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Note

Efficacy and toxicity of Eudragit-coated chitosan–succinyl-prednisolone conjugate microspheres using rats with 2,4,6-trinitrobenzenesulfonic acid-induced colitis

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ABSTRACT

A targeted delivery system for inflammatory bowel disease (IBD), Eudragit L100 (EuL)-coated chitosan (Ch)–succinyl-prednisolone (SP) conjugate microspheres (Ch–SP-MS/EuL), were designed and examined in vivo for efficacy and toxicity. Their preparation was conducted in the same manner as previously; that is, by synthesis of the conjugate by carbodiimide coupling of Ch and SP, conversion into microspheres (Ch–SP-MS), and coating of Ch–SP-MS with EuL. Experimental colitis was induced by instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS) into the colon in rats. Drugs were administered once or twice a day intragastrically for three consecutive days. Visible colitis severity, colon/body weight ratio and myeloperoxidase activity were measured as inflammatory indices. Toxicity was examined from the decrease in the thymus/body weight ratio. Efficacy was dose-dependent and the greatest in the order Ch–SP-MS/EuL > Ch–SP-MS > prednisolone (PD) alone, and Ch–SP-MS/EuL showed excellent recovery of colitis states. Toxicity was the greatest in the order PD ≫ Ch–SP-MS > Ch–SP-MS/EuL. Ch–SP-MS and Ch–SP-MS/EuL reduced significantly the thymic atrophy caused by PD. It was demonstrated that Ch–SP-MS/EuL enhanced effectiveness of PD and reduced toxic side effects of PD greatly. Also, these results established the prediction by previous in vitro and in vivo studies.

1. Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, is getting a critical problem as a severe, chronic and refractory disease with the increase in the westernized style in Japan. IBD appears to be induced by various factors such as the individual genetic background and environment affecting enteric flora and the intestinal immune system (Fiocchi, 2002; Danese and Fiocchi, 2006). Anti-inflammatory drugs are used for ordinary treatment (Ardizzone and Bianchi Porro, 1998). 5-Aminosalicylic acid-related drugs such as salazosulfapyridine are used under mild or moderate disease conditions, while steroids and immunosuppressive drugs are required for the treatment of more severe inflammation. However, the use of latter types of drugs is seriously restricted because of their toxic side effects (Campieri et al., 1997), which are related to systemic absorption. Therefore, targeted drug delivery systems to the diseased sites have been developed to improve the efficacy and to reduce toxic side effects (Kesisoglou and Zimmermann, 2005). Micro- or nanoparticles have been examined as a delivery system to improve efficacy and adverse effects. Micro- and nanoparticles with a diameter of less than 10 μm are retained well at colitis sites with a thicker mucous layer, but dosage forms such as tablets and pellets are excreted easily with diarrhea (Watts et al., 1992; Lamprecht et al., 2001a).

Since chitosan (Ch) is a biocompatible and biodegradable polymer (Tozaki et al., 2002; Azab et al., 2006), we have developed its microparticles containing prednisolone (PD) as a targeted oral delivery system against IBD. Simple microparticles with chitosan as a matrix polymer could not control release rate easily (Onishi et al., 2005); therefore, we synthesized chitosan–succinyl-prednisolone conjugate (Ch–SP) as a macromolecular prodrug of PD for release control, prepared the microspheres (Ch–SP-MS) using Ch–SP, and then produced Eudragit L100-coated Ch–SP-MS (Ch–SP-MS/EuL). Until now, we have characterized Ch–SP-MS/EuL in vitro and in vivo. Namely, Ch–SP-MS/EuL suppressed release at stomach pH, but exhibited gradual release at intestinal pH (Oosegi et al., 2008a). After oral administration to rats with 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis, Ch–SP-MS/EuL realized drug distribution mainly in the lower intestine, especially around the cecum, over 24 h, while PD alone showed drug distribution only in the stomach and small intestine and fairly rapid elimination (Oosegi et al., 2008b). Ch–SP-MS/EuL allowed PD to be released mainly in the

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cecum and colon over 24 h, resulting in very low drug absorption (Oosegi et al., 2008b). In this study, Ch–SP-MS/EuL were examined for their efficacy and toxic side effects using rats with TNBS-induced colitis in order to elucidate their usefulness as a specific drug delivery system for the treatment of IBD.

2. Materials and methods

Chitosan (viscosity grade, 1000 (5 g/l, 20 °C); deacetylation degree, 80% (mol/mol)) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Prednisolone (PD), prednisolone 21-hemisuccinate (SP) sodium salt and 2,4,6-trinitrobenzenesulfonic acid (TBBS) were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Eudragit L100 was purchased from Rohm GmbH Chemische Fabrik (Darmstadt, Germany). Sorbitan sesquioleate (SO-15) was purchased from Nikko Chemicals Co. Ltd. (Tokyo, Japan). All other chemicals were of reagent grade.

Male Wistar rats (6 weeks old, 200–210 g) were purchased from Tokyo Laboratory Animals Science Co. Ltd. (Tokyo, Japan), and bred on the breeding diet MF (Oriental Yeast, Tokyo, Japan) with water ad libitum at 23 ± 1 °C, a relative humidity of 60 ± 5% and 12 h light–dark cycle. They were used for the experiments soon after purchase. The experimental protocol was approved by the Committee on Animal Research of Hoshi University, Japan. The animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, Japan.

The Ch–SP conjugate (Ch–SP) was prepared as reported previously (Oosegi et al., 2008a). Ch (120 mg) was dissolved in water (85 ml) with the pH adjusted to 5.5 with 1 M HCl aqueous solution and 1 M NaOH aqueous solution, water (5 ml) containing SP sodium (40 mg) was added, 5 ml of aqueous solution containing EDCI (200 mg) was added, and the mixture was stirred under ice cooling for 5 h, and at room temperature for 19 h. Moreover, water (5 ml) containing EDCI (200 mg) was added, and the mixture was at room temperature for 24 h. The product was precipitated by addition of acetone, and washed using a mixture of acetone and water (4:1, v/v) and lyophilized to yield Ch–SP powder. Ch–SP-MS and Ch–SP-MS/EuL were also prepared according to the previous method (Oosegi et al., 2008a). Briefly, 1% (v/v) acetic acid aqueous solution (10 ml) containing Ch–SP (50 mg) was added to 150 ml of liquid paraffin with 1% (w/v) SO-15 solution. The mixture was stirred at 1200 rpm at 70 °C for 20 min and at 80 °C for another 10 min. The emulsion was sonicated at 80 °C under 28 kH (100 W) for 10 min, stirred at 400 rpm at 100 °C for 1 h, and cooled to room temperature. After addition of *n*-hexane, the particles was separated by centrifugation, washed with *n*-hexane and dried in a desiccator to yield Ch–SP-MS powder. Ch–SP-MS (130 mg) were added to 1 ml of methanol solution containing Eudragit L100 (130 mg), and the suspension was emulsified in liquid paraffin (50 ml) containing 2% (w/v) SO-15. The emulsion was stirred at 600 rpm at 40 °C under reduced pressure until the complete removal of methanol, and cooled to room temperature. After the addition of *n*-hexane, the particles were separated by centrifugation, washed with *n*-hexane, and dried in a desiccator to yield Ch–SP-MS/EuL powder.

Ch–SP-MS and Ch–SP-MS/EuL were analyzed for particle size and morphology using a JEOL JSM-5600LV scanning electron microscope (JEOL, Tokyo, Japan) after thinly coating with platinum using a JEOL JFC-1600 Auto Fine Coater. The drug contents of Ch–SP, Ch–SP-MS and Ch–SP-MS/EuL were measured according to the previous reports (Oosegi et al., 2008a). Briefly, the sample (2 mg) was put into a 1 M NaOH aqueous solution (10 ml), incubated for 10 min at 45 °C and centrifuged. The UV absorbance of the supernatant was measured at 246 nm to determine the amount of PD. The incorporation efficiency of the drug was calculated as the ratio of the observed drug content to the ideal drug content.

The animal experiment schedule used is shown in Fig. 1. After the rats were fasted for 48 h, TNBS (20 mg) dissolved in 0.25 ml of 50% (v/v) ethanol was instilled to each rat into the colonic part 7 cm from the anus with a catheter in order to induce ulcerative colitis (Tozaki et al., 1999, 2002; Lamprecht et al., 2001a; Yano et al., 2002). Three days after TNBS treatment, rats that weighed 80–100% of the weight immediately before TNBS instillation were selected as the ulcerative colitis-developing animals, and other rats were removed from the in vivo studies (Yano et al., 2002).

The above ulcerative colitis-developing rats were divided into eight groups (n = 3–4 in each group) as follows: Control (saline), Eudragit L100-coated chitosan microspheres with no drug (Ch-MS/EuL; 30 mg/d), PD (5 mg/(kg d)), PD (10 mg/(kg d)), Ch–SP-MS (5 mg PD equiv./(kg d)), Ch–SP-MS (10 mg PD equiv./(kg d)), Ch–SP-MS/EuL (5 mg PD equiv./(kg d)), and Ch–SP-MS/EuL (10 mg PD equiv./(kg d)). The substance corresponding to 5 mg PD equiv./kg was suspended in 1.5 ml of saline. It was administered via gastric intubation once daily for three consecutive days except for 10 mg PD equiv./(kg d) at which administration was conducted twice daily every 12 h for three consecutive days. Healthy rats (n = 4) were bred for comparison with diseased rats. Five days after the final administration, the body

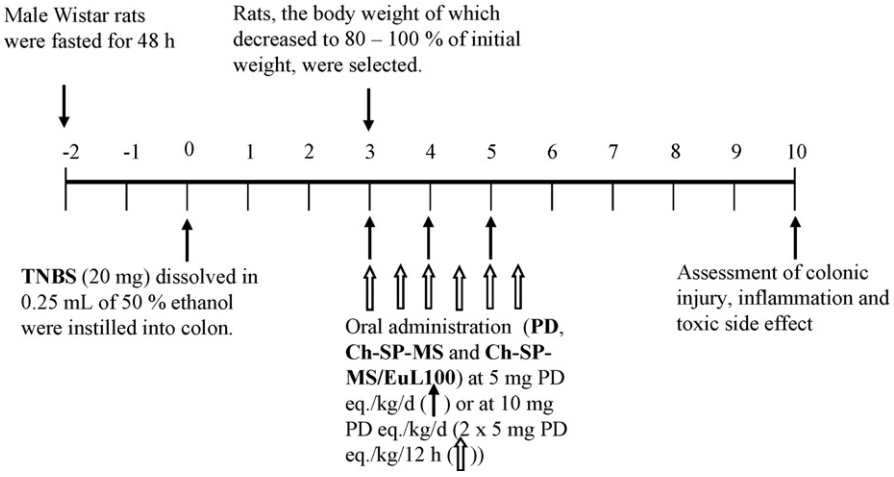

Fig. 1. Induction of ulcerative colitis and schedules of administration and assessment.

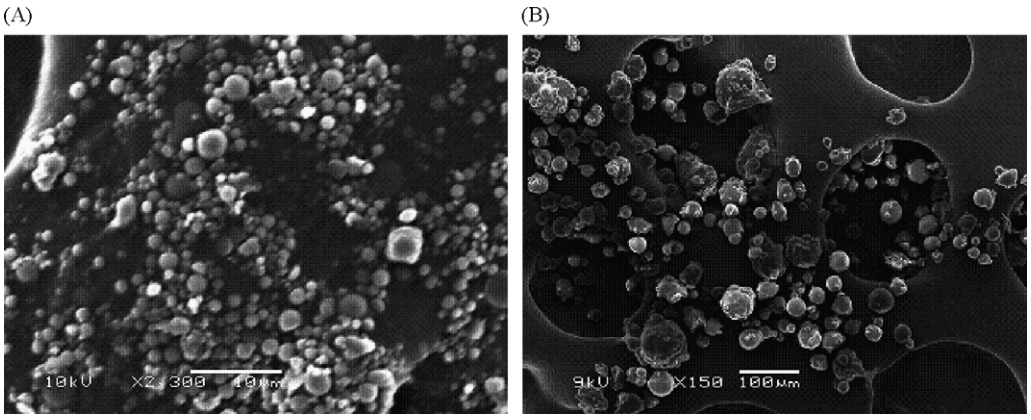

Fig. 2. Scanning electron micrographs of Ch–SP-MS (A) and Ch–SP-MS/EuL (B). The white bar length is 10 μm in (A) and 100 μm in (B).

weight was measured. Then, the rats were sacrificed by excessive ether anesthesia, and the colon and thymus were excised.

In vivo evaluation of efficacy and toxicity was performed as follows: (1) For colitis severity, visible damage to the distal colon, stool consistency and rectal bleeding were observed. The distal colon damage was scored on a 0–5 basis, according to the report by Tozaki et al. (2002). Briefly, a score of 0 indicated no damage, 1 was for localized hyperemia with no ulcer, 2 was given to linear ulcers with no significant inflammation, 3 was for linear ulcers with inflammation on one site, 4 indicated two or more sites of ulceration and/or inflammation, and 5 was for two or more major sites of inflammation and ulceration or one major site of inflammation and ulceration extending more than 1 cm along the length of the colon. Scores of stool consistency and rectal breeding were determined referring to the report by Lamprecht et al. (2001b). For stool consistency, a score of 0 was given to well-formed pellets, 2 was for pasty and semiformed stools that did not stick to the anus, and 4 indicated liquid stools that stuck to the anus. Rectal bleeding was scored as 0 for no blood, 2 for a finding of blood and 4 for gross bleeding. The visible colitis severity degree (VCSD) was calculated as the mean of all scores. (2) The ratio of proximal colon weight (Cp) to body weight (B), the ratio of distal colon weight (Cd) to B, and the thymus weight (T) to B were determined in the following manner. 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) (Lamprecht et al., 2001a). These segments were rinsed with saline to remove colon contents, their weights (Cp and Cd, respectively) were measured, and Cp/B and Cd/B were calculated. In addition, the thymus was removed and rinsed with saline. Then, the thymus weight (T) was measured, and T/B was calculated. (3) The MPO activity, used as a reliable index of inflammation caused by infiltration of activated neutrophils (Lamprecht et al., 2001a), was measured to quantify the colitis state using an MPO assay kit (Cytostore, Alberta, Canada). Briefly, after the distal colon was rinsed with saline to remove its contents, a specimen (200 mg) was minced and homogenized in 0.5% (w/v) hexadecyltrimethylammonium bromide (HTAB) aqueous solution on ice, and diluted to 4 ml by the addition of HTAB aqueous solution. The suspension was sonicated for 10 s, freeze–thawed three times, stirred with a vortex mixer, and centrifuged at 5000 rpm for 2 min. At 25 °C, the supernatant (200 μl) was added to 2 ml of the kit development reagent containing *O*-dianisidine dihydrochloride at 0.167 mg/ml and hydrogen peroxide at 0.0005%. Immediately after that, absorbance was measured at 450 nm. Furthermore, after the mixture was kept at 25 °C for 60 s, absorbance was measured again at 450 nm. The difference in absorbance (450 nm) between 0 s and 60 s was used as the MPO activity.

For statistical analysis, comparison of two groups followed the unpaired *t*-test. The results of more than two groups were compared using ANOVA followed by the Scheffe post hoc test. In all cases, significant difference was set as $P < 0.05$.

3. Results and discussion

The scanning electron micrographs of Ch–SP-MS and Ch–SP-MS/EuL are shown in Fig. 2, which displayed the particle size and shape. The drug content could be obtained by the exposure to a strong alkaline solution (1 M NaOH), which was based on the following features (Onishi et al., 2005, 2007; Oosegi et al., 2008a). Namely, as the alkaline aqueous solution quickly permeates powder and microparticles, the ester bond between PD and the polymer support is rapidly exposed to that alkaline pH medium, resulting in rapid hydrolysis of the ester bond. Then, the regenerated free PD diffuses quickly to the outer solution due to the swelling of the powder or macroparticles, leading to the quick achievement of the plateau level of the regenerated PD. Also, for Eudragit-coated particles, since their coating layer dissolves quickly in the strong alkaline solution, the same phenomena occur. Characteristics of the products are summarized in Table 1. The significant difference ($P < 0.001$) in size between Ch–SP-MS and Ch–SP-MS/EuL indicated that Ch–SP-MS got bigger by Eudargit coating. The drug contents of Ch–SP-MS and Ch–SP-MS/EuL were $4.6 \pm 0.7\%$ (w/w) ($n = 3$) and $3.2 \pm 0.7\%$ (w/w) ($n = 3$), respectively, which were considered acceptable because PD is used at a fairly low dose. The lower incorporation efficiency of Ch–SP-MS represented a loss of PD, which was considered to be caused by ester hydrolysis in the preparation of microspheres. The incorporation efficiency of Ch–SP-MS/EuL was much more than 100%, indicating that the recovery of Eudragit L100 was lower than that of Ch–SP-MS. The drug content was not significantly different between Ch–SP-MS and Ch–SP-MS/EuL ($P > 0.05$). The drug release profiles of both particles had already

Table 1
Characteristics of Ch–SP, Ch–SP-MS and Ch–SP-MS/EuL

Substance	Particle diameter[a] (μm)	Drug content[b] (%, w/w)	Incorporation efficiency[c] (%)
Ch–SP	–	7.7 ± 1.8	–
Ch–SP-MS	1.3 ± 0.4	4.6 ± 0.7[*]	60
Ch–SP-MS/EuL	31.8 ± 11.1[#]	3.2 ± 0.7[*]	139

[a] Results are expressed as the mean ± S.D. ($n = 160$).
[b] Results are expressed as the mean ± S.D. ($n = 3$).
[c] Calculated as the ratio of mean observed content to mean ideal content.
[#] $P < 0.001$ vs. Ch–SP-MS.
[*] $P < 0.05$ vs. Ch–SP.

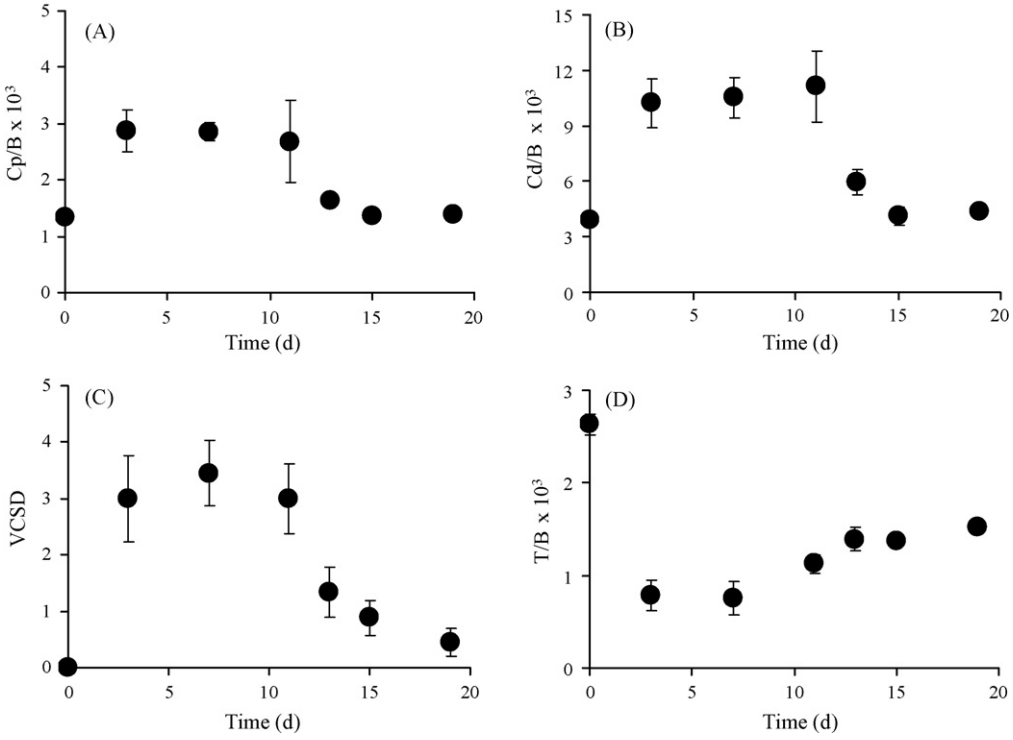

Fig. 3. Change in Cp/B (A), Cd/B (B), VCSD (C) and T/B (D) in rats after the colonic instillation of TNBS (20 mg) in 50% (v/v) ethanol aqueous solution (0.25 ml) per rat. The results are expressed as the mean ± S.E. (n = 3).

been reported before (Oosegi et al., 2008a); the release was suppressed almost completely at pH 1.2 (JP 14 first fluid), and PD was released gradually at pH 6.8 (JP second fluid). In addition, the drug release profiles from Ch–SP-MS and Ch–SP-MS/EuL in the suspension (pH 6.8) containing 20% (w/v) cecal and colonic contents, which were taken from rats with TNBS-colitis, were reported previously (Oosegi et al., 2008b). The large intestinal contents hardly influenced the release patterns, that is, the drug release was ruled mainly by pH, which was considered to be because the ester linkage between PD and a polymer support should be protected from hydrolytic enzymes due to the steric hindrance by the bulky polymer support. Furthermore, the release profile at pH 6.8 was hardly affected by the addition of 10% (w/v) homogenate of cecum or colon tissue (data not shown), suggesting that the ester bond should be stable in these tissues, which was also considered to be due to the steric hindrance by the polymer support.

Gastrointestinal transit and pharmacokinetics for PD alone and Ch–SP-MS/EuL had been clarified before (Oosegi et al., 2008b). Namely, after PD alone was administered intragastrically, PD hardly reached cecum and colon. However, Ch–SP-MS/EuL delivered Ch–SP-MS efficiently to the lower intestine, and PD was released gradually over 24 h. Although PD alone gave high plasma concentration rapidly and eliminated fast, while the plasma level of PD was suppressed almost completely after administration of Ch–SP-MS/EuL. These in vivo results were consistent with in vitro release and in vitro regeneration of Ch–SP-MS from Ch–SP-MS/EuL (Oosegi et al., 2008a). Thus, it had been predicted that Ch–SP-MS/EuL should be a good system to deliver PD to the IBD diseased site (Oosegi et al., 2008b). In the present study, the in vivo efficacy and toxicity were examined to prove the availability of Ch–SP-MS/EuL.

First, the experimental ulcerative colitis was induced in rats, and the body weight 3 d after TNBS injection was used as a criterion for

Table 2
Effect of substances on visible colitis severity in rats with TNBS-induced colitis

Group	Dose (mg PD equiv./kg)	Visible colitis severity			
		Colonic damage score [0–5]	Stool consistency [0–4]	Rectal bleeding [0–4]	VCSD [0–4.3]
Healthy	–	0.0 ± 0.0[#,†]	0.0 ± 0.0[#]	0.0 ± 0.0	0.0 ± 0.0[#]
Control	–	5.0 ± 0.0	4.0 ± 0.0	0.7 ± 0.7	3.2 ± 0.7
Ch-MS/EuL	–	5.0 ± 0.0	4.0 ± 0.0	0.7 ± 0.7	3.2 ± 0.7
PD	5	3.7 ± 0.9	0.7 ± 0.7[#]	0.0 ± 0.0	1.4 ± 0.7
	10	2.3 ± 0.5[#]	0.0 ± 0.0[#]	0.0 ± 0.0	0.8 ± 0.4[#]
Ch–SP-MS	5	2.3 ± 0.3[#]	1.3 ± 0.3[#]	0.0 ± 0.0	1.2 ± 0.4
	10	1.7 ± 0.7[#]	0.0 ± 0.0[#]	0.0 ± 0.0	0.6 ± 0.3[#]
Ch–SP-MS/EuL	5	2.0 ± 0.0[#]	0.0 ± 0.0[#]	0.0 ± 0.0	0.7 ± 0.3
	10	1.0 ± 0.0[#,†]	0.0 ± 0.0[#]	0.0 ± 0.0	0.3 ± 0.2[#]

Score range is shown in brackets. For colonic damage score, stool consistency and rectal bleeding, the results are expressed as the mean ± S.D. (n = 3–4). VCSD was calculated as the mean of all the scores, and expressed as the mean ± S.D. (n = 9–12).

[#] $P < 0.05$ vs. control and Ch-MS/EuL.

[†] $P < 0.05$ vs. PD (5 mg PD equiv./kg).

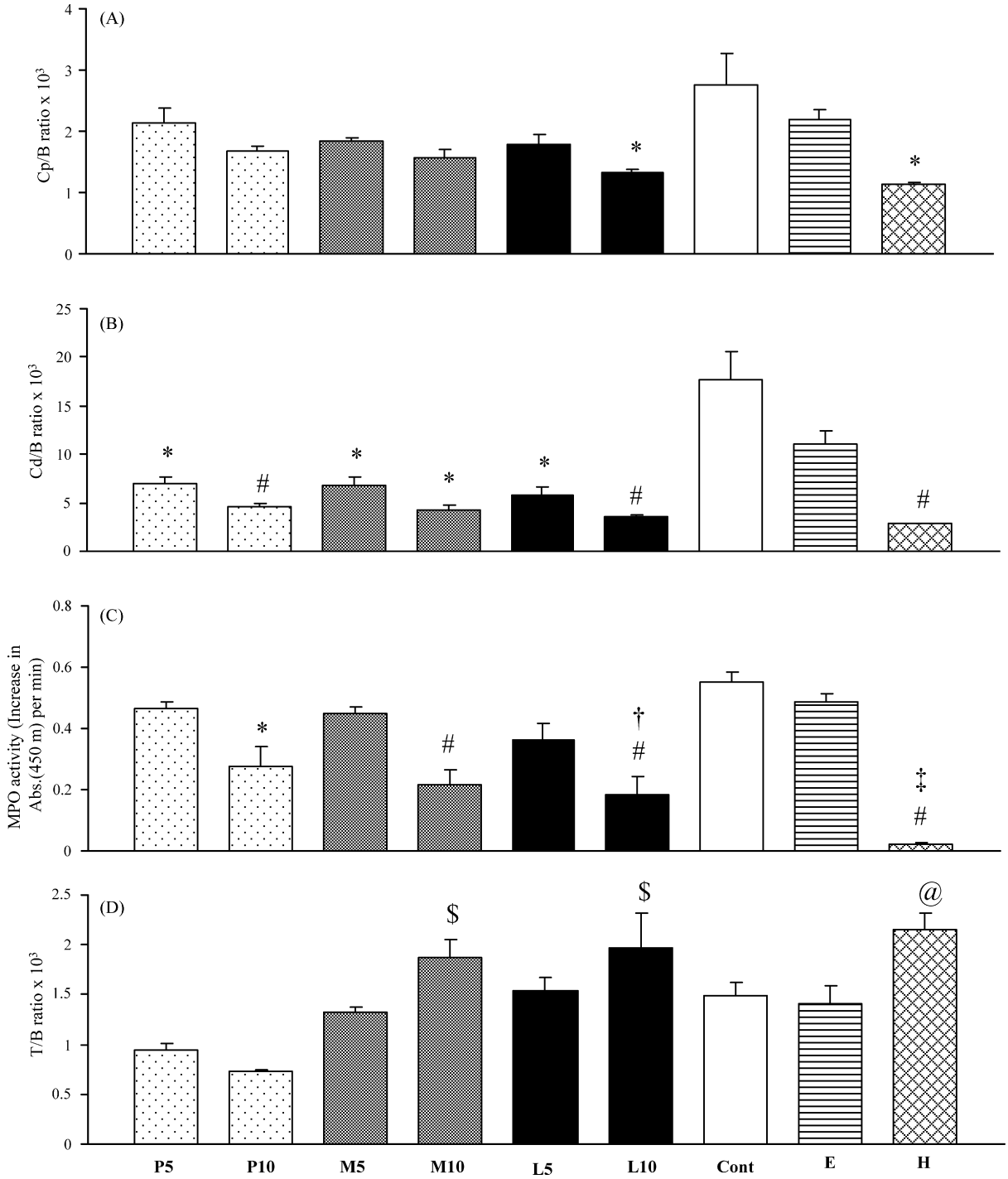

Fig. 4. Effect of administered substances on Cp/B (A), Cd/B (B), MPO activity (C) of distal colon, and T/B (D) in rats with TNBS-induced colitis. P5: PD alone (5 mg/(kg d)), P10: PD alone (10 mg/(kg d)), M5: Ch–SP-MS (5 mg PD equiv./(kg d)), M10: Ch–SP-MS (10 mg PD equiv./(kg d)), L5: Ch–SP-MS/EuL (5 mg PD equiv./(kg d)), L10: Ch–SP-MS/EuL (10 mg PD equiv./(kg d)), Cont: Control (saline 1.5 ml/d), E: Ch-MS/EuL (30 mg/d), H: Healthy group. The results are expressed as the mean ± S.E. (n = 3–4 for Cp/B and Cd/B; n = 3 for MPO activity). $^{*}P < 0.05$ vs. Cont; $^{\#}P < 0.05$ vs. Cont and E; $^{\dagger}P < 0.05$ vs. P5; $^{\ddagger}P < 0.05$ vs. P5, M5 and L5; $^{\$}P < 0.05$ vs. P10; $^{@}P < 0.05$ vs. P5 and P10.

the induction, that is, the rats with 80–100% of the weight immediately before TNBS instillation was adopted as the animals surely developing colitis (Yano et al., 2002). For the resultant rats with the ulcerative colitis, the time courses of Cp/B, Cd/B, VCSD and T/B were checked as the indices of colitis symptoms (Fig. 3). Cp/B, Cd/B and VCSD increased after the injection of TNBS, and reached a plateau on 3 d. The plateau state continued until 11 d. In particular, the values of Cd/B and VCSD increased to a large extent, while T/B fell down by TNBS injection and the plateau continued from 3 d until 11 d. These results proved that the efficacy and toxicity could be evaluated by the drug administration on 3 d and the examination of Cp/B, Cd/B and VCSD and T/B on 10 d Fig. 1.

The scores of visible colitis state are summarized in Table 2. Each treated group showed a significant decrease in the colonic damage score and VCSD except PD (5 mg/kg) and Ch-MS/EuL. For stool consistency, each drug was significantly effective at 5 and

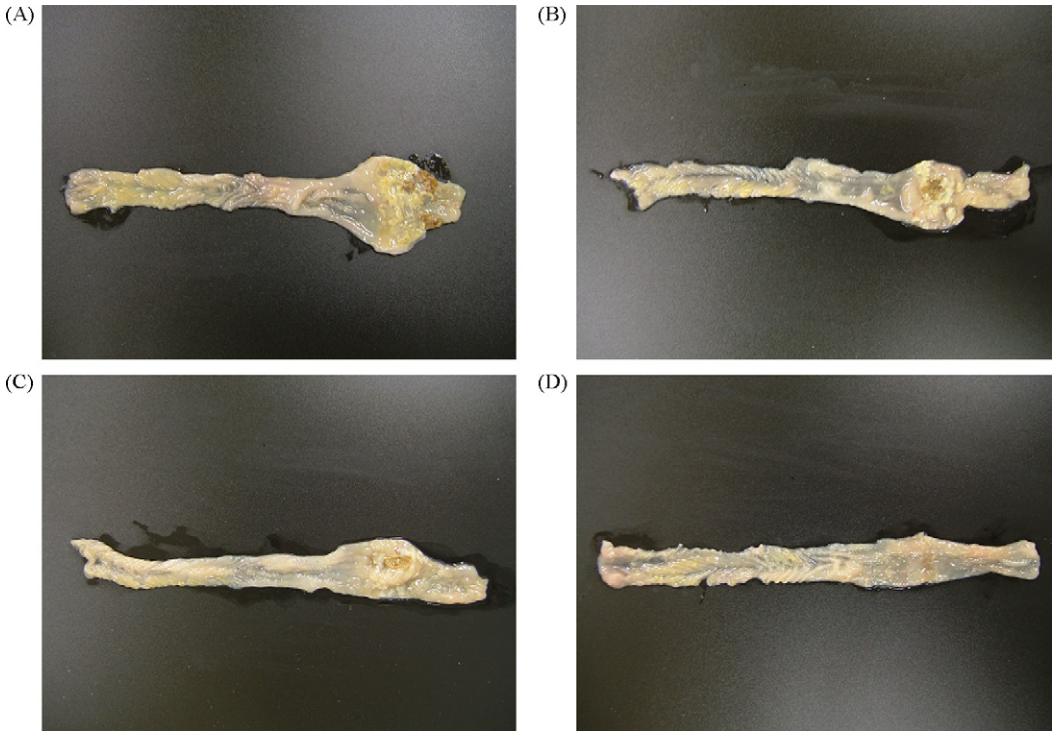

Fig. 5. Colitis states in the assessment after the treatment of rats with TNBS-induced colitis: (A) Control (saline), (B) PD alone (10 mg/(kg d)), (C) Ch–SP-MS (10 mg PD equiv./(kg d)), (D) Ch–SP-MS/EuL (10 mg PD equiv./(kg d)). Each substance was intragastrically administered 3, 4 and 5 d after TNBS injection, and the colon was excised 10 d after TNBS injection. The whole colon was rinsed and cut open lengthwise. Left side (4 cm) of the sample is proximal colon, and its right side (8 cm) is distal colon.

10 mg PD equiv./kg. Ch-MS/EuL (carrier alone) exhibited almost the same colitis state as the control (saline alone), which was the most severe. Visible colitis severity was suppressed the most greatly in the order Ch–SP-MS/EuL > Ch–SP-MS > PD in each score. Cp/B, Cd/B, MPO activity and T/B are shown in Fig. 4. Only Ch–SP-MS/EuL (10 mg PD equiv./kg) reduced Cp/B significantly, and the value was close to that of healthy rats. All the groups except for Ch-MS/EuL showed significant reduction of Cd/B against the control. The healthy group, Ch–SP-MS/EuL (10 mg PD equiv./kg) and PD (10 mg/kg) showed a significantly lower Cd/B than the control and Ch-MS/EuL. MPO activity was reduced significantly at 10 mg PD equiv./kg in every group, not at 5 mg PD equiv./kg. Overall, Cp/B, Cd/B and MPO activity were suppressed the most greatly in the order Ch–SP-MS/EuL > Ch–SP-MS > PD, and the suppression was more at 10 mg PD equiv./kg than at 5 mg PD equiv./kg. PD reduced T/B most greatly, which was lower than the control. The healthy group, Ch–SP-MS (10 mg PD equiv./kg) and Ch–SP-MS/EuL (10 mg PD equiv./kg) exhibited significantly larger T/B than PD (10 mg/kg). Ch–SP-MS/EuL (10 mg PD equiv./kg) displayed almost the same T/B as that of the healthy group, indicating that Ch–SP-MS/EuL would be very useful to reduce the toxic side effects of PD. Ch–MS/EuL showed almost the same values as the control in all the assessment parameters. Thus, it was suggested that Ch–SP-MS/EuL should enhance the efficacy of PD the best and reduce the toxic side effect the most. As shown in Fig. 5, the colitis states of control and treated groups also supported that Ch–SP-MS/EuL exhibited the highest efficacy.

As reported in the previous paper (Oosegi et al., 2008b), PD alone was distributed and absorbed mainly in the stomach and small intestine for several hours, while Ch–SP-MS/EuL allowed Ch–SP-MS to localize specifically in the cecum and to release PD mainly around the colon over 24 h. These features were considered to be the reason that Ch–SP-MS/EuL led to the higher efficacy and reduced toxicity of PD. Ch–SP-MS also displayed good results, but Ch–SP-MS/EuL exhibited more excellent results than Ch–SP-MS. This was probably because some Ch–SP-MS were impaired at stomach pH or entrapped on stomach mucosa, resulting in the loss of particle features and the reduction of targeting efficiency. The greater reduction of toxicity in Ch–SP-MS/EuL was considered to be due to less transfer of PD into the systemic circulation, which had been reported previously (Oosegi et al., 2008b).

4. Conclusion

Ch–SP-MS and Eudragit-coated Ch–SP-MS (Ch–SP-MS/EuL) showed good particle characteristics for size, drug content and drug release. The inflammatory indices, Cp/B, Cd/B, MPO activity and VCSD, were smaller in Ch–SP-MS and Ch–SP-MS/EuL than in PD alone. The toxic side effect index, reduction of T/B, was smaller in Ch–SP-MS and Ch–SP-MS/EuL than in control and PD alone. In addition, Ch–SP-MS/EuL tended to show a better efficacy and less toxic side effects than Ch–SP-MS. These results were considered to be because Ch–SP-MS/EuL allowed PD to be delivered efficiently to the lower intestine, retained well there and released gradually as having been reported before. Thus, the present study proved that Ch–SP-MS/EuL should be a useful colonic delivery system with the capability to enhance efficacy of PD and reduce toxicity of PD for the treatment of IBD.

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