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DEXTRAN SULFATE SODIUM INDUCES PAN-GASTROENTERITIS IN RODENTS: IMPLICATIONS FOR STUDIES OF COLITIS

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Dextran sulfate sodium is widely used to induce colitis in rodents. Though given orally in drinking water, this agent is widely believed to produce injury through direct toxic effects on the epithelium, and it has been assumed to produce damage and inflammation only in the colon. Given the apparent toxic effects of dextran sodium sulfate on epithelial cells, its administration orally, and the anticoagulant properties of this agent, we hypothesized that significant damage and inflammation would be produced in regions of the digestive tract proximal to the colon. Groups of rats or mice received DSS (5%) in the drinking water for up to 7 days. Tissues were harvested at various time-points for blind evaluation of damage, and measurement of several markers of inflammation. In both rats and mice given DSS, significant damage and inflammation was produced in the stomach, small intestine and colon. Significant granulocyte infiltration was apparent in all tissues by day 3 of DSS ingestion. Bleeding was evident throughout the small intestine and colon. These studies clearly demonstrate that DSS, when administered orally in drinking water, produces a pan-gastroenteritis, rather than the damage and inflammation being limited to the colon. The damage and inflammation in the stomach and small intestine could contribute to changes in body weight, stool consistency and bleeding, all of which are commonly used as indices of severity of colitis. Beneficial or detrimental effects of therapeutic interventions could be attributable, at least in part, to modulation of injury and inflammation proximal to the colon.

Key words: colitis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, animal model, inflammation

INTRODUCTION

Since first described in 1985 for use in hamsters (1) and subsequently for use in mice (2), dextran sulfate sodium (DSS)-induced colonic injury and inflammation has grown to be the most widely employed model for studies of the pathogenesis and treatment of inflammatory bowel disease (IBD). It is the simplicity and reproducibility of the DSS model that makes it so attractive to IBD researchers. Animals are provided with drinking water supplemented with DSS and over the course of the days that follow, depending on the concentration of DSS used, colonic injury and inflammation develop.

Another attractive feature of the DSS model is the simplicity of the markers of colitis that are commonly employed. For example, scoring for decreases in body weight, stool consistency and fecal blood are very commonly used, and quite often are the sole end-points. The length of the colon is another commonly used end-point, as shortening of the colon appears to correlate with severity of colonic inflammation (2).

Some of the most common criticisms of the model, or at least of its application, include the failure, in many studies, to monitor consumption of drinking water by the animals. Interventions (e.g., administration of drugs, special diets, gene deletions, etc.) may alter the amount of water the animal consumes, and therefore the amount of DSS they consume. This can sometimes lead to invalid conclusions being drawn. Another criticism is the over-reliance on qualitative end-points that may be influenced by factors unrelated to colonic inflammation; indeed, it appears to be widely held that DSS produces inflammation specifically in the colon, and changes in qualitative end-points (body weight, stool consistency, fecal blood, etc.) are therefore a consequence of colonic inflammation.

DSS is an anti-coagulant (3) that appears to damage the colonic epithelium through a direct corrosive action. Indeed, in one of the earliest descriptions of this model (4), it was reported that damage to the epithelium preceded the onset of inflammation in the colon, and it was later reported that the colitis could be produced in the absence of B or T lymphocytes (5). One of the original descriptions of the model suggested that the inflammation was limited to the distal colon (2). We hypothesized that it would be unlikely that an agent with such corrosive properties, ingested orally, would produce damage that would be limited to the colon. We therefore examined the effects of DSS on mucosal integrity throughout the gastrointestinal tract. Any damaging effects of DSS in the gastrointestinal tract proximal to the colon would likely contribute to some of the changes that are attributed, in many studies, to colitis (e.g., changes in body weight, stool consistency, fecal blood).
Table 1. Criteria for scoring colonic damage.

<table>
<thead>
<tr>
<th>Appearance/ulceration</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Ulceration with inflammation at 1 site</td>
<td>1</td>
</tr>
<tr>
<td>Ulceration/inflammation at 2 or more sites</td>
<td>2</td>
</tr>
<tr>
<td>Major damage throughout the small intestine</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Criteria for scoring small intestinal damage.

<table>
<thead>
<tr>
<th>Appearance/adhesions</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No adhesions</td>
<td>0</td>
</tr>
<tr>
<td>Minor adhesions (intestine can be separated from other tissue with effort)</td>
<td>1</td>
</tr>
<tr>
<td>Major adhesions</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Appearance/hyperemia</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No hyperemia</td>
<td>0</td>
</tr>
<tr>
<td>Focal hyperemia (at 1 site)</td>
<td>1</td>
</tr>
<tr>
<td>Hyperemia multiple sites</td>
<td>2</td>
</tr>
<tr>
<td>Hyperemia throughout small intestine</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Appearance/bleeding</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No bleeding</td>
<td>0</td>
</tr>
<tr>
<td>Blood at 1 site</td>
<td>1</td>
</tr>
<tr>
<td>Blood in multiple sites</td>
<td>2</td>
</tr>
<tr>
<td>Blood throughout the small intestine</td>
<td>3</td>
</tr>
</tbody>
</table>

| Total Score                           |       |

The score was increased by 1 or 2 if there were mild or severe adhesions, respectively, by 1 if diarrhea was evident, and by 1 if rectal bleeding was evident. The maximum colon thickness (in mm) was also added to the score.

Table 2. Criteria for scoring small intestinal damage.

MATERIAL AND METHODS

Animals

Male, Wistar Rats (175–200 g) and male, Balb/c mice (20–25 g) were obtained from Charles River Breeding Farms (Montreal, QC, Canada). The animals were housed in plastic cages in the McMaster University Central Animal Facility with controlled temperature, humidity and light cycle. They were fed standard laboratory chow ad libitum. All experiments were conducted in accordance with the guidelines established by the Canadian Council of Animal Care. The Animal Care Committee at McMaster University approved all protocols.

Dextran sulfate sodium administration and assessment of injury and inflammation

Groups of 8–12 rats or mice were provided drinking water supplemented with DSS (5% wt/vol; 36-50 kDa) ad libitum. Control animals received normal drinking water. The animals were euthanized 3 or 7 days later. Body weight and the volume of drinking water (±DSS) consumed were measured daily. In addition, the presence or absence of diarrhea and fecal blood was blindly evaluated each day. After euthanization, the stomach, small intestine and colon were removed and opened by a longitudinal incision. The extent of damage was blindly evaluated. For the colon, damage was assessed using previously described criteria (6) (Table 1). For the stomach, the lengths of all lesions were measured (in millimeters) and were summed for each animal to give a total gastric damage score. This scoring system has been widely used for quantifying damage in the stomach (7). For the small intestine, a scoring system was developed that consisted of several elements: presence of blood in the lumen, mucosal hyperemia, adhesions, and change in length of the small intestine (Table 2). The scores for each criterion were summed to provide an overall score of intestinal damage.

After scoring the macroscopic damage, tissue samples were taken for measurement of myeloperoxidase (MPO), a biochemical marker of granulocyte infiltration, using a method adapted (8) from that originally described by Bradley et al (9). Additional tissue samples were fixed in neutral-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin, then blindly examined by light microscopy.

Real-time reverse transcription polymerase chain reaction

Samples from the stomach, mid-jejunum and distal colon were excised, snap-frozen in liquid nitrogen, and stored at –80°C. RNA extraction was performed using the RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. Two-step, real-time, reverse transcription polymerase chain reaction was utilized, as described previously (10). Validated primer sets for rat and mouse TNF-α, COX-2 and β-actin were used (Qiagen).

Statistical analysis

All data are expressed as mean ±S.E.M. Comparisons among groups of data were performed using a one analysis of variance followed by the Neuman-Keuls test. An associated probability (P value) of <5% was considered significant.

RESULTS

Dextran sulfate sodium-induced symptoms and damage

There were no differences in daily water consumption between the DSS and controls groups, for both rats and mice. By
day 3, rats in the DSS group exhibited occult blood in the feces. By 7 days, all rats exhibited diarrhea, rectal bleeding, occult blood, lethargy and a cessation of weight gain. In mice, diarrhea and bleeding were not evident after 3 days of DSS administration. However, by day 7 diarrhea, rectal bleeding and occult blood in their feces were evident in most mice. Mice receiving DSS for 7 days also exhibited a significant decrease in body weight.

Macroscopically visible colonic damage followed a similar time-course as the above-mentioned symptoms. By day 3, rats had developed significant colonic damage, and this damage was markedly worse by day 7 (Fig. 1A). In mice, significant colonic damage was observed at day 7, but not day 3 (Fig. 1B). Macroscopically, hyperemia was evident throughout the entire colon, and blood was present in the lumen. In many rats and mice there were adhesions between the colon and other organs. Histologically, as has been described previously (1-3), the colonic damage was characterized by focal mucosal necrosis and substantial granulocyte infiltration (Fig. 2). The necrosis was largely limited to the mucosal layer.

Many of the above-mentioned changes were not limited to the colon. Significant increases in the small intestinal damage scores were observed for rats by day 3 and for mice by day 7 (Fig. 3). By day 3, all DSS-treated rats exhibited intestinal hyperemia, and in half of the rats the hyperemia extended throughout the small intestine. Blood was present in the lumen throughout the small intestine of most rats at day 7 of DSS consumption, and the cecum was black and was full of blood in half of the rats. However, no rats had adhesions between the small intestine and other organs. Only one mouse exhibited hyperemia in the jejunum at day 3, but most mice had blood throughout the small intestinal lumen. By day 7, the entire small intestine in all mice was hyperemic, and ~50% of the mice had significant adhesions between the small intestine and the surrounding tissues. Histologically, rats and mice receiving DSS exhibited extensive mucosal necrosis and granulocyte infiltration (Fig. 4).

The stomachs of mice and rats receiving DSS exhibited no changes from controls by day 3. However, by day 7 the stomachs were hyperemic. Blood was not observed in the lumen and there

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**Fig. 1.** Colonic damage scores in rats (A) and mice (B) receiving normal drinking water (control) or DSS-supplemented drinking water for up to 7 days. Damage was blindly scored using the criteria outlined in Table 1. ***p<0.001 vs. the control group; *p<0.05 vs. the day 3 group (ANOVA and Neuman-Keuls test).

**Fig. 2.** Micrographs showing the appearance of colonic tissue from (A) control rat, (B) day 7 DSS-treated rat, (C) control mouse, and (D) day 7 DSS-treated mouse. Both in rats and mice, DSS consumption resulted in extensive mucosal necrosis and granulocyte infiltration. Granulocyte infiltration of the submucosa was also extensive, and sometimes accompanied by necrosis. Magnification bar =100 µm.
were no macroscopically visible hemorrhagic erosions or ulcers. In 50% of the mice and one rat receiving DSS, there were adhesions between the stomach and liver. Histologically, damage to the surface epithelium was observed in several mice and rats and granulocyte infiltration was evident in the mucosa and submucosa (Fig. 5).

Dextran sulfate sodium-induced inflammation

Rats and mice receiving DSS in the drinking water developed significant inflammation in the stomach, small intestine and colon by day 3, as indicated by the marked elevation of myeloperoxidase activity (Fig. 6). In the rat, the myeloperoxidase activity in the small intestine and colon (but not the stomach) were further increased at day 7, while in the mouse, the myeloperoxidase activity was further increased at day 7 in all tissues studied (Fig. 6).

The ability of DSS to increase pro-inflammatory gene expression in the colon of mice was confirmed in the present study, as expression of mRNA for both TNF-α and COX-2 were significantly elevated over control levels at day 7 (Fig. 7). Moreover, similar increases (2- to 4-fold) in TNF-α and COX-2 expression were observed in the stomach and small intestine of mice receiving DSS for 7 days (Fig. 7).

Fig. 3. Small intestinal damage scores in rats (A) and mice (B) receiving normal drinking water (control) or 5% DSS-supplemented drinking water for up to 7 days. Damage was blindly scored using the criteria outlined in Table 2. *p<0.05; ***p<0.001 vs. the control group; †p<0.05 vs. the day 3 group (ANOVA and Neuman-Keuls test).

Fig. 4. Micrographs showing the appearance of jejunal tissue from (A) control rat, (B) day 7 DSS-treated rat, (C) control mouse, and (D) day 7 DSS-treated mouse. Both in rats and mice, DSS consumption resulted in extensive destruction of villi and granulocyte infiltration of the submucosa. Magnification bar =100 µm.

DISCUSSION

Addition of DSS to the drinking water is a very commonly used method for inducing colitis in rodents. Changes in body weight, the appearance of blood in the stool and diarrhea are symptoms often used as surrogate markers of the severity of colitis produced by DSS, and of the effectiveness of interventions aimed at reducing the severity of colitis. The present study...
demonstrates that DSS given to rats or mice does indeed produce severe colitis. However, with a similar time-course of onset, DSS also produces significant tissue injury, bleeding and inflammation in the stomach and small intestine. In addition to epithelial damage, which was more severe in the small intestine than in the stomach, marked increases in granulocyte infiltration and up-regulation of expression of pro-inflammatory genes (for TNF-α and COX-2) were observed in all tissues examined.

DSS has been reported by several groups to produce epithelial injury as a primary effect, with mucosal inflammation following and leading to more extensive damage (2, 3). Significant changes in the colonic microflora have also been reported, which may contribute significantly to DSS-associated tissue injury and inflammation (2). We observed slight differences in the onset of colonic damage between rats and mice. In mice, myeloperoxidase activity (indicative of granulocyte inflammation) was significantly increased by day 3, but we did not observe epithelial injury at that time. However, we cannot rule out the possibility that some epithelial injury had occurred prior to granulocyte infiltration.

Increased expression mRNA for TNF-α and COX-2 was not observed until day 7 in both rats and mice, subsequent to the onset of damage and granulocyte infiltration. With respect to the small intestine, virtually the same time-course of development of damage and inflammation was observed as in the colon, with the same small differences between rats and mice.

In the stomach, injury was much less severe than in the other tissues studied, with the damage being limited to the surface epithelium. Myeloperoxidase levels were significantly elevated, but not to the same extent as seen in the small intestine and colon. The significant increase in expression of COX-2 and TNF-α in the stomach and small intestine.

**Fig. 5.** Micrographs showing the appearance of gastric tissue from (A) control rat, (B) day 7 DSS-treated rat, (C) control mouse, and (D) day 7 DSS-treated mouse. Both in rats and mice, DSS consumption resulted in damage to the superficial epithelium, with some infiltration of granulocytes. Deeper erosions and ulcers were not observed, and significant granulocyte infiltration was not evident in the submucosa. Magnification bar = 100 µm.

**Fig. 6.** Myeloperoxidase activity, a biochemical marker of granulocyte infiltration, in stomach, small intestine (jejunum) and distal colon of (A) rats and (B) mice that received normal drinking water (controls) or drinking water supplemented with 5% DSS for up to 7 days. *p<0.05 vs. the control group; °p<0.05 vs. the day 3 group (ANOVA and Neuman-Keuls test).
stomach by day 7 is consistent with previous studies in which gastritis was induced with iodoacetamide (11, 12). TNF-α has been shown to contribute to tissue injury in the stomach associated with use of nonsteroidal anti-inflammatory drugs (14, 15) and Helicocacter pylori infection (16), while COX-2 is up-regulated in response to tissue injury (17, 18) and plays an important role in maintenance of mucosal integrity and repair of damage (18-21).

There have been over 5,000 publications on DSS-induced colonic injury since the model was first described in 1985 (PubMed search). Given the widespread use of the model, it is surprising that damage and inflammation in the stomach and intestine have not been reported more widely. Despite a marked increase in granulocyte infiltration, the damage we observed in the stomach was quite mild, involving only the superficial epithelium. Holzer et al. (11) had similarly observed damage induced by DSS in the stomach, and though quite mild compared to the injury observed in the colon, it was sufficient to result in significant impairment of the gastric barrier to acid back-diffusion. Yazbeck et al. (13) reported that in mice consuming 2% DSS for 6 days, inflammation extended proximally from the colon into the small intestine. In addition to elevated myeloperoxidase in the jejunum, they observed significant increases in villus height, but no overt damage.

The popularity of the DSS model of colitis for testing the effects of novel therapeutics or genetic manipulations is most likely related to its simplicity and reproducibility. However, like any experimental model, it has limitations, and care should to be taken in interpretation of the results generated. In many studies, symptoms (diarrhea, bleeding, weight loss) and indirect markers of “inflammation” (colon length) have been used as the primary endpoints for the effectiveness of a pharmacological or genetic intervention. The present study shows that inflammation, bleeding and injury were produced throughout the gastrointestinal tract and therefore could have contributed to the loss of body weight and the diarrhea and fecal blood that was observed. For example, malabsorption of nutrients as a result of the extensive small intestinal injury could have contributed significantly to the generation of diarrhea, as well as to a loss of body weight. The intestine also appeared to be a major site of bleeding in rats and mice treated with DSS.

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REFERENCES


