

The Severity of Dextran Sodium Sulfate-Induced Colitis Can Differ Between Dextran Sodium Sulfate Preparations of the Same Molecular Weight Range

Shigeki Bamba · Akira Andoh · Hiromitsu Ban · Hirotugu Imaeda · Tomoki Aomatsu · Ayako Kobori · Yosuke Mochizuki · Makoto Shioya · Takashi Nishimura · Osamu Inatomi · Masaya Sasaki · Yasuharu Saitoh · Tomoyuki Tsujikawa · Yoshio Araki · Yoshihide Fujiyama

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Abstract

Background We hypothesized that the severity of dextran sodium sulfate (DSS)-induced colitis could differ between DSS preparations of the same molecular weight, and that this difference may be affected by the sulfur content. To test this, we used three DSS preparations of similar molecular weights but with different sulfur contents.

Methods Three DSS preparations with molecular weights of 40,000 to 50,000 were tested: MP Biomedicals (MP Bio), USB (USB), and The Lab Depot (The Lab). Epithelial cell lines were used to assess the levels of poly (ADP-ribose) polymerase (PARP) in the presence of 2.0% DSS in vitro. Eight-week-old female C57/B6 mice were fed 2.0% DSS in water for 1 week, and then sacrificed to investigate the effects of the DSS preparations in vivo.

Results In vitro experiments using CaCo-2 and CMT-93 cells revealed decreased PARP levels from all DSS preparations. Notably, the PARP level was significantly decreased in CaCo-2 cells treated with DSS from USB as

compared to The Lab Mice treated with The Lab DSS had significantly decreased body weight losses on day 7 as compared to mice receiving DSS from MP Bio and USB. This result was supported by their DAI score, colon weight/length ratio, and histological scores.

Conclusion The severity of colitis can differ between similar DSS preparations of the same molecular weight range. This difference in colitogenic properties may be affected by the total sulfur content of each DSS preparation.

Keywords Inflammatory bowel disease · Dextran sulfate · Colitis

Introduction

Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, still have an unknown etiology.

S. Bamba (✉) · H. Ban · H. Imaeda · A. Kobori · M. Shioya · T. Nishimura · O. Inatomi · Y. Fujiyama
Division of Gastroenterology, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga 520-2192, Japan
e-mail: sb@belle.shiga-med.ac.jp

A. Andoh
Division of Mucosal Immunology, Graduate School of Medicine, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga 520-2192, Japan

T. Aomatsu
Department of Pediatrics, Osaka Medical College, Takatsuki, Osaka, Japan

Y. Mochizuki · Y. Saitoh
Division of Endoscopy, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga 520-2192, Japan

M. Sasaki
Division of Clinical Nutrition, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga 520-2192, Japan

T. Tsujikawa
Comprehensive Internal Medicine, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga 520-2192, Japan

Y. Araki
Department of Pathology, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga 520-2192, Japan

Many experimental colitis models are now widely used to elucidate the pathogenesis of IBD, and to test the effects of newly-developed drugs. Dextran sodium sulfate (DSS)-induced colitis is one of the most common experimental models. Marcus and Watt first produced an ulcerative colitis (UC)-like model by the oral administration of a sulfated polysaccharide, carrageenan, to guinea pigs and rabbits in 1969 [1]. Furthermore, they discovered that related and unrelated high molecular weight sulfated products, such as sulfated amylopectin [2, 3] and sodium lignosulphonate [4, 5], are able to produce a high incidence of ulcerative disease in the colon following oral feeding. Interestingly, some of these preparations, such as carrageenan, dextran sulfate, lignosulphonates and sulfated amylopectin, are known to prevent or provide a measure of protection against several types of experimentally induced gastroduodenal ulcerations [6–10]. Subsequently, in 1985, Ohkusa induced enteritis in hamsters by oral DSS feeding with a molecular weight of 54,000, and reported that these lesions showed erosion, ulceration, inflammatory cell infiltration, crypt abscesses and epithelioglandular hyperplasia as in human UC [11].

Murine DSS-induced colitis models are known to have altered disease severity between: (1) different strains (Balb/c vs. C57BL) [12], (2) different conditions (SPF or conventional) [13], and (3) different DSS molecular weights [14]. However, there are no previous studies reporting that the severity of DSS-induced colitis can differ between different DSS preparations of the same molecular weight range. The precise mechanisms responsible for the ulcerogenic properties of DSS are unknown. Nevertheless, one possible explanation is that the powerful electronegativity of these polyanions has cytotoxic actions on the epithelial cells [15]; however, this mechanism needs to be proven. In our previous report, increased apoptosis and decreased proliferation of the colonic epithelium were observed in DSS-induced colitis in mice [16], suggesting that the cytotoxic properties of DSS were associated with epithelial apoptosis.

In this study, we hypothesized that the severity of DSS-induced colitis can differ between DSS preparations of the same molecular weight range, and that this difference may be affected by the sulfur content of the DSS. To test this, we used three different DSS preparations of similar molecular weights between 40,000 and 50,000 with different percentages of sulfur content. First, we checked the chemical properties of each DSS preparation. Second, the levels of poly (ADP-ribose) polymerase (PARP) were assessed as an apoptotic marker to investigate the cytotoxic properties of DSS *in vitro*. Third, the severity of the DSS-induced colitis was assessed for each DSS preparations *in vivo*.

Methods

DSS Preparations


There are three commercially-available DSS preparations with molecular weights of 40,000 to 50,000; [1] MP Biomedicals: MP Bio (lot number: 9135 J), [2] USB: USB (lot number: 124156), and [3] The Lab Depot: The Lab (lot number: WU0269). The details of these DSS preparations are shown in Fig. 1.

High-Performance Liquid Chromatography (HPLC) Conditions

A HPLC LC6A apparatus (Shimadzu, Kyoto, Japan) was used in this study. Strong interactions between DSS and the stationary phase eluant are thought to take place in this system, since DSS is a strongly negatively charged polysaccharide in aqueous solution. Therefore, 0.2 M phosphate buffers (pH 3.0) were used as the mobile phases to provide counter ions [17]. The mobile phases were delivered isocratically at a flow rate of 1.0 ml/min. The retention times of PA-DSS were determined using a size-exclusion column, Cosmosil 5Diol-120 Packed Column (7.5 × 300 mm; Nacalai Tesque, Kyoto, Japan). The column temperature was maintained at 60°C. For the detection of DSS, a SPD-10A fluorescence detector (Shimadzu) was used at 209 nm UV absorption.

Electrophoresis and Metachromasia

A solution of 2.0% DSS was applied onto a 2% agarose-gel with 1× TAE buffer at 100 V for approximately 60 min. The gel was stained with 0.01% toluidine blue for 3 h, and then washed. A mixture of the DSS solution and toluidine blue exhibits a color change from blue to reddish purple, a phenomenon called metachromasia [18].



	MP Biomedicals, LLC (9135J)	USB Corporation (124156)	The Lab Depot Inc. (WU0269)
Molecular weight	36,000 – 50,000	40,000 – 50,000	40,000 – 50,000
Glucose content	43.0%	40.1%	45.8%
pH of a 1% solution	6.2	7.3	7.1
Residue of ignition	40.0%	41.4%	42.0%
Specific rotation	+104°	N/A	+102°
Total sulfur content	19.0%	19.0%	17.2%
Loss of drying	5.6%	5.6%	4.5%
Free inorganic sulfate	not detected	N/A	<0.2%

Fig. 1 The detailed preparation of DSS

Poly (ADP-Ribose) Polymerase (PARP)/Apoptosis Assay

CaCo-2 and CMT-93 cells were cultured in Dulbecco's minimal essential medium (DMEM) containing 10% fetal bovine serum (FBS). All culture media were supplemented with 50 U/ml penicillin and 50 µg/ml streptomycin. Approximately 90% confluent, 6-well plates were used. A solution of 2.0% DSS was then added to the culture medium. The CaCo-2 cells were cultured for 3 days at 37°C, and then the cells were harvested for analysis. The PARP/apoptosis assay was performed using a HT colorimetric PARP/Apoptosis assay (TREVIGEN Inc., MD).

Induction of Colitis

Eight-week-old female C57/B6 mice were purchased from Charles River Japan (Kanagawa, Japan). They were housed individually in a room maintained at 22°C under a 12-h day-night cycle throughout the experiments. The mice were fed normal chow (MF; Oriental Yeast, Tokyo, Japan) and water containing 2.0% (wt/wt) DSS ad libitum for 1 week, and were then sacrificed. The study protocol was approved by the Animal Care and Use Committee of the Shiga University of Medical Science (Otsu, Japan). The experiments were repeated at least three times.

Assessment of Inflammation in DSS-Induced Colitis

Daily food and water intake was monitored from each cage. The disease activity index (DAI) score of the DSS-induced colitis was measured, including the body weight, an evaluation of the stool consistency, and the presence of blood in the stools by a guaiac paper test. The stool consistency was assessed using the following 4-point scale: 0, normal; 1, soft; 2, very soft but formed; and 3, liquid. The intensity of the guaiac test was scored by the following scale: 0, negative; 1, faintly blue; 2, moderately blue; 3, dark blue; and 4, blood visible. A validated clinical disease activity index ranging from 0 to 4 was calculating using the following parameters: stool consistency, the presence of fecal blood, and changes in body weight [19]. The mice were sacrificed on day 7, and the length and weight of the colons were measured.

Histological Assessment

A histological examination was performed on three samples of the distal colon from each animal. The samples were fixed in 10% buffered formalin, dehydrated in ethanol, and then embedded in paraffin. Four-micrometer-thick sections were then prepared, and were stained with hematoxylin and eosin. All histological evaluations were performed in a

blinded fashion using a validated scoring system [20]. Apoptotic cells were detected using an in situ Apoptosis Detection kit (Takara Bio, Shiga, Japan). The number of apoptotic cells per crypt in the proximal colon was counted. A total of 150 crypts were counted per mouse.

Statistical Analysis

The statistical significance of the differences was determined using a Kruskal–Wallis test (Statview version 4.5). Differences resulting in *P* values less than 0.05 were considered to be statistically significant.

Results

The molecular weights and electronegativity were confirmed using the HPLC system and electrophoresis. As shown in Fig. 2b, the molecular weights of the three DSS preparations were almost identical. However, the negative charge of the DSS from The Lab was weaker (Fig. 2c), which is in line with the decreased sulfur content of the attached product documents (Fig. 1).

An in vitro experiment using CaCo-2 and CMT-93 cells revealed decreased PARP levels in all DSS preparations (Fig. 3a, c). Notably, the PARP levels were significantly lower in CaCo-2 cells treated with the DSS from USB than in cells treated with the DSS from The Lab (Fig. 3b, c). The PARP levels of the MP Bio-treated cells were also decreased as compared to The Lab DSS, but did not reach statistical significance.

To evaluate the severity of the DSS-induced colitis, the three DSS preparations and healthy controls (H/C) were studied. During the 7 days of DSS administration, the food and water intake from each cage was monitored. The trends in food and water intake were similar in each group (Fig. 4). The mice treated with The Lab DSS had significantly decreased body weight loss by day 7 as compared to mice treated with DSS from MP Bio and USB (Fig. 5). This result was supported by the DAI score and colon weight/length ratio (Fig. 6a, d). Similar results were observed in two more repeats of these experiments.

The DSS-induced colitis was characterized by histological findings such as edema, the infiltration of inflammatory cells into both the mucosa and submucosa, the destruction of epithelial cells, and mucosal thickening. Our histological analysis indicated that mice treated with The Lab DSS showed a decreased number of infiltrating cells and an amelioration of the mucosal damage, especially in the mid- to distal colon, as compared to mice treated with DSS from MP Bio and USB (Fig. 7). The histological scores of the colitis were also significantly decreased in mice treated with The Lab DSS (Fig. 8). Furthermore, the

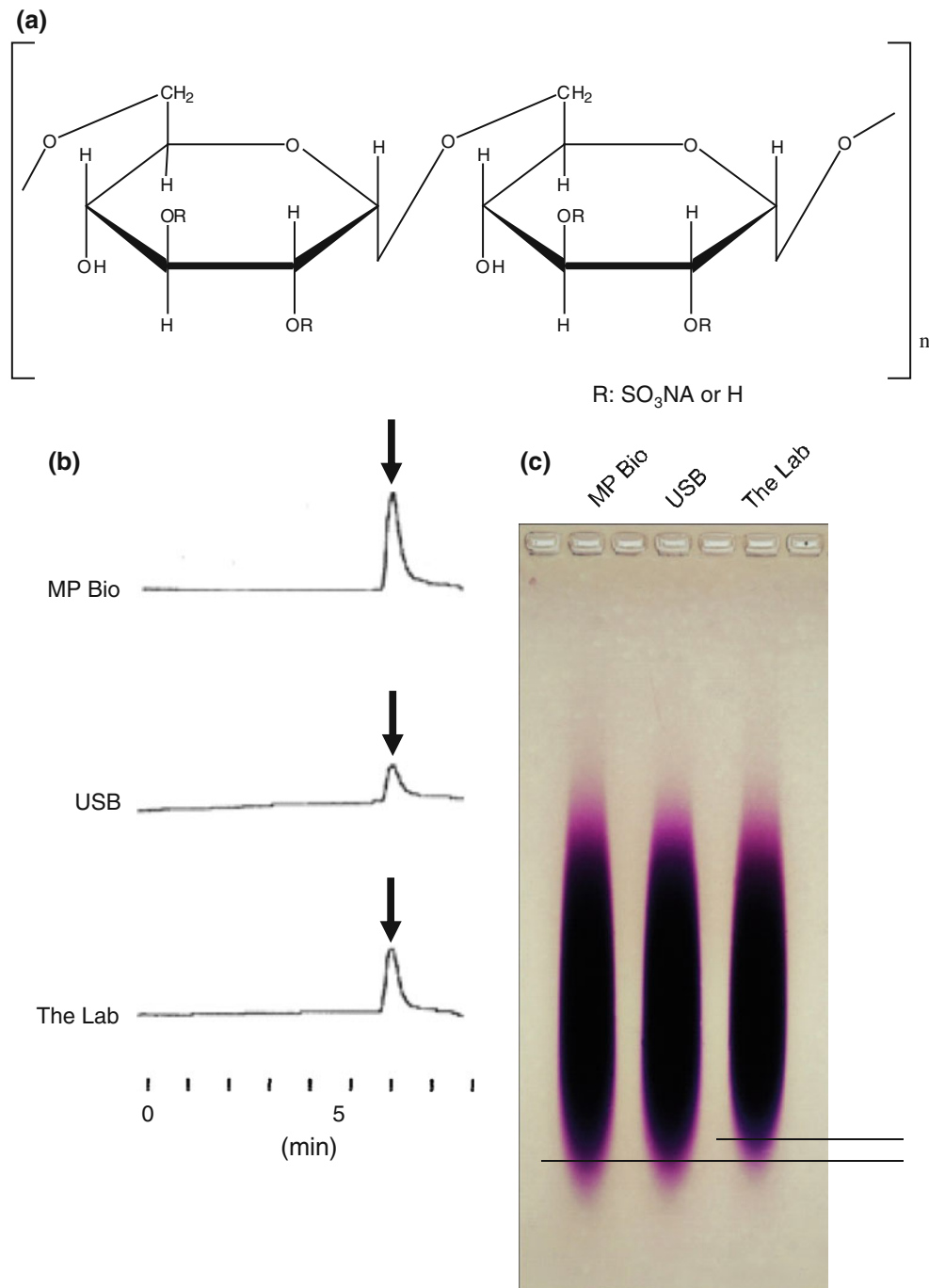


Fig. 2 **a** Structure of DSS. **b** HPLC revealed similar molecular weights for the three DSS preparations. **c** Electrophoresis and metachromasia of the three DSS preparations

numbers of apoptotic cells were significantly increased in all DSS treated groups (Fig. 9).

Discussion

DSS-induced colitis is one of the most widely-used experimental models of colitis. However, the precise

mechanisms responsible for DSS-induced colitis remain to be elucidated [19, 21]. DSS produces an acute onset of colitis within 5 days of the oral administration. The histology of the acute lesion is characterized by epithelial injury with sparse inflammatory cell infiltration, unlike ulcerative colitis, and is more similar to a graft-versus-host disease. The earliest histological changes occurring by the third day are crypt shortening with preservation of the

Fig. 3 **a** Results of the PARP/Apoptosis assay on CaCo-2 cells treated with medium and all DSS preparations. Error bar represents standard deviation (SD). $**P < 0.01$. **b** Magnified bar graph of all DSS preparations. Error bar SD. $**P < 0.01$. **c** Results of the PARP/Apoptosis assay on CMT-93 cells treated with medium and all DSS preparations. Error bar SD. $**P < 0.01$, $*P < 0.05$

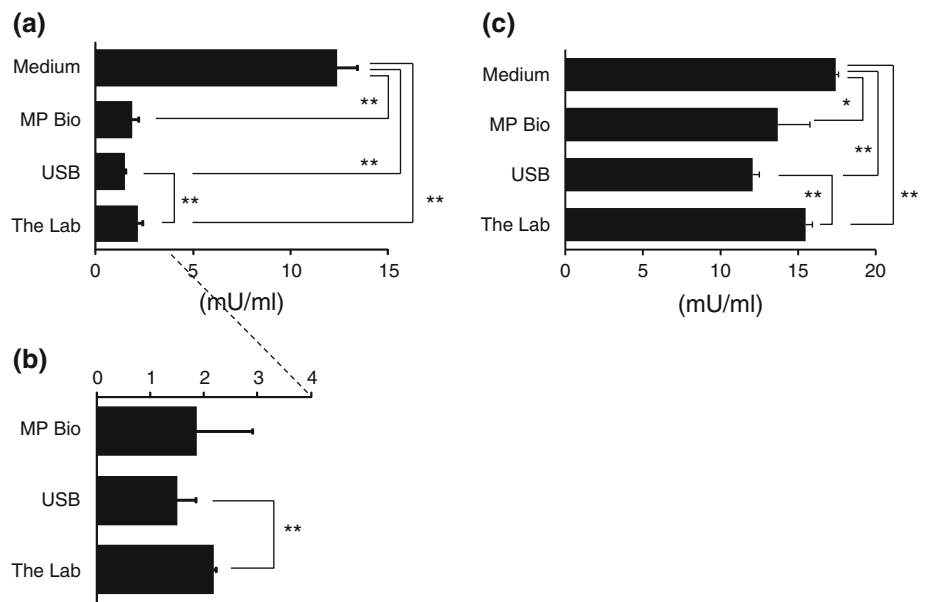


Fig. 4 Transition of the food and water intake during DSS administration. The food and water intake of each cage were monitored daily

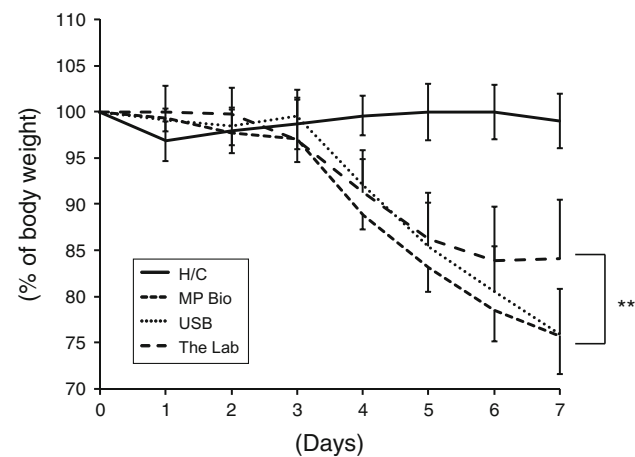
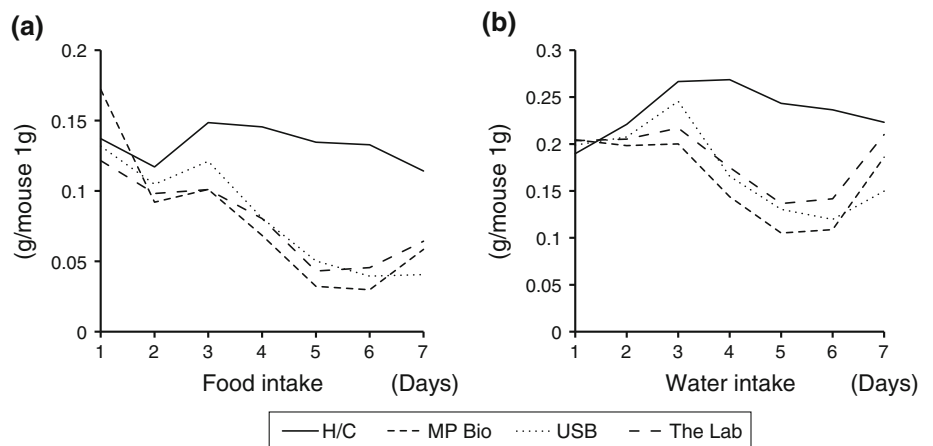


Fig. 5 Changes in the body weight of DSS-induced colitis mice. The mice were fed normal chow and 2.0% (wt/wt) DSS in water ad libitum for 1 week, and then sacrificed. The weights of the individual mice were monitored daily. The data represent means \pm SD. $**P < 0.01$; The Lab vs. MP Bio and USB

surface epithelium [19]. As the acute lesion progresses, much of the epithelial layer is lost in the affected areas.

Several mechanisms for DSS-induced colitis have been proposed in the literature. These include a defect in bacterial phagocytosis due to the accumulation of DSS in the lamina propria macrophages, and direct epithelial injury leading to early crypt shortening and erosions [22, 23]. The rapidity of the acute model suggests that acquired immunity is not pivotal to the genesis of these lesions. Furthermore, no immune mechanisms have been identified which contribute to the development of the acute lesions. The development of these lesions by DSS is also similar in severe combined immunodeficient mice, suggesting that the induction of the colitis in this model does not require T cells or B cells [20]. DSS-induced colitis can also be reproduced in athymic mice and in CD4, NK-deficient mice [24].

In this context, the pathogenic mechanism(s) responsible for this colitis can be attributed to its direct toxic effects on

Fig. 6 Effects of the DSS-induced colitis on the DAI score (a), colon length (b), colon weight (c) and colon weight/length ratio (d). The data represent means + SD. * $P < 0.05$, ** $P < 0.01$

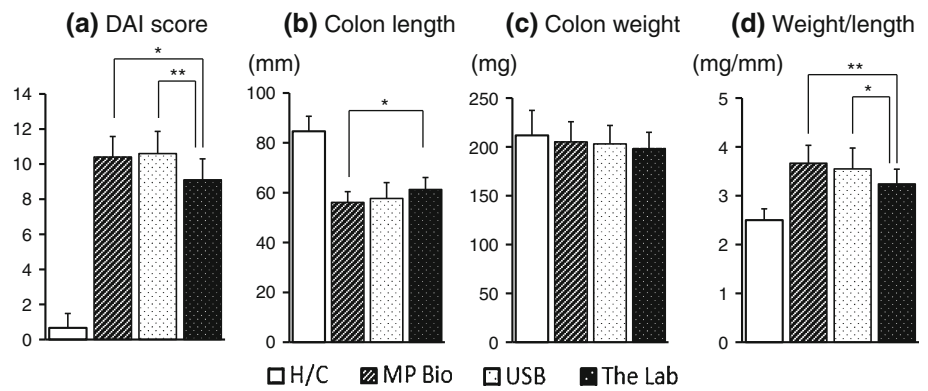


Fig. 7 Histology of the colon was evaluated by hematoxylin and eosin staining ($\times 100$)

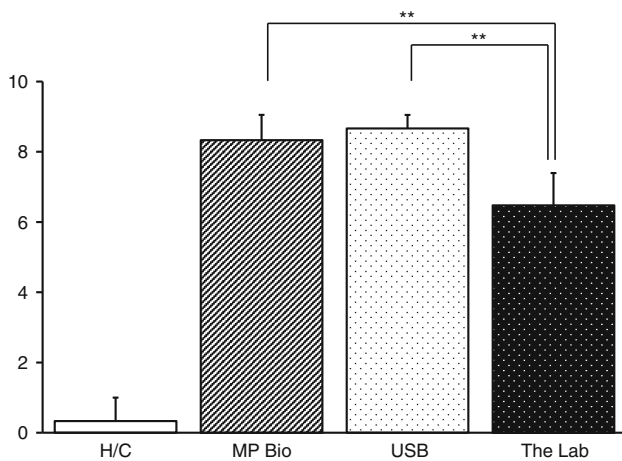
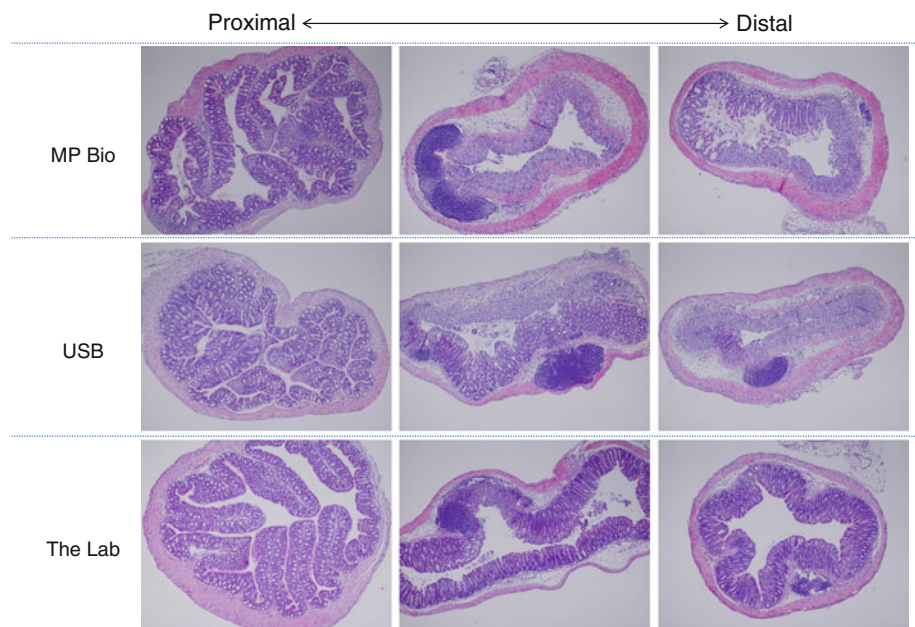
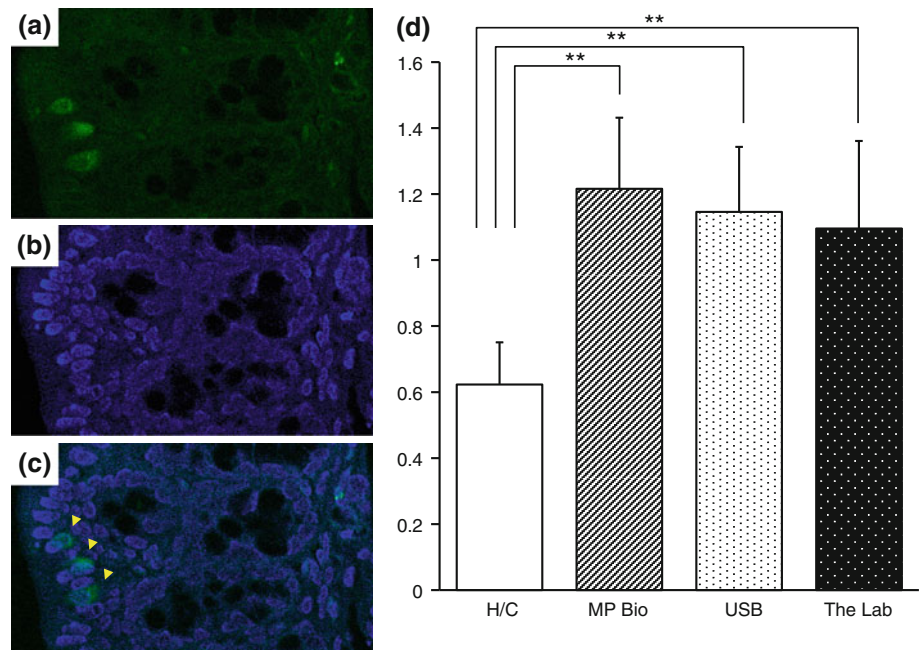


Fig. 8 Histological findings of the DSS-induced colitis. The colons were excised after 7 days of DSS treatment, and were stained with hematoxylin and eosin ($\times 100$). The data represent means + SD. ** $P < 0.01$; The Lab versus MP Bio and USB

colonic epithelial cells. First, we investigated the chemical properties of DSS, including HPLC and electrophoretic analyses. Since controlling the total sulfur content is known to be difficult in the process of chemical synthesis, the total sulfur content can be different between different lots, even from the same company. The total sulfur content used in this study is shown in Fig. 1. The DSS from The Lab included less total sulfur than the other two DSS preparations. Although the molecular weights are the same (Fig. 2b), the electronegativity of the DSS from The Lab was weaker (Fig. 2c). Based on the structure of DSS, the weak electronegativity of The Lab DSS may reflect its lower sulfur content (Fig. 2a).

As previously reported, increased apoptosis was observed in murine DSS-induced colitis [16]. To assess the cytotoxic effects of DSS in vitro, we investigated the levels of PARP as a marker of apoptotic activity [25], in the presence of 2.0% DSS. As shown in Fig. 3, the DSS from The Lab had weaker apoptotic properties as

Fig. 9 **a** The apoptotic cells were labeled with fluorescein isothiocyanate (FITC: *green*). **b** 4',6-diamino-2-phenylindole (DAPI: *blue*) was used as a counter-stain for the chromosomes. **c** Merged image. The *arrow heads (yellow)* indicate apoptotic cells in the epithelium. **d** The number of apoptotic cells per crypt. The data represent means + SD. ****** $P < 0.01$



compared to the DSS from the USB. These sulfated preparations are thought to induce apoptosis in the colonic epithelium, and this difference can alter their colitogenic properties. In line with the *in vitro* studies, the *in vivo* experiments confirmed the attenuated severity of colitis caused by the DSS from The Lab. These results were also consistent in terms of body weight transition, DAI score, colon weight/length, and histological scores. We also confirmed that the apoptotic cell numbers were increased in DSS-induced colitis.

In conclusion, we showed that the severity of colitis can differ between similar DSS preparations over the same range of molecular weights. The difference in colitogenic properties may result from the apoptotic properties of DSS. Furthermore, our results suggest that the total sulfur content in each DSS preparation is a vital in determining the severity of DSS-induced colitis.

References

- Marcus R, Watt J. Seaweeds and ulcerative colitis in laboratory animals. *Lancet*. 1969;2:489–490.
- Watt J, Marcus R. Ulceration of the colon in rabbits fed sulphated amylopectin. *J Pharm Pharmacol*. 1972;24:68–69.
- Marcus R, Watt J. Ulcerative disease of the colon in laboratory animals induced by pepsin inhibitors. *Gastroenterology*. 1974;67:473–483.
- Marcus R, Watt J. Colonic ulceration in guinea-pigs and rabbits fed lignosulphonate. *Vet Rec*. 1974;94:580.
- Watt J, Marcus R. Proceedings: effect of lignosulphonate on the colon of guinea-pigs. *Proc Nutr Soc*. 1974;33:65A–66A.
- Anderson W, Watt J. Inhibition of peptic activity, protection against histamine ulceration in the guinea pig, and combination with gastric mucin by an algal polyanion. *J Pharm Pharmacol*. 1959;11:318.
- Barnes WA, Redo SF, Ecker RR, Wenig J. Dextran sulfate. A new and potent antiulcer agent. *Am J Surg*. 1967;113:27–31.
- Vocac JA, Alphin RS. Effects and mechanism of action of a lignosulphonate on experimental gastric ulceration in rats. *Eur J Pharmacol*. 1968;4:99–102.
- Rosen H, Townsend P, Seifter J. Effect of sodium polyanhydromannuronic acid sulfate on incidence of ulcers in the Shay rat. *Proc Soc Exp Biol Med*. 1956;92:439–440.
- Bianchi RG, Cook DL. Antipeptic and antiulcerogenic properties of a synthetic sulfated polysaccharide (Sn-263). *Gastroenterology*. 1964;47:409–414.
- Ohkusa T. Production of experimental ulcerative colitis in hamsters by dextran sulfate sodium and changes in intestinal microflora. *Nihon Shokakibyō Gakkai Zasshi*. 1985;82:1327–1336.
- Melgar S, Karlsson A, Michaelsson E. Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. *Am J Physiol*. 2005;288:G1328–G1338.
- Hahm KB, Im YH, Parks TW, et al. Loss of transforming growth factor beta signalling in the intestine contributes to tissue injury in inflammatory bowel disease. *Gut*. 2001;49:190–198.
- Araki Y, Mukaisyo K, Sugihara H, Fujiyama Y, Hattori T. *Proteus mirabilis* sp. intestinal microflora grow in a dextran sulfate sodium-rich environment. *Int J Mol Med*. 2010;25:203–208.
- Watt J, Marcus R. Experimental ulcerative disease of the colon in animals. *Gut*. 1973;14:506–510.
- Araki Y, Mukaisyo K, Sugihara H, Fujiyama Y, Hattori T. Increased apoptosis and decreased proliferation of colonic epithelium in dextran sulfate sodium-induced colitis in mice. *Oncol Rep*. 2010;24:869–874.
- Araki Y, Andoh A, Fujiyama Y, et al. Application of 2-aminopyridine fluorescence labeling in the analysis of *in vivo* and *in vitro* metabolism of dextran sulfate sodium by size-exclusion high-performance liquid chromatography. *J Chromatogr*. 2001;753:209–215.

18. Araki Y, Katoh T, Urabe M, Kishi Y, Ishizuka I, Fujiyama Y. The analysis of pyridylamino-dextran sulfate oligomers by high-performance liquid chromatography and a novel detection system for sulfated polysaccharides. *Oncol Rep.* 2004;12:363–367.
19. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest.* 1993;69:238–249.
20. Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. *Gastroenterology.* 1994;107:1643–1652.
21. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology.* 1990;98:694–702.
22. Vlodavsky I, Miao HQ, Medalion B, Danagher P, Ron D. Involvement of heparan sulfate and related molecules in sequestration and growth promoting activity of fibroblast growth factor. *Cancer Metastasis Rev.* 1996;15:177–186.
23. Ohkusa T, Okayasu I, Tokoi S, Araki A, Ozaki Y. Changes in bacterial phagocytosis of macrophages in experimental ulcerative colitis. *Digestion.* 1995;56:159–164.
24. Axelsson LG, Landstrom E, Goldschmidt TJ, Gronberg A, Bylund-Fellenius AC. Dextran sulfate sodium (DSS) induced experimental colitis in immunodeficient mice: effects in CD4(+)-cell depleted, athymic and NK-cell depleted SCID mice. *Inflamm Res.* 1996;45:181–191.
25. Yu SW, Wang H, Poitras MF, et al. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science.* 2002;297:259–263.