Effects of Chondroitin Sulfate on Colitis Induced by Dextran Sulfate Sodium in Rats

Yusuke Hori*, Jiro Hoshino, Chihiro Yamazaki, Tomoko Sekiguchi, Satoshi Miyauchi and Katuyuki Horie
Tokyo Research Institute, Seikagaku Corporation, 3-1253 Tateno, Higashiyamato, Tokyo 207-0021, Japan
Received June 13, 2000 Accepted November 9, 2000

ABSTRACT—Chondroitin sulfate (CS) is currently marketed as a therapeutic drug for neurodynia, lumbago and arthrodynia. Recently, many clinical studies have demonstrated the therapeutic effects of orally administered CS against diseases with inflammation. Furthermore, these reports suggest CS plays an important role in the protection of the base of ulcers and has anti-inflammatory activity. We investigated the effects of CS against dextran sulfate sodium (DSS)-induced rat colitis. Rats were given 3% DSS solution for 10 days ad libitum. CS and 5-aminosalicylic acid (5-ASA) were orally administered daily. The doses of the CS groups were 20 or 100 mg/kg and that for the 5-ASA group was 100 mg/kg. Evaluations were made of bloody stools, areas of erosion and hematological data. CS improved the symptoms of bloody stools, erosion and increase of white blood cells. Especially, CS (100 mg/kg) group showed markedly more improvement than the 5-ASA group. We think that the major mechanism of the therapeutic effects of CS are the prevention of tissue damage by the protection of digestive mucosa and anti-inflammatory effects. Therefore, CS may have therapeutic value for alimentary tract diseases such as inflammatory bowel disease or ulcer.

Keywords: Chondroitin sulfate, Anti-inflammation, Alimentary tract diseases, Dextran sulfate, Colitis

Chondroitin sulfate (CS) is a typical glycosaminoglycan distributed in a wide variety of tissues including the cartilage, bone, aorta, teeth and cornea in all animals. It is a sulfated linear polysaccharide constructed of two or three kinds of mono-saccharides: N-acetylgalactosamine, L-iduronic acid and D-glucuronic acid.

CS is currently marketed as a therapeutic and safe drug for neurodynia, lumbago and arthrodynia. Evidence is accumulating that sulfated glycosaminoglycans such as CS and dermatan sulfate regulate the diffusion of colloid and water (1). Owing to their hydrophilic nature, sulfated glycosaminoglycans form high-volume gels, which help to maintain tissue turgor and the three-dimensional structure of the intestinal wall (1 – 3). Recently, a clinical study demonstrated therapeutic effects of orally administered CS in osteoarthritic patients, specifically an improvement of articular function and reduction of pain (4). It is also reported that the mucopolysaccharides play an important role in protecting the base and accelerating the healing of acetic-acid-induced gastric ulcers in rats (5).

In the present study, we investigated the mucoprotective and anti-inflammatory activity of CS against dextran sulfate sodium (DSS)-induced rat colitis.

MATERIALS AND METHODS

Test substances and reagents
In this study, we used chondroitin sulfate sodium (sulfur content: 6.8%, degree of sulfation (number of sulfate groups per sugar unit): 0.5; Seikagaku Co., Tokyo) made from shark and cow cartilage. It had a mean molecular weight of 24,000 as determined through gel filtration analysis with high performance liquid chromatography. CS was diluted in distilled water and administered at a dose of 20 or 100 mg/kg. 5-Aminosalicylic acid (5-ASA; Fluka Chemie AG Co., Buchs, Switzerland), a therapeutic drug for inflammatory bowel disease (IBD), was suspended in 0.5% carboxy methyl cellulose sodium (Maruishi Pharmaceutical Company, Tokyo) solution, and administered at a dose of 100 mg/kg. DSS (mean molecular weight: 5000, sulfur content: 18.5%, degree of sulfation: 2.4; Wako Pure Chemical Industries, Ltd., Osaka) was dissolved in distilled water to produce a 3% solution. Eroded areas were stained with Carazzi’s hematoxylin (Muto Pure Chemicals, Ltd., Tokyo).
**Animals and housing conditions**

Healthy 7-week-old male Wistar rats (SPF) (Charles River Japan, Inc., Yokohama) were used in this experiment. These rats were housed in wire mesh bottom cages in a room with controlled temperature, humidity and lighting, with food and water available ad libitum. At breeding, plain tap water was supplied. At examination, the normal group received distilled water supplied in bottles and the other groups were fed 3% DSS solution supplied in bottles.

**Colitis model (6) and group composition**

A total of 77 rats were randomly placed into five groups and were housed individually. The normal group (n = 5) was given distilled water, and the other groups were fed 3% DSS for 10 days. CS (20 or 100 mg/kg) and 5-ASA (100 mg/kg) were orally administered daily from day 0 to day 9. The control group was orally administered distilled water (2 ml/kg, 18 rats) daily during the same period.

**Parameters**

All assessments were done in a blind fashion.

**Bloody stool, body weight and quantity of DSS ingested:**

The body weight and stool conditions were monitored daily. The quantity of 3% DSS ingested was determined and 3% DSS was supplied every second day. The symptom of bloody stool was scored based on observations made in a pilot study: Grade 0, stool without blood; Grade 1, stool with blood; Grade 2, constant bleeding from the anus.

**Hematological analysis:** On day 10, blood was withdrawn from the abdominal vena cava of the rats under anesthesia using a syringe with heparin. For hematological examination, the erythrocyte count (RBC), hematocrit (Ht) and leukocyte count (WBC) were determined with a blood cell counter (Sysmex K2000; Sysmex Co., Tokyo).

**Erosion area:** The rectums and descending colons (7 cm) were excised promptly. They were opened longitudinally, and fixed to be dilated for more than one week. After staining with Carrazi’s hematoxylin, the area of erosion stained with dark purple was determined using an image analyzer (Image Pro Plus; Media Cybernetics, Silver Spring, MD, USA).

**Table 1.** Time course of the bloody stool score in the rat colitis model treated with chondroitin sulfate

| Drug     | 1      | 2      | 3      | 4      | 5      | 6      | 7      | 8      | 9      | 10     |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Control  | 0 ± 0  | 0.06 ± 0.06 | 0.11 ± 0.08 | 0.33 ± 0.11 | 0.44 ± 0.06 | 0.78 ± 0.13 | 0.83 ± 0.12 | 1.00 ± 0.11 | 1.33 ± 0.14 | 1.67 ± 0.11 |
| CS 20 mg/kg | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0 ± 0  |
| CS 100 mg/kg | 0 ± 0 | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0.06 ± 0.06* | 0.06 ± 0.06* | 0.17 ± 0.09* | 0.44 ± 0.12 | 1.00 ± 0.18 | 1.11 ± 0.20 |
| 5-ASA 100 mg/kg | 0 ± 0 | 0 ± 0  | 0 ± 0  | 0 ± 0  | 0.06 ± 0.06 | 0.11 ± 0.08 | 0.28 ± 0.11 | 0.39 ± 0.12 | 0.61 ± 0.14 | 0.94 ± 0.15 | 1.06 ± 0.13* |

Each value represents the mean ± S.E.M. of 18 animals. *P<0.05 vs Control.

**Statistical analyses**

Results are presented as values of the mean ± S.E.M. All results obtained from this experiment were analyzed with Dunnett’s multiple comparison test or the Pearson correlation analysis (SAS; SAS Institute Japan, Ltd., Tokyo) after an analysis of variance.

**RESULTS**

During this experiment, there was no significant difference between the groups in body weight and quantity of 3% DSS or distilled water ingested (data not shown). No toxic effects were observed when normal rats were treated with 100 mg/kg CS for 10 days (data not shown).

**Bloody stool**

Blood in the stool in the control group appeared on day 3 after DSS administration and had increased by day 10 in all of the animals. Blood was observed on days 5 – 6 in the rats administered CS. Treatment with CS significantly inhibited the presence of blood in the stools. Furthermore, on day 4 for the CS 20 mg/kg group and days 4 – 7 for the CS 100 mg/kg group, the bloody stool score was significantly reduced (Table 1). Treatment with 5-ASA also significantly reduced the bloody stool score on day 10 (Table 1).

**Hematology**

The WBC in the control group (293.1 ± 38.7 × 10^9/μl) given 3% DSS increased to approximately threefold that in the normal group (107.6 ± 6.8 × 10^9/μl). Moreover, RBC and Ht in the control group (RBC: 662.2 ± 24.8 × 10^9/μl, Ht: 42.2 ± 1.3%) were about 20% lower than those in normal rats (RBC: 828.2 ± 4.6 × 10^9/μl, Ht: 52.4 ± 0.1%) (Fig. 1: A and B). Treatment with CS inhibited the increase of WBC (20 mg/kg group: 242.5 ± 24.4 × 10^9/μl, 100 mg/kg group: 225.8 ± 25.7 × 10^9/μl) and decrease of RBC and Ht (RBC: 20 mg/kg group, 695.0 ± 28.2 × 10^9/μl; 100 mg/kg group, 715.6 ± 32.2 × 10^9/μl; Ht: 20 mg/kg group, 44.9 ± 2.1%; 100 mg/kg group, 45.2 ± 2.0%) (Fig. 1: A, B and C). CS at 100 mg/kg significantly inhibited the increase of WBC and the decrease of RBC and Ht. Treatment with 5-ASA at 100 mg/kg significantly inhibit-
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ed the increase of WBC (228.6 ± 47.1 × 10^9/µl) and decrease of RBC and Ht (RBC: 734.4 ± 33.7 × 10^6/µl, Ht: 47.3 ± 1.7%) (Fig. 1: A, B and C).

**Erosion area**

Erosion was observed on the rectum and descending colon. It was found in all animals except one rat from the CS 20 mg/kg group and two rats from the CS 100 mg/kg group (Fig. 2). The erosion area of the control group was 226.7 ± 24.7 mm². Treatment with CS inhibited the formation and growing of ulcer. Treatment with CS (100 mg/kg) and 5-ASA significantly reduced the erosion area (CS 20 mg/kg: 171.1 ± 26.5 mm², CS 100 mg/kg: 70.4 ± 12.5 mm², 5-ASA: 129.6 ± 20.0 mm²) (Fig. 2).

**Correlation with analysis parameters**

The relationships between all parameters of each rat were analyzed. RBC was significantly concerned with total counts of bloody stool score (total score of 10 days) (Fig. 3). There was no correlation between the other data excluding RBC and the bloody stool score.

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**Fig. 1.** Effect of chondroitin sulfate on blood constituents in DSS-induced rat colitis model. A) WBC (leukocyte count in blood). B) RBC (erythrocyte count in blood). C) HCT (hematocrit value). Each value represents the mean ± S.E.M. of 18 animals. *P<0.05, **P<0.01 vs control.

**Fig. 2.** Effect of chondroitin sulfate on erosion area on the rectum and descending colon. Each value represents the mean ± S.E.M. of 18 animals. *P<0.05 vs control.
DISCUSSION

DSS induced colitis predictably through an unknown mechanism. Several other models of colitis have been investigated using sulfated polysaccharides as well, including carrageenan and amylopectin. DSS (3%)-induced rat colitis was reported to be a useful model of ulcerative colitis for the evaluation of therapeutic effect, since erosion occurs frequently in the rectum, depending on the period of ingestion of DSS and is reproducible (6 – 8). On the other hand, it is reported that the molecular weight and the sulfur content per molecule of DSS might be important factors in the animal model of colitis (9, 10). Kunou and Hatanaka showed that the degree of sulfation greater than 1.0 caused a decrease in cell viability (11), and they suggest that low sulfa content substance such as CS may not have the toxicity to induce colitis.

In this study, we examined the therapeutic effects of CS on this model. CS significantly suppressed the progression of bloody stools, the formation of erosion areas, and the decrease of RBC and Ht. Furthermore, it also reduced the increase of WBC, which is a characteristic of the acute fulminating type of ulcerative colitis. In a recent study, we suggested that an allergic-like reaction occurred in this model because neutrophils and eosinophils infiltrated the colonic mucosa (8). Bleeding was observed in the area of erosion. It is considered that erosion leads to a progression of bloody stools and a decrease of RBC and Ht. A correlation between RBC and the total bloody stool score was observed. This result suggests that the decrease of RBC was dependent on the loss of blood from the ulcers, and CS will not have effect on the production of erythrocytes. 5-ASA, which is the active form of salazosulfapyridine, significantly prevented the appearance of blood in stools and erosion.

Inflammatory conditions such as necrotizing enterocolitis and Crohn’s disease result in a loss of CS in the mucosa (12, 13). This degradation has been attributed to reactive oxygen species and nitrogen species such as superoxide, hydroxyl radical and nitric oxide (12 – 14). It is reported

Fig. 3. Correlation between RBC (erythrocyte count in blood) and total counts of bloody stool score. Each point shows the value analyzed. The correlation between the RBC and the total counts of bloody stool score for 10 days. R = correlation coefficient value. 18 animals were used in each group.
that inflammatory disruption of sulfated glycosaminoglycan such as CS in vascular and connective tissue causes leakage of protein and fluid, thrombosis, and inhibition of tissue remodeling seen in IBD (12, 15 – leakage of protein and fluid, thrombosis, and inhibition of glycan such as CS in vascular and connective tissue causes inflammatory disruption of sulfated glycosaminoglycans (19). As well as having effects through cytokine secretion, activated inflammatory cells may cause tissue damage through the release of proteases and glycanases (20 – 22). Recently, Ito et al. reported that chitin and chitosan, which are negatively charged polysaccharides, had potent gastric cytoprotective and ulcer healing-promoting actions (23). It is also reported that a part of CS, orally administered, was excreted intact in stool (24). These reports suggest that negatively charged sulfated glycosaminoglycans such as CS or degraded CS has mucoprotective and healing-promoting potency. In other words, the exogenous CS may have therapeutic effects on IBD and alimentary tract disease directory. The reports of Barnes et al. (25) and Kunou and Hatanaka (11) suggest the cytotoxicity of DSS was stronger than antiulcer potency. Ronca et al. reported that CS inhibited the directional chemotaxis induced by zymosan-activated serum and is able to decrease the phagocytosis and the release of lysozyme induced by zymosan and to protect the plasma membrane from oxygen reactive species (26). They reported that compared with nonsteroidal anti-inflammatory drugs, CS appeared to be more effective against the cellular events of inflammation than edema formation. Furthermore, oral administration of CS had anti-inflammatory effects on rats.

In summary, in the 3% DSS-induced rat colitis model, orally administered CS improved the symptoms of bloody stools, erosion and increase of WBC. The major mechanism of the therapeutic effect of CS is the prevention of tissue damage by the protection of digestive mucosa and anti-inflammatory effects. Therefore, CS may have therapeutic value for alimentary tract diseases such as IBD or ulcer.

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