Evaluation of acute colitis induced by dextran sulfate sodium in C57BL/6

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Abstract. Dextran sulfate sodium (DDS)-induced acute colitis in C57BL/6 mice is essential for several research aspects, but the inducing condition should be optimized with the purpose of different study. In this study, C57BL/6 mice drank water with 5 (w/v) % DSS dissolved for 7 days to induce acute colitis, which were evaluated from three aspects, including body weight change, colon length and histopathological changes. On day 8, the inflammation response to DSS treatment showed in the colon of C57BL/6 mouse. Compared with the control group, 5% DSS treatment decreased the weight of mice after drinking for 3 days, with Disease activity index (DAI) score corresponding to the pathological manifestations ($p < 0.001$). The colons were shorter significantly ($p < 0.001$) compared with control group. Partial colon of DSS-treated mice under H&E staining displayed severe inflammatory manifestations. In conclusion, this study constructed a successful acute colitis model, which can be a reference for others to do some studies related.

1. Introduction

Chronic diseases are a heavy burden for people. Some of them suffer from Inflammatory bowel diseases (IBDs), commonly in two forms with inflammation of the intestine, which are ulcerative colitis (UC) and Crohn’s disease (CD) [1]. Patients with IBD present some clinically challenging problems for physicians. Despite related research were performed for many years, the etiopathogenesis of IBD was unclear. To delineate the molecular mechanisms of IBD and explore the potential therapy for patients, researchers developed numerous animal models to simulate human’s IBD [2]. IBD models can be divided according to their establishment method, such as treated with chemical compounds (e.g., dextran sulfate sodium (DSS)), or induced by genetic targeting of specific genes (e.g., regulatory cytokines) [3]. Among those models, DSS-induced model is easy to carry out, and condition of immediate and controllable inflammation could be shown. After feeding the mice with low-molecular weight DSS in water, there existed epithelial damage in the colon, showing a robust inflammation response and could be lasting for several days [4]. Bloody feces, diarrhea, weight loss and the inflammation of tissue always existed in this model [5]. Different severity of models depends on the actual DSS administration, for example various concentration, duration and frequency would induce various models, including acute or chronic colitis, sometimes dysplastic lesions even. And the severity of colitis and carcinogenic were related to the molecular weights of DSS. And the inflammation severity of the mouse model were always distinct among animal models, which were unsuitable for following experiments. And environmental factors like lab environments matter. Changes in protocols may also make results different [6]. In this study, we observed the 5% DSS-treated mice, and focused
on the specific changes of its acute colitis, and analyzed the mouse ulcerative colitis model with multiple aspects, including body weight, DAI, colon length, and H&E staining of colon tissue. And a successful mice model of acute colitis induced by 5% DSS was established.

2. Materials and methods

2.1. Materials and reagents

Dextran Sulfate Sodium (36-50 kD) was purchased from MP Biomedicals (France). Paraformaldehyde was purchased from Solarbio Science & Technology Co., Ltd (Beijing, China). And urine-faeces occult blood qualitative detection kit (o-toluidine method) was from Leagene Biotechnology (Beijing, China).

2.2. Animals and acute colitis model

Female C57BL/6 mice (18-20 g) were obtained from Shanghai Xipul-bikai experimental animal Co., Ltd. (SCXK (Shanghai) 2018-0006) (SPF level). They were acclimatized to the lab environment for one week before the start of the experiment, which was featured by a cycle of 12:12-h light-dark and housed temperature at 22 °C and 60% humidity. Lab rodent diet was given to mice and water ad libitum. All mice were randomly kept in two cages, one cage one group. Mice in the control group (n = 10) drank distilled water and in the DSS group (n = 10) constantly drank water with 5% (w/v) DSS dissolved for 7 days in order to develop colitis. Mice weight was recorded, with stool consistency checked and occult blood tested daily. By cervical dislocation, mice were sacrificed at day 8, and we extract their colons for the following analysis.

2.3. Evaluation of colitis severity

2.3.1. Disease Activity Index (DAI)

From three aspects, composed of body weight change, stool consistency and fecal occult blood, DAI was scored and the total score was calculated to assess colitis severity (Table 1). We monitored mice in two groups daily, measuring their body weight, recording condition of diarrhea and fecal occult blood. The occult blood was tested by the urine-faeces occult blood qualitative detection kit following the instructions.

| Score | Body weight change* | Stool consistency | Occult blood |
|-------|---------------------|-------------------|--------------|
| 0     | No loss or increase| Normal            | Occult blood (-) |
| 1     | -1% ~ -5%           | Shaped and loose  | Occult blood (+ ~ 2+) |
| 2     | -5% ~ -10%          | Shaped and slime  | Occult blood (3+~4+) no gross blood |
| 3     | -10% ~ -15%         | Mushy             | Gross blood   |
| 4     | ≤-15%               | Diarrhea          | Anus bleeding |

Note: * the day before DSS treatment was day 0, and the weight was recorded as BW0; After DSS treatment, the weight daily was recorded as BWN (N = 1, 2, 3, 4, 5, 6, 7), and the daily weight loss value was = (BWN - BW0) /BW0 *100 %

2.3.2. Colon length and H&E staining

By cervical dislocation, mice were all sacrificed at day 8. Entire colon of every mouse was gently separated by longitudinal anatomy upward along the anus up to ileocecal junction. After rinse with the
precooling of saline, the colon was blotted by filter paper and its length was measured, then 0.8 cm colon near the anus 0.5 cm was gently sheared, fixed with 4% paraformaldehyde solution for 24h and then delivered to Department of pathology, affiliated hospital of southeast university to perform hematoxylin and eosin (H&E) staining. Those sections were observed and photographed.

2.3.3. Histopathological features
The sections were analysed from three aspects: (1) inflammation severity; (2) inflammation scale, from none to penetrating the basal layer; (3) the extent of crypt damage, from none to the disappearance of crypt and epithelial cells.

2.4. Ethics
All the experiment about mice was under the guidelines and ethics of the animal care and use committee of southeast university (Nanjing, China).

2.5. Statistical analysis
The statistics were performed with SPSS and the comparison between data of two groups was analysed by students-t test, and p value below 0.05 was set as statistical significance.

3. Results and discussion

3.1. Body weight change and DAI score
By monitoring, weight loss could be measured from mice in the 5% DSS group, which appeared diarrhea, anus bleeding and emaciated rapidly, accompanied with vitality lack, shaggy hair at the end of experiment. As shown in Figure 1A, from day 0 to 3, the body weight of mice in the two group were increasing, and rapid decrease of mice weight existed in the DSS-induced group, which was significantly different from another group after day 4 (p < 0.001). After 5% DSS administration, stool of mice presented loose or even diarrhea. Occult blood tested positive existed from day 1, and every day thereafter, occult blood became severe gradually and thus occult blood score increased. The inflammatory manifestations were lasting throughout the experiment, with a negative correlation body weight, an increased presence of diarrhea and appearance of blood in faeces, demonstrated in Figure 1B by DAI score (p <0.001).

![Figure 1](image)

**Figure 1.** Change of body weight and DAI score of mice from two groups. (A) Body weight change (%) of mice; (B) DAI score. *P < 0.05, **P < 0.01 and ***P < 0.001.

3.2. Changes in colon length of mice in two groups after DSS treatment
Colon length was a notable index to reflect the severity of colorectal inflammation. Sacrifice of mice was carried out after the 7 days administration, and their entire colon was gently separated by longitudinal anatomy upward along the anus up to ileocecal junction. The entire colon length was measured and photographed. In Figure 2A, twenty images displayed that the colons of mice drinking
water daily were healthy and no ulceration. While the mice fed 5% DSS water were all with an acute inflammation, characterized by the blood stool. Moreover, the macroscopic scores were also consistent with the change of the colon length. Mice in the control group had longer colons than those drinking 5% DSS. As shown in the graph Figure 2B, the length in the DSS group (4.8 ± 0.41 cm) and that in the control group (7.97 ± 0.42 cm) were significantly different \( (p < 0.001) \). These results indicated that the model of acute colitis was constructed successfully.

Figure 2. The length of mice colons from two groups. (A) Macroscopic appearance of mice colon samples, \( n = 10 \); (B) Colon length (cm) of mice from the control and the DSS-treated. Data of 10 mice per group presented as Mean ± SD, and significantly difference was set as \( * P < 0.05 \), \( ** P < 0.01 \) and \( *** P < 0.001 \) versus control group.

3.3. Histopathological features of mice colons after 7-days DSS treatment

H&E staining was carried out to show the histological features of mice colons for next analyses. And slices of colon sections in two groups with H&E staining were applied to exam histological changes in the epithelium and stroma of the colon under a microscope and photographed. As shown in Figure 3, in normal mice, the colons displayed that the mucosal layer, submucosal layer, muscular layer and serous membrane layer of the colon were complete, with the crypts intact and clear, and no obvious inflammatory cell infiltration was observed. Whereas, 5% DSS group showed significant inflammatory response, with large scale loss and necrosis of colonic mucosa, disappearance of crypts and epithelial cells, and large scale of infiltration of inflammatory cells, and a large number of plasma
cells, which were different from the control group. All these indicated that the model was successfully induced.

Figure 3. H&E staining slices from colorectal sections, here each group with a representative slice, the top magnification (100 ×) and the bottom (200 ×). (Control: the control group; DSS: the DSS group).

4. Conclusion

In this study, 5% DSS induced a successful acute colitis model in C57BL/6. From three notable indexes, the evaluation was performed with main features, based on these features, the following changes existed in 5% DSS-treated acute colitis mouse model:

1. Under the administration of drinking 5% DSS, C57BL/6 started to lose body weight on day 4, and the results showed statistically significant in next 3 days, different from mice in the control group.

2. The colons were shorter significantly than those of the control mice, meaning that 5% DSS for 7 days is a good way to create the acute colitis model.

3. Histopathological features with large scale loss and necrosis of colonic mucosa, disappearance of crypts and epithelial cells, large scales of infiltration of inflammatory cells, and large numbers of plasma cells were observed.

Based on these results, the acute colitis model was induced successful.

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