Changes in Colonic Mucosal Permeability in Mouse Colitis Induced with Dextran Sulfate Sodium

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Abstract: In this study we examined changes in colonic mucosal permeability induced by dextran sulfate sodium (DSS) during the acute phase of mouse colitis. To induce colitis, the mice were given drinking water containing 5% (w/v) DSS (MW=40,000) ad libitum. Colonic mucosal permeability was evaluated by the permeation of Evans blue (EB) from the lumen into the wall of the colon on 1, 2, 3 and 7 days postadministration of DSS. Mucosal changes were also histologically examined daily for 7 days postadministration. The permeation of EB increased significantly by days 3 and 7 postadministration. Histological analysis showed that crypt loss was the initial change, with no inflammatory process and the surface mucosal epithelial cells remained morphologically intact. These histological changes developed on 2 to 3 days postadministration. Erosion was first recognized at 5 days postadministration. These findings indicated that the increase in colonic mucosal permeability may have occurred in 3 days postadministration, and the increase in mucosal permeability occurred before the appearance of the inflammatory process. This suggests that an increase in colonic mucosal permeability, leading to the destruction of mucosal barrier function, may play an important role in the induction of DSS-induced murine colitis.

Key words: colitis, DSS, mouse, mucosal permeability

Introduction

Many experimental animal models have been used for the study of human inflammatory bowel disease. Recently, Okayasu et al. [15] reported the use of a model for acute and chronic colitis with ulceration by orally administering dextran sulfate sodium (DSS) to mice. This model is considered to be a useful animal model for human ulcerative colitis (UC). The end result of this treatment shows marked loss of body weight, rectal bleeding, reduction of colonic length, and destruction of the epithelial layer and glandular architecture of the large intestine [4, 15]. One of the key mechanisms of DSS-induced colitis in this animal model is thought to be a direct toxic effect on mucosal epithelial cells [5, 11, 13], but the exact mechanisms of DSS-induced colitis are still unknown.

In recent studies concerning host defense, the intestinal mucosa has been described as constituting an important barrier, protecting the body from a large number of external antigens and microorganisms such as food derived antigens, viruses, bacteria, parasites and the products of these organisms within the intestinal lumen [2, 7]. In certain intestinal diseases, the epithe-

(Received 11 September 1998 / Accepted 25 December 1998)
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lial barrier function is impaired even though the epithelial cells remain morphologically intact [2, 7]. For example, in human UC, polymorphonuclear neutrophils (PMNs) and lymphocytes are known to migrate through the epithelial cell to form crypt abscesses. With a large number of transmigrating PMNs and lymphocytes, the function of tight junctions is greatly impaired [18].

As DSS is considered to produce toxic effects on mucosal epithelial cells, the dysfunction of the mucosal barrier may be an initial event leading to mucosal inflammation in this model. In the present study we examined the changes in mucosal permeability during the acute phase of a DSS-induced mouse colitis model. From the results of the study with this mouse model we may gain further insight into human UC.

### Materials and Methods

#### Animals

Female BALB/c mice, purchased from Japan SLC Co., Shizuoka, Japan and maintained by brother-sister mating at our laboratory, were used in this study. All mice were 6–7 weeks of age at the beginning of the experiment. The mice were fed an autoclaved commercial diet (CA-1; Japan CLEA, Tokyo, Japan) and given drinking water ad libitum. They were housed 6 per cage and kept under constant temperature (22 ± 2°C) and humidity (55 ± 5%). The light cycle was 12 hr light and 12 hr dark.

#### Induction of colitis

The mice were given 5% (w/v) DSS (ICN Biomedicals Inc., CA, U.S.A.), MW=40,000, in their drinking water and allowed to drink ad libitum. The control group received distilled water without DSS. The study design was approved by the Saga Medical School committee on animal experimentation and performed following their guidelines for animal experimentation.

#### Surgical procedure

On 1, 2, 3 and 7 days postadministration of DSS, the mice (n=8 each) were anesthetized intravenously with 50 mg/kg sodium pentobarbital (Nembutal®; Abbot Laboratories, North Chicago, U.S.A.). Colonic mucosal permeability was evaluated following a modified version of Lange’s method [9]. A fine catheter connected to a 5-ml syringe, filled with phosphate-buffered saline (PBS), was introduced to the distal rectum via the anus. The proximal colon was incised at the junction with the cecum, and the fecal contents in the colon were washed out gently with 2–3 ml of PBS. Then the proximal colon and distal rectum were ligated, and 0.2 ml of 1.5% (w/v) Evans blue (EB; Wakoal Tesque, Inc., Kyoto, Japan) in PBS was injected into the lumen of the colon with a 1-ml syringe.

After the surgery the mice were warmed with an incandescent lamp until they recovered from the anesthesia, which appeared to be within 90 to 120 min of surgery. After 120 min of exposure to EB, the mice were sacrificed by cervical spine dislocation. The colon was rapidly dissected out, and the amount of EB that had permeated into the gut wall was assayed.

#### Assay of EB permeation

The dissected colon was opened and rinsed three times in 6 mM acetylcysteine (Katayama Chemical, Osaka, Japan) in PBS at room temperature. With the last rinse there was only minimal coloring of the solution. The colon was dried on filter paper at 37°C for 24 hr. The colon was then weighed and incubated with 1 ml of formamide (Katayama Chemical, Osaka, Japan) at 50°C for 24 hr. The amount of dye eluted was estimated with a Model 550 Microplate reader (Bio-Rad Laboratories, CA, U.S.A.) at a wavelength of 655 nm. The amount of EB permeating the gut wall (μg EB/mg colonic tissue) was calculated based on the standard curve of EB in formamide.

#### Histological analysis

An histopathological study was performed to evaluate the progression of mucosal injury. For histological analysis, the mice (n=3 each) were sacrificed by cervical spine dislocation daily for 7 days postadministration of DSS. The entire colon was dissected out and fixed in a 10% neutral-buffered formalin solution. Then the specimens were embedded in paraffin wax, and transverse sections were cut and stained routinely with haematoxylin and eosin (H-E) stain. Twelve transverse sections were made from each colon and evaluated histologically. The severity of mucosal injury was graded in the same way as described in a previous report [4] as follows: grade 0, intact crypt; grade I, loss of the one-third of the crypt; grade II, loss of the basal two-thirds of the crypt; grade III, loss of entire crypt with the
surface epithelial cells remaining intact; grade VI, loss of both the entire crypt and the surface epithelial cells (erosion). Grade 0, I, II, III and VI were given scores of 0, 1, 2, 3 and 4, respectively. Then the histological index was calculated as the mean of the scores for each section of the entire colon. In addition to the histological index, erosion was also calculated as a percentage of grade 4 sections in the number of sections examined. All slides were reviewed blindly.

**Statistical analysis**

Data were expressed as the mean ± SEM. Statistical analysis was performed by Student’s t-test. A value of p<0.05 was considered statistically significant.

**Results**

**Changes in colonic mucosal permeability**

Compared to that in control mice, the amount of EB that had permeated into the colonic wall increased significantly by 3 and 7 days postadministration of DSS (Fig. 1). On 3 and 7 days postadministration the amount of EB was approximately twice (p<0.05) and four times (p<0.01) as high as that in the control animals, respectively. The amount of EB (μg EB/mg colonic tissue) that had permeated into the colonic wall in the control samples, by 3 and 7 days postadministration was 0.60 ± 0.14 μg/mg, 1.46 ± 0.40 μg/mg and 2.56 ± 0.30 μg/mg, respectively. On 1 and 2 days postadministration, the amount of EB was similar to that in the control mice, and there were no significant differences.

**Histological findings**

Loss of the crypt was the earliest histological feature observed and this was followed by separation of the crypt base from the muscularis mucosa. These changes developed by 2 to 3 days postadministration of DSS, but the surface epithelial cells still remained morphologically intact and no inflammatory infiltration was apparent at this time (Fig. 2C). By 4 days postadministration, the loss of the crypts became more extensive throughout the entire colon, and a slight inflammatory infiltration consisting of macrophages, PMNs and lymphocytes was present in the lamina propria and submucosa. On 5 days postadministration, the loss of the crypt was more pronounced, and the crypts were replaced by various amounts of inflammatory cells, and focal erosion was observed at these sites (Fig. 2C). By 6 to 7 days postadministration, the erosion and inflammation in the mucosa had become more extensive (Fig. 2D). In control mice without DSS, no lesions such as crypt loss, inflammatory infiltration or erosion were observed (Fig. 2A).

**Histological index and the percentage of erosion occurrence**

Mucosal damage was evaluated by the histological grading method described above in materials and methods. The histological index began to increase at 2 days and gradually increased until 4 days postadministration of DSS (Fig. 3). Then the histological index increased rapidly to between 5 and 7 days postadministration.

Erosion was first observed at 5 days postadministration of DSS. The percentage of erosion that had occurred in DSS-treated mice by 5, 6 and 7 days postadministration was 8.3 ± 8.3%, 47.2 ± 12.1% and 72.2 ± 2.8%, respectively (Fig. 4). Both the severity and occurrence of erosion tended to be more frequent in the distal portion than in the proximal portion of the colon.
Fig. 2. Histopathological findings in DSS-treated mice. H-E stain. Bar = 50 μm. Control mice given water without DSS (A). On 3 days postadministration of DSS, the separation of the crypt base from muscularis mucosa and crypt loss was observed, but surface epithelial cells remain morphologically intact (B). On 5 day postadministration, crypt loss was more prominent, and moderate inflammatory infiltrate consisting of macrophages, PMNs and lymphocytes was seen in the submucosa and in the lamina propria. Focal erosion (*) was observed (C). By 7 days postadministration, the erosion and inflammatory cell infiltration had become more extensive (D).

Discussion

There are several methods available to evaluate mucosal permeability in vivo. For example, $^{51}$Cr-labeled ethylenediaminetetraacetic acid [12], ethylene glycol polymers [12], lactulose [6, 12] and mannitol [6] have been used as test markers for intestinal permeation. These markers are administered orally as a single dose. Intestinal permeation is then determined by measuring radioactivity or detecting the test markers in urine by gas or high-pressure liquid chromatography [2, 6, 12]. Other methods included horseradish peroxidase used as a marker substance for the study of macromolecular transport across the mucosal epithelial cells, and ultrastructural examination by electron microscopy [3]. In the present study, we used EB, an azo dye (MW=960.83), as a marker for intestinal permeation by using a modified version of Lange’s method [9]. EB is
widerly used as a non-R1 marker for demonstrating pathologically increased microvascular permeability. Moreover, permeability can be easily assessed indirectly by quantifying the amount of the dye eluted with formamide and by spectrophotometric determination [16]. Lange et al. [9] also demonstrated that the EB is a useful non-R1 marker substance for evaluating mucosal permeability in the small and large intestines.

Fluid and solute transport across the intestinal mucosal barrier are considered to occur both transcellularly and paracellularly. The latter route, mediated via tight junctions, is more important for the permeation of medium-sized and larger compounds [7, 18]. The macromolecules are pathophysiologically significant because of their potentially antigenic, toxic, or carcinogenic action. Therefore determinants of tight-junction permeability are of significant theoretic and clinical interest [10]. In certain diseases such as Celiac disease, Crohn’s disease and human UC, mucosal permeability is known to be increased [2, 7, 18].

In the present study, by using a murine colitis model for human UC, we report that the permeation of EB increased significantly on 3 days postadministration of DSS. At this time, the superficial mucosal epithelial cells remained morphologically intact and no inflammatory process was apparent, but small changes in basal crypt loss were detected in the histological analysis. These findings suggest that the increase in colonic mucosal permeability might have occurred in 3 days postadministration, prior to the erosion or ulcer formation. Moreover, the fact that an increase in mucosal permeability occurred prior to the appearance of the inflammatory process indicates that dysfunction of the mucosal barrier may be involved in the induction of the inflammatory reaction in this model. In this study, however, it was not possible to clarify which route, paracellular or transcellular, chiefly contributed to colonic mucosal permeation.

In previous reports, the mechanisms of induction of DSS-induced colitis have been explained by a toxic effect on the intestinal epithelial cells [5, 11, 13], local
immunological disturbances [8], the dysfunction of lamina propria macrophages [14], and changes in the intestinal microflora population [15]. The toxic effect of DSS on colonic mucosal epithelial cells is considered to be a likely mechanism. In support of this idea, Dieleman et al. [5] reported that DSS inhibits proliferation of the murine colon carcinoma cell line and causes a decrease in cell viability and by preventing some cells entering the G2 + M phase of the cell cycle in vivo. This may further indicate that the disappearance of the basal crypt cell as an initial change is caused by an inhibitory effect of DSS on cell proliferation [5], because the basal crypt cell is an active cell in cell division in the intestinal mucosa, but the exact mechanisms involved in the induction and pathogenesis of DSS-induced colitis are still unclear.

In a clinicopathological study of DSS-induced mouse colitis, Cooper et al. [4] reported that the disappearance of the basal crypt was followed by an inflammatory process in the mucosa. In severe combined immunodeficient (SCID) mice, which lack functional T and B lymphocytes, Axelsson et al. [1] and Dieleman et al. [5] reported that histologically similar changes were induced with the oral administration of DSS. Our histological findings regarding the initial changes in acute colitis were similar to the findings in these reports. From these data, it was apparent that inflammation was not the primary event, and that the inflammatory process or immunocompetent cells did not play an important role in the induction of DSS-induced mouse colitis.

Okayasu et al. [15] reported a significant increase in the population of members of the families Enterobacteriaceae and Bacteroidaceae and Clostridium spp., but a decrease in Eubacterium spp. and Enterococcus spp., after administering a 5% DSS solution, but the significance of the changes in the intestinal microflora on the pathogenesis of DSS-induced colitis remains unclear.

A possible mechanism of the induction and pathogenesis of DSS-induced colitis is as follows: DSS may cause mild injury to the colonic epithelial cells resulting in an increase in colonic mucosal permeability and toxic luminal bacterial products such as endotoxin and peptidoglycan-polysaccharide polymers which permeate into the colonic mucosa [17]. These permeated substances may cause destruction of the epithelial cells of basal crypts and induce an inflammatory reaction in colonic mucosa. The present study suggests that the increase in colonic mucosal permeability, resulting in the destruction of mucosal barrier function, may play an important role in the induction of DSS-induced mouse colitis.

Acknowledgments

We thank Dr. Takumi Sasaki of the Chemo-Sero-Therapeutic Research Institute, for his useful suggestions. We are also grateful to Mr. Kiyoitsu Tomoda of the Center for Laboratory Animals, Saga Medical School, for his technical assistance.

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