative colitis.

Table 2: Antiinflammatory effects and toxic side effect of PD alone (PD), Ch-SP1-MP, Ch-SP1-MP/EuL, Ch-SP2-MP/EuS andPD/EuS in intragastric administration at 5 mg PD eq./kg x 3 and 10 mg PD eq./kg x 6 rats with TNBS-induced colitis* [39,43].

Group code	Dose (mg PD eq./kg/day)	Cp/B (ratio to control, %)	Cd/B (ratio to control, %)	MPO activity (ratio to control, %)	CDS (ratio to control, %)	CAS (ratio to control, %)	TB (ratio to control, %)
PD (5)	5	64.5	38.3	83.6	79.0	58.6	70.0
PD (10)	10	65.8	37.3	96.3	72.5	44.4	53.2
Ch-SP1-MP (5)	5	66.1	38.8	81.1	46.7	37.9	89.1
Ch-SP1-MP (10)	10	56.7	23.9	39.22	33.3	17.2	124.6
Ch-SP1-MP/EuL (5)	5	64.4	32.7	65.5	40.0	20.7	103.1
Ch-SP1-MP/EuL (10)	10	47.5	20.5	33.8	20.0	10.3	132.0
Ch-SP2-MP/EuS (5)	5	57.6	30.5	89.2	30.8	27.6	131.3
Ch-SP2-MP/EuS (10)	10	83.1	36.2	51.7	23.1	24.1	101.1
PD/EuS (5)	5	53.7	46.8	106.1	84.6	86.2	72.7
PD/EuS (10)	10	59.1	52.1	126.0	84.6	58.6	35.0
Ch/EuL	-	79.2	63.0	88.5	100.0	100.0	94.3
Healthy	-	45.1	19.5	3.0	0.0	3.4	156.2
Control		100.0	100.0	100.0	100.0	100.0	100.0

Gastrointestinal Disposition Profiles

After intragastric administration of PD alone, Ch-SP1-MP/EuL, Ch-SP2-MP/EuS and PD/EuS to rats with TNBS-induced colitis, the distribution of free PD (released PD) was examined at early (3 – 4 h) and late (24 h) time. As shown in Figure 4, PD was found in stomach and small intestine to a large extent at early periods after administration, but not detected in cecum and colon. No PD was found at late time. In the case of PD/EuS, PD was found in stomach, small intestine and cecum at early period, and no PD was detected at late time. On the other hand, as to Ch-SP1-MP/EuL and Ch-SP2-MP/EuS, PD was not found in stomach and upper intestine, but detected in lower intestine at both early and late time. Although the disposition of PD in Ch-SP1-MP is not shown, PD was found in stomach, upper intestine and lower intestine at 8 h after administration [47]. As Ch-SP1-MP absorb acidic gastric medium and swelled quickly, they can interact with

the gastrointestinal mucosa. This was the reason for long retention of Ch-SP1-MP to the upper intestine. Considering these phenomena, microparticles showing very slow release was superior for the effective localization in lower intestine, and enteric coating enhanced the specific delivery to cecum and colon. Simple coating of PD with EuS was not sufficient to specifically deliver PD to lower intestine. The distribution of total drugs containing conjugated and free PD was investigated for Ch-SP1-MP and Ch-SP1-MP/EuL; the results indicated conjugated PD was also found in the area where free PD was detected, and not found at the site where no free PD was detected [38].

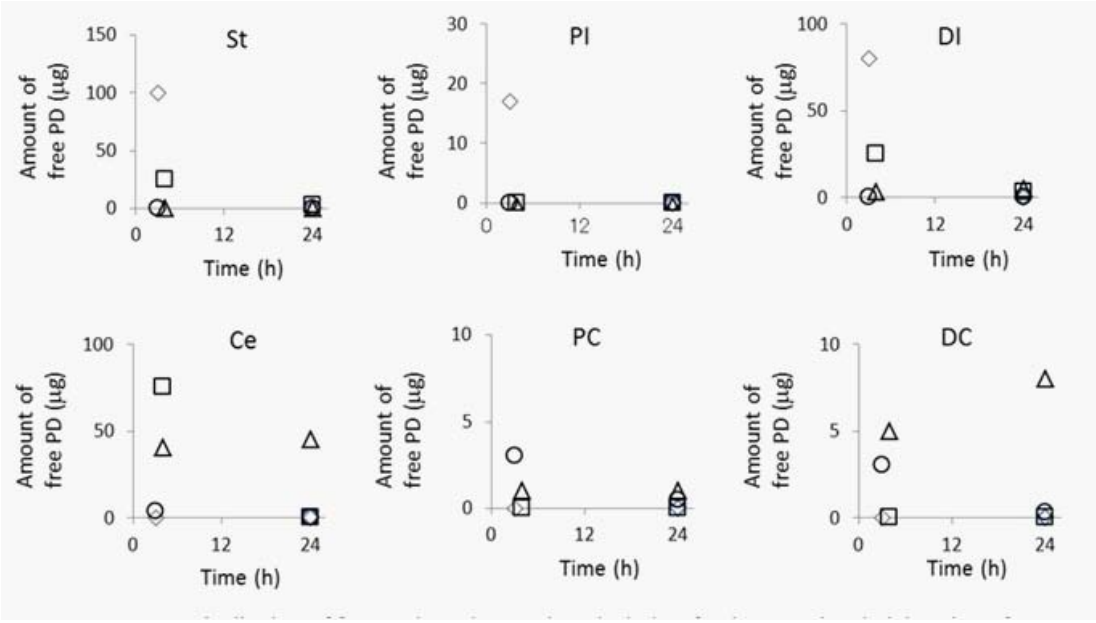

Figure 4: Distribution of free PD in each gastrointestinal site after intragastric site after administration of PD alone (◊), Ch-SP1-MP/EuL (O), Ch-SP2-MP/EuS (Δ) and EuS/PD (□) at 5mg PD eq./kg in rats with TNBS-induced colitis*[38,43].

ST: Stomach, PI: Proximal small intestine, DI: Distal small intestine, Ce: Cecum. PC: Proximal colon, DC: Distal colon.

From these results, microparticles, showing very slow release and undergoing enteric coating, were suggested to be superior for the specific delivery to the colonic region. Although such microparticles did not give high drug distribution, the PD level appeared to be maintained over the minimal effective concentration for a long period [48-50], which was considered as an essential point of the delivery system.

Plasma Concentration-Time Profiles

The plasma concentration of PD after intragastric administration to rats with TNBS-induced colitis was also investigated for PD alone, Ch-SP1-MP/EuL, Ch-SP2-MP/EuS and PD/EuS [38,43]. The results are shown in Figure 5. After the administration of PD, the plasma concentration

increased rapidly and gave high concentration (more than 1.5 μg/mL) at early time, but declined fairly fast and not detected after 7 h. PD/EuS suppressed the plasma levels to some extent, but the PD concentration was maintained for a prolonged period. This observation was consistent with the results of its release profile (Figure 3) and gastrointestinal distribution (Figure 4). Ch-SP1-MP/EuL gave a very small concentration of PD at early time, and no PD was detected after 4 h. As to Ch-SP2-MP/EuS, no PD was detected for the observation period (0 – 24 h). These findings were considered to be due to very slow drug release and good localization to the lower intestine of Ch-SP1-MP/EuL and Ch-SP2-MP/EuS. Although the plasma concentration-time profile was not shown for Ch-SP1-MP, they could suppress the plasma levels as observed in Ch-SP1-MP/EuL. However, Ch-SP1-MP (data not shown) gave the gradual increase in plasma concentration [47], which was considered to be due to their features of long retention in the upper intestine. The plasma concentration was related to the toxic side effect of PD. The thymus atrophy was examined by calculating the T/B values (Table 2). The smaller T/B value represents more severe diseased state. The results in T/B values were consistent with the plasma concentration-time profiles. PD alone and PD/EuS exhibited large reduction of T/B, showing more severe toxicity. On the other hand, Ch-MP1-SP, Ch-SP1-MP/EuL and Ch-SP2-MP/EuS showed higer T/B values rather than conrol; healthy group displays the highest T/B value. Overall, Since Ch-SP1-MP/EuL and Ch-SP2-MP/EuS were found to suppress the systemic absorption of PD, they were evaluated to be superior to improve the drug toxicity caused by systemic absorption.

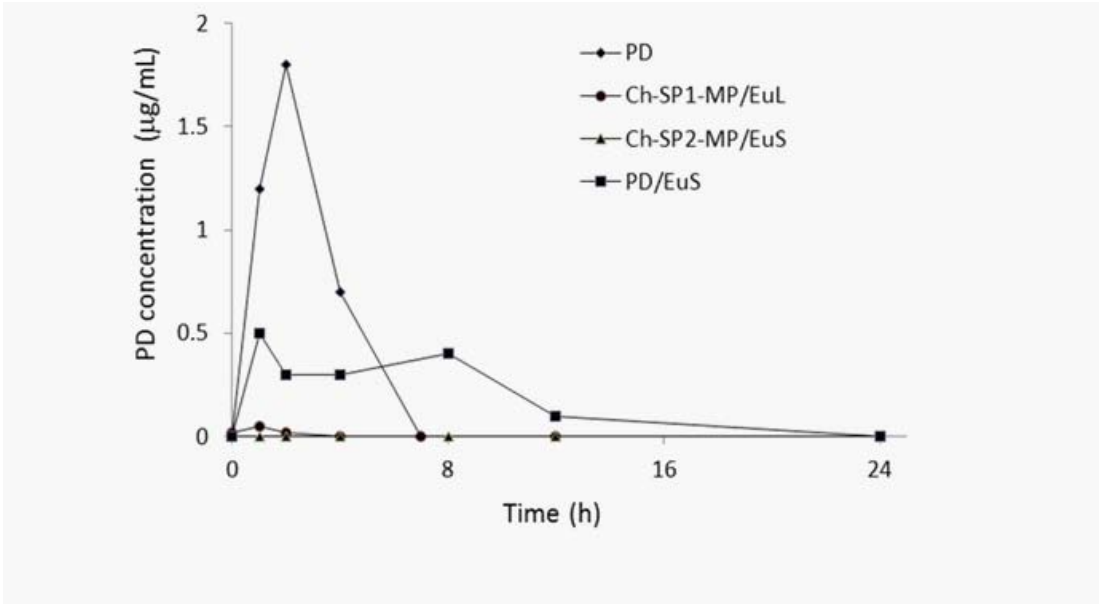

Figure 5: Plasma concentration of PD after intragastric administration of PD alone (PD), Ch-SP1-MP/EuL, Ch-SP2-MP/EuS and PD/EuS at 5 mg PD eq/kg in rats with TNBS-induced colitis* [38,43].

CONCLUSION

Microparticles loaded with PD have been developed for the treatment of ulcerative colitis, and evaluated *in vitro* and *in vivo*. The strategy was mainly composed comprised very slow continuous release and specific delivery to lower intestine. Since ester prodrugs generally exhibit suppression of release at acidic pH and gradually generate a parent drug at neutral or weakly basic pHs, succinyl-prednisolone (SP) was used as an intermediate compound. As chitosan (Ch) is highly safe material in oral ingestion, it was used as base material for a macromolecular prodrug. Chitosan-SP conjugate was obtained by carbodiimide coupling, and was used to obtain its microparticles, named Ch-SP-MP. Since it was necessary to protect Ch-SP-MP from deformation such as swelling at gastric acidic environments, they were coated with enteric-coating polymer Eudragit (Eu) to obtain Eu-caoted Ch-SP-MP (Ch-SP-MP/Eu); actually, Eu L100 or S100, called EuL and EuS, respectively, were used as an enteric coating polymer. In addition, simple Ch microparticles loaded with PD (PD/Ch) and simple Eu microparticles (PD/Eu) were prepared. Ch-SP-MP, Ch-SP-MP/Eu, PD/Ch and PD/Eu were examine *in vitro* (particle chracateristics, release rate) and *in vivo* (efficacy and toxicity, pharmacokinetic features). Ch-SP-MP, Ch-SP-MP/Eu and PD/Eu displayed sufficiently small size and acceptable drug content, and showed suppressed release at gastric conditions and gradual release at intestinal pH. Ch-SP-MP were distributed in the wide range of the gastrointestinal tract due to swelling and interaction with mucosa; although they only gave a low plasma concentration, the PD plasma level increased gradually., suggesting Ch-SP-MP was not sufficient for the specific delivery to the colonic area. On the other hand, Ch-SP-MP/EuL and Ch-SP-MP/EuS achieved a specific delivery of PD to the lower intestine and long retention there. Furthermore, both microparticles promoted antiinflammaory effects and improved toxic side effects *in vivo* using rats with TNBS-induced colitis. From these examinations, enteric-coated Ch-SP conjugate microparticles are suggested found to be superior as an integrated delivery system which can complete prolonged drug supply specifically to the diseased colonic sites.

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