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Characterization of Acute Murine Dextran Sodium Sulfate (DSS) Colitis: Severity of Inflammation is Dependent on the DSS Molecular Weight and Concentration

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ABSTRACT

***Background*:** Colitis murine models have become essential tools to investigate the molecular and cellular mechanisms that lead to inflammatory bowel disease (IBD), such as ulcerative colitis (UC). DSS-induced colitis model faithfully reproduces many of the clinical presentation and immunological disturbances observed in UC. Notwithstanding mice can show differential susceptibilities and responsiveness to dextran sodium sulfate (DSS), and varying DSS concentration and molecular weights appear to be associated with the severity of inflammation. The aim of this study was to analyze the features of mice induced colitis using different DSS concentrations and molecular weights.

***Materials, Methods and Results*:** C57BL/6 mice received 2% of high molecular weight DSS (36 000 - 50 000) in drinking water (HDSS2%) or 5% of the same molecular weight (HDSS5%); other group received 5% of low molecular weight DSS (10 000) (LDSS5%). During the 7 days of DSS administration, animals were observed for weight loss, stool consistency and presence of blood feces to determine the disease activity index (DAI). On day 8, colons were removed, measured and weighed for indirect assessment of inflammation. The tissue samples were processed for histological analysis and blood samples were collected for hematological analysis. Our results demonstrated that HDSS5% group began to show significant clinical signs starting from day 1, HDSS2% on day 2 and LDSS5% on day 3 ($P < 0.05$). However, from day 3, HDSS5% group presented DAI significantly higher than other groups ($P < 0.001$). In addition, DSS administration for 7 days was associated with significant ($P < 0.05$) changes in mice body weight compared to control animals. Group HDSS2% showed a weight loss of 23.8%±3.0, and HDSS5% and LDSS5% groups, presented weight loss of 32.65%±0.0 and 8.7%±1.7, respectively. From day 6, HDSS5% group presented weight loss significantly greater than HDSS2% and LDSS5% groups ($P < 0.05$). In colon macroscopic analysis, high molecular weight DSS groups showed a significantly macroscopic colon changes ($P = 0.001$) and hematological parameters alteration ($P < 0.005$) compared to control group. In histological features of colitis, these groups presented a higher histological score compared to normal colon ($P < 0.001$), with crypt damage, mucosal ulceration and cell inflammatory infiltration. Mice from group LDSS5% did not present significant macroscopic colon changes, hematological parameters alteration, and histological score compared to control group.

***Discussion*:** Results of the present study evidenced that acute colonic mucosal injury induced by DSS is dependent on the concentration and molecular weight of DSS administered in drinking water, and these findings are important consideration for reproducible induction of experimental colitis with this model. Moreover, DSS with high molecular weight and high concentration can initiate a severe colitis, which may not be an appropriate model for studies of therapeutic regeneration of the colonic mucosa. Thus, identification of differences in mice response to DSS could provide the basis for investigations of susceptibility or resistance to colitis. DSS-induced colitis model study contributes to the understanding of IBD and in the finding for new therapies targeting the reduction of inflammation.

Keywords: dextran sodium sulfate, ulcerative colitis, DSS-induced colitis model, DSS molecular weight, DSS concentration.

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INTRODUCTION

Inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) are chronic and relapsing inflammation of the gastrointestinal tract [11,19]. Although the causes of IBD are still unknown, recent advances in the understanding of molecular pathogenesis have been made.

Given the variety of etiological factors and complex genetic heterogeneity, much of the knowledge of IBD pathogenesis has come from studies of animal models [1,2]. Colitis murine models have become essential tools to investigate the molecular and cellular mechanisms leading to IBD. These models are frequently used to develop novel alternative treatments [4].

Dextran sodium sulfate (DSS) damage is believed to result primarily from an acute chemical toxicity into colonic epithelium [14]. Colitis mice models are fed with DSS in drinking water, developing diarrhea, rectal bleeding and weight loss. The exact mechanism of induction and pathogenesis of DSS-induced colitis is unknown [15,17].

It is known that DSS-induced colitis faithfully reproduces many of the clinical presentation and immunological disturbances observed in UC [19]. Notwithstanding mice can show differential susceptibilities and responsiveness to DSS, and varying DSS concentration [6,12,13,15,16,18] and molecular weights [8,9,15] appear to be associated with the severity of inflammation. To understand the mechanisms of colitis in this model, it is important to elucidate the relationship between molecular weight/concentration and features of colitis. Thus, the aim of this study was to analyze the features of mice induced colitis using different DSS concentrations and molecular weights.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice, 8-12 weeks old, were purchased from the Unidade de Experimentação Animal (UEA) of the Hospital de Clínicas de Porto Alegre (HCPA) - Universidade Federal do Rio Grande do Sul (UFRGS). Mice were kept at the house facilities, with humidity (50%) and temperature controlled (20-22°C), 12-12h light-dark cycles, and fed standard diet and drinking water *ad libitum*. All procedures were performed in accordance to UFRGS guidelines for animal experimentation and the Brazilian Federal Law 11.794/08 that establishes procedures for the scientific use of animals and regulates the registration of experimentation centers.

Mouse DSS-induced colitis

DSS at two different molecular weights, 36 000 - 50 000 Da[1] and 10 000 Da[2], were used in this study. Mice C57BL/6 were divided into four groups each one containing 6 animals. The control group received normal drinking water.

Animals that received 2% of high molecular weight DSS (36 000 - 50 000 Da) in drinking water were named HDSS2% group; the group that received 5% of the same molecular weight DSS was named HDSS5%. Animals that received 2% of low molecular weight DSS (10 000 Da) were called LDSS2%. The protocol was followed by 7 days, and water was changed each 48 hours.

Clinical evaluation

The disease activity index (DAI) score was determined by an investigator blinded to the protocol. Daily, animals were observed for weight loss, stool consistency and presence of gross blood feces and anus [1]. For each parameter a score from 0 to 4 was attributed, resulting in the total DAI score ranging from 0 (unaffected) to 12 (severe colitis) [Table 1].

Colon Macroscopic evaluation

After 7 days of DSS administration, mice were euthanized by cervical dislocation of spine, and colons were removed from the cecum to the anus. The samples were measured and weighed for indirect assessment of inflammation.

Histological evaluation of colitis

Colons were fixed in 10% buffered formalin, processed and embedded in paraffin to obtain longitudinal medial cuts. Colon section (4 μm) was stained with haematoxylin-eosin (H&E) and analyzed using halogen light microscope. Histological score was blindly determined as per Dieleman *et al*. [5] (Table 2). Each parameter of histological score, such as severity of inflammation (0-3), depth of inflammation (0-3), regeneration (0-4) and crypt damage (0-4), was multiplied by the percentage of compromised tissue (1 point for 25%, 2 points for 26-50%, 3 points for 51-75%, and 4 points for 76-100%). Therefore, inflammation and extent have a range from 0 to 12, and regeneration and crypt damage have a range from 0 to 16.

Hematologic analysis

Following isoflurane-induced anesthesia, blood samples were collected by retro-orbital puncture for hematological analysis. The hematologic parameters such as white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), hemoglobin (Hb), and percentage of lymphocytes (Lym), monocytes (Mon) and granulocytes (Gran) were analyzed using an electronic hematologic analyzer.

Statistical Analysis

Results were shown as mean ± standard error of mean (SEM) for each group. Statistical analysis was performed using SPSS (Version 18.0) statistical software. Generalized Estimated Equations (GEE) was used for DAI and weight loss analysis. For multiple comparisons (colon weight and length, and hematologic analysis) non parametric Kruskal-Wallis test was used. In case significant differences, post hoc analysis was performed with Bonferroni test. $P < 0.05$ was considered to be statistically significant.

Table 1. Disease activity index (DAI) score.

Score	Weight loss	Stool consistency	Bleeding
0	none	normal	no bleeding
1	1-5%	-	-
2	5-10%	loose stools	slight bleeding
3	10-15%	-	-
4	more than 15%	watery diarrhea	gross bleeding

Table 2. Histological grading of colitis.

Feature graded	Grade	Description
Inflammation	0	None
	1	Slight
	2	Moderate
	3	Severe
Extent	0	None
	1	Mucosa
	2	Mucosa and submucosa
	3	Transmural
Regeneration	4	No tissue repair
	3	Surface epithelium not intact
	2	Regeneration with crypt depletion
	1	Almost complete regeneration
	0	Complete regeneration or normal tissue
Crypt damage	0	None
	1	Basal 1/3 damaged
	2	Basal 2/3 damaged
	3	Only surface epithelium intact
	4	Entire crypt and epithelium lost
Percent involvement	1	1-25%
	2	26-50%
	3	51-75%
	4	76-100%

RESULTS

Disease Activity Index (DAI)

During DSS administration, the disease activity index presented different degrees of severity in all three groups. As expect compared to control group all DSS administered animals presented a significant DAI increase, characterized by bloody diarrhea, rectal blood and sustained weight loss (Figure 1A).

Group HDSS5% began to show significant clinical signs compared to control group starting from day 1 (0.5 ± 0.2 control group and 1.58 ± 0.14 HDSS5%, $P < 0.001$), HDSS2% started to differ from control group on day 2 (0.5 ± 0.31 control group and 2.2 ± 0.33 HDSS2%, $P = 0.001$) and LDSS5% presented the difference on day 3 (0.17±0.15 control group and 1.75 ± 0.34 LDSS5%, $P < 0.01$). From day 3, HDSS5% group presented DAI significantly higher than HDSS2% and LDSS5% groups ($P < 0.001$).The disease activity index is presented on Figure 1B.

DSS administration for 7 days was associated with significant ($P < 0.05$) changes in mice body weight compared to control mice. Group HDSS2% showed a weight loss of 23.8%±3.0, and HDSS5% and LDSS5% groups, 32.65%±0.0 and 8.7%±1.7, respectively. From day 6, HDSS5% group presented weight loss significantly greater than HDSS2% and LDSS5% groups ($P < 0.05$) [Figure 1C].

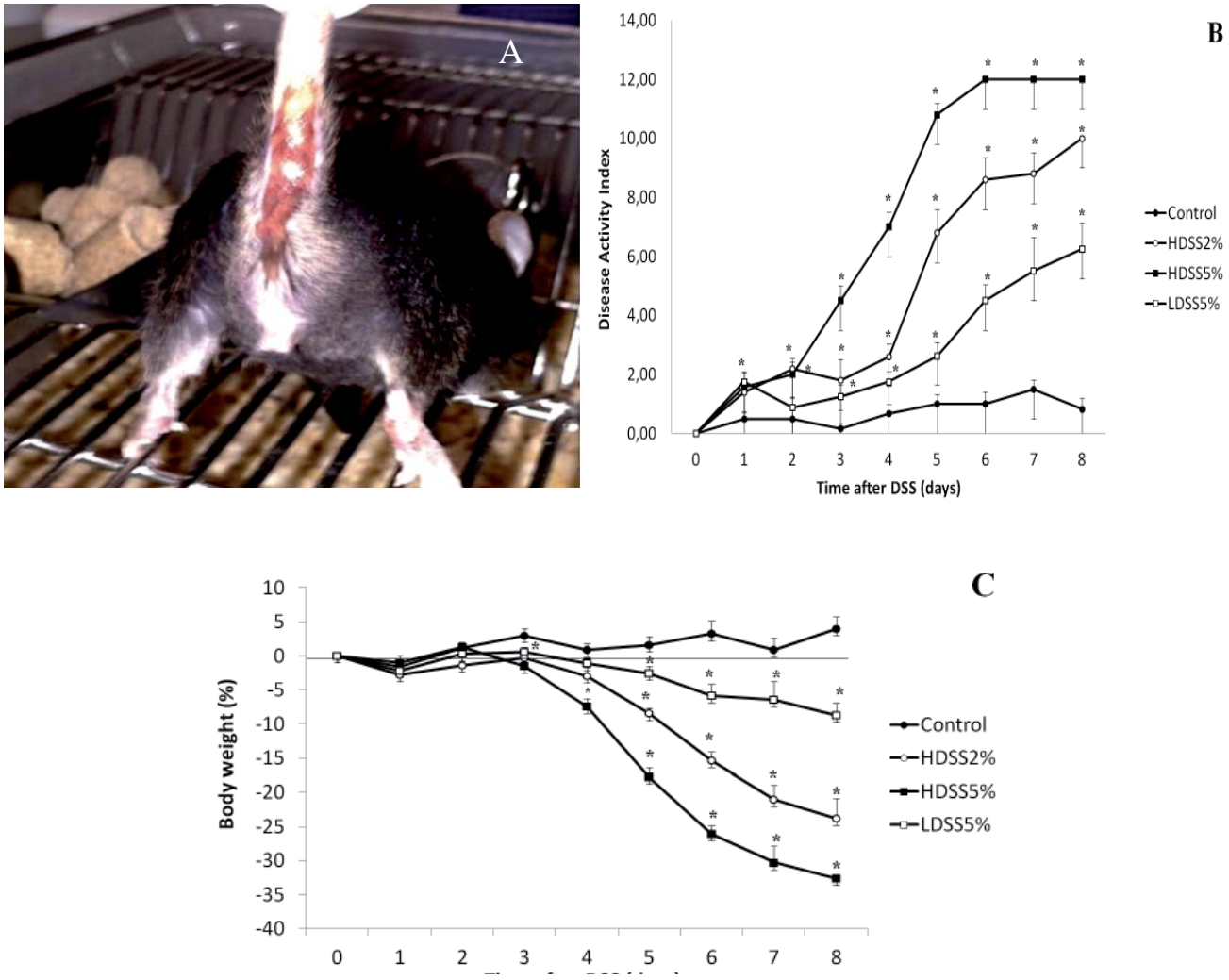

Figure 1. DSS with different concentrations and molecular weight induce clinical signs of acute colitis in mice. (A) Representative photograph showing clinical sign of colitis in HDSS2% mice after 5 days. (B) Disease activity score. Group HDSS5% began to show significant clinical signs starting from day 1, HDSS2% on day 2 and LDSS5% on day 3($*P < 0.05$ vs control group). From day 3, HDSS5% group presented DAI significantly higher than HDSS2% and LDSS5% groups. (C) Weight loss. Treatment of mice with DSS for 7 days was associated with significant changes in body weight compared to control mice ($*P < 0.05$ vs control group). From day 6, HDSS5% group presented weight loss significantly greater than HDSS2% and LDSS5% groups. n = 6 mice/group.

Macroscopical changes of colon

Mice that received high molecular weight DSS over 7 days presented macroscopical changes, associated with DSS-induced colitis. These changes included shortening of the colon length and decreased weight. HDSS2% and HDSS5% showed a colon length and weight significantly lower than the control group ($P = 0.001$ and $P = 0.045$, respectively). Mice from group LDSS5% did not present significant macroscopical changes compared to control group. (Figures 2A, 2B and 2C).

Histophatology

Groups HDSS2% and HDSS5% showed histological pattern similar to acute colitis different from control group ($P < 0.001$). Colonic inflammation was mostly confined to the mucosa with loss of goblet cells, crypt damage, and mucosal ulceration, but in some areas extensive edema of the submucosa was observed. In areas of local lesions inflammatory cell infiltration was seen, including neutrophils and mononuclear cells (Figure 3A). Histological score data from different groups are presented on Figure 3B. Mice from LDSS5% group did not show signs of colitis in histological analysis.

Effect on hematological parameters

A statistically significant increase in the WBC count was seen in HDSS5% when compared to control group ($P = 0.038$). Also there was a statistically significant increase in the total Gran (%) count on HDSS2% and HDSS5% compared to control group ($P = 0.015$). DSS treatment did not lead to a statistically significant change in other hematological parameters (Table 3).

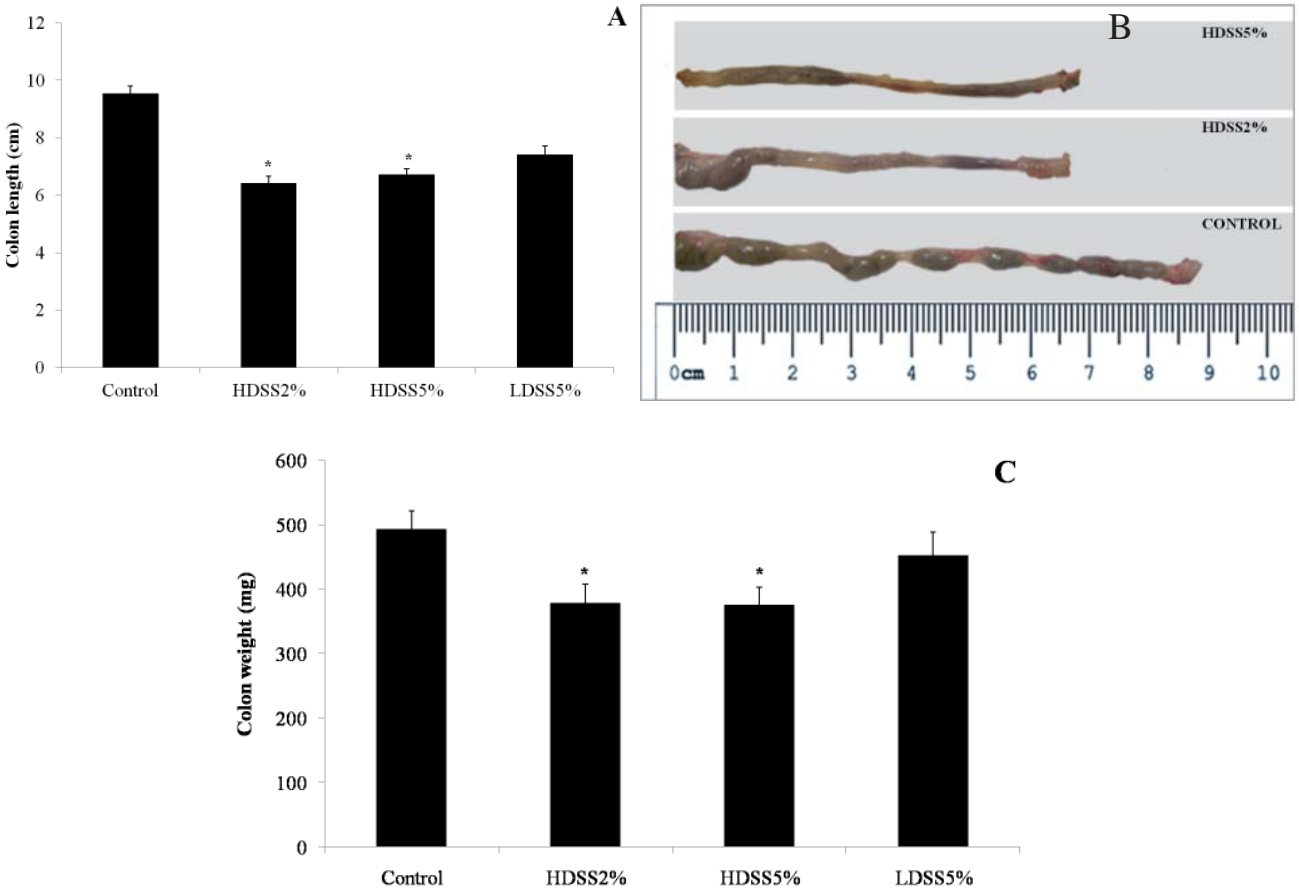

Figure 2. Colon macroscopic analysis. (A) Colon length. Groups HDSS2% and HDSS5% showed a significantly lower colon length compared to control group (*$P = 0.001$). (B) Representative photograph showing the shortening of the colon in mice treated with DSS. (C) Colon weight. Groups HDSS2% and HDSS5% showed a significantly lower colon weight compared to control group ($P = 0.045$). n = 5-6 mice/group.

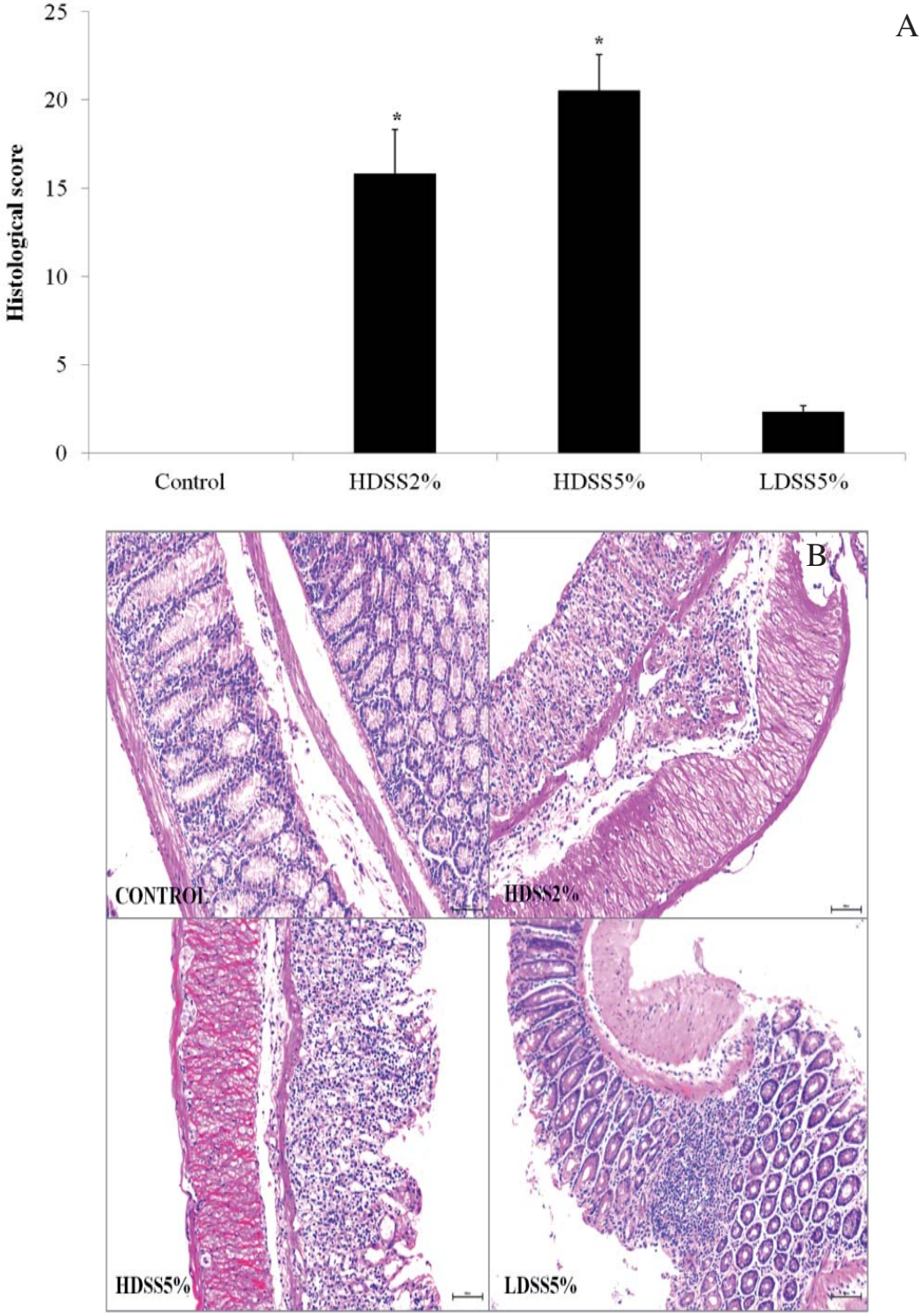

Figure 3. Histological features of colitis induced by DSS. (A) Histological score. HDSS2% and HDSS5% groups presented a higher histological score compared to normal colon (*$P < 0.001$). LDSS5% group showed no statistically significant difference compared to the control group.(B) Representative photomicrographs of mice colon sections stained with hematoxylin and eosin (H&E, x100). n = 5-6 mice/group. [Scale bars represent 100μm.]

Table 3. Effect of DSS treatment on the hematological parameters in mice.

Parameters	Groups			
	Control	HDSS2%	HDSS5%	LDSS5%
WBC (×103/mm3)	4.95 (2.7;6.6)	7.60 (5.6;26.6)	17.55 (14.2;21.9)*	10.90 (5.2;12.1)
RBC (×106/mm3)	8.45 (4.2;9.2)	8.75 (7.2;9.0)	4.02 (3.1;6.4)	7.97 (6.2;8.2)
MCV (fl)	45.00 (44.5; 49.2)	47.00 (46;49.5)	44.00 (44;46)	47.00 (46;47)
Hb (g/dl)	12.60 (9.7;12.8)	12.70 (11.2;13.4)	6.10 (4.7;9.5)	11.60 (9.6;12.7)
Lym (%)	85.45 (82.2;88.1)	80.60 (58.8;87.6)	73.45 (68;76.2)	87.20 (83.7;88.6)
Mon (%)	8.55 (5.9;9.0)	4.90 (3.8;8.8)	13.80 (11.7;14.9)	7.00 (5.7;8.4)
Gran (%)	6.05 (4.3;10.1)	15.50 (8.5;32.5)**	14.20 (10.7;18.2)**	6.30 (5.6;7.1)

All the values are expressed as percentiles (n = 5-6). *P = 0.019 and **P = 0.016 vs. Control.

DISCUSSION

DSS-induced colitis model has been widely employed to improve current understanding and treatment of IBD. This model has some advantages when compared to other colitis models, due to convenient induction of intestinal inflammation such as DSS in drinking water *ad libitum*, low mortality rate, and high reproducibility [4,8,17]. Furthermore, acute and chronic colitis model can be induced by changing concentration and cycle of DSS administration [7]. However, the exact mechanism of colitis induction in this model is unknown. Some authors suggest that the increased apoptosis and decreased proliferation of epithelial cells might lead to a breakdown of the epithelial barrier function, and thus facilitating the mucosal invasion of intraluminal microorganisms in DSS-induced colitis [19].

DSS-induced colitis studies suggest that the inflammation severity is dependent on the concentration of DSS, the molecular weight of DSS, the duration of DSS supplementation, and the inbred mouse strain [15]. Egger *et al*. [6] administered four different concentrations of DSS (0, 2.5, 5, 7.5%) for 7 days. Their results showed that acute colonic mucosal injury induced by DSS is directly dependent on the concentration of DSS administered in drinking water, since an increase was seen in crypt damage and pro-inflammatory cytokine with increasing concentration. Kitajima *et al*. [9] administered 5% DSS at three different molecular weights (5, 40 and 500 kD). Colitis induced by 40 kD DSS was more severe than when induced by 5kD DSS, but no colitis was observed in the mice given 500 kD DSS. Authors suggested that the lack of colitis in mice treated with 500 kD DSS is due to high molecular weight preventing passage of the molecules through the mucosal membrane. These findings suggest the molecular weight of DSS to be an important factor in colitis murine model. In the present investigation, oral administration of high molecular weight DSS for 7 days induced several symptoms including diarrhea and weight loss, and histological changes of the colonic mucosa including inflammation and crypt loss, especially in the distal colon. In addition we showed the severity of colitis when administrating HDSS2% and HDSS5% in macroscopical changes of colon and in hematological parameters. In contrast mice that received low molecular weight DSS did not present significant macroscopical and hematological parameters changes (compared to control group), and showed less severe sign of disease than other experimental groups. Thus, the molecular weight of DSS is an important consideration for reproducible induction of experimental colitis in this model. However, high molecular weight DSS should be tested in different concentrations due to the severity of the disease, because it may cause low capacity for tissue regeneration (data not shown) and high mortality rate. Our results evidenced that acute colonic mucosal injury induced by DSS is dependent on the concentration of the DSS administered in drinking water. HDSS5% group showed clinical signs earlier and greater weight loss than HDSS2% group (with the same molecular weight) during 8 days of observation. Histological analysis indicates that HDSS5% produces more severe disease than LDSS5% group. DSS with high molecular weight and high concentration can initiate severe colitis, which may not be an appropriate DSS-induced colitis model for studies of therapeutic regeneration of the colonic mucosa.

Another important question regarding de development of colitis animal model is the mice strain.

Some authors have demonstrated that C57BL/6 strain is resistant to cecum inflammation, but susceptible in the colon. In contrast with other strains tested, such as DBA/2J, which showed intermediate susceptibility in the cecum while being most resistant in the colon [10]. These differences in disease expression have been attributed to genetic differences in the ability of the mucosa to withstand inflammatory damage. In our high molecular weight DSS-induced colitis model, the acute phase of colitis was characterized by crypt damage and mucosal and submucosal inflammation with inflammatory cells extending along the colon. HDSS2% group presented an acute inflammation of the colon, followed by a slow regeneration of the colonic epithelium. However, HDSS5% group showed no regenerative capacity of the epithelium. Thus, identification of differences in the response of mice to DSS could provide the basis for investigations of susceptibility or resistance to colitis. Since the impaired colon of C57BL/6 mice has a capacity of tissue regeneration in HDSS2% model, it becomes more suitable for the study of therapeutic alternatives in UC.

We concluded that the severity of acute colitis was distinct for the different concentration and molecular weights of DSS demonstrating the importance of reagent characteristics in the development of a reproducible experimental animal model of colitis.

SOURCES AND MANUFACTURES

[1]MP Biomedicals, Solon, OH, USA.

[2]Sigma, St. Louis, MO, USA.

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Ethical approval. This study was approved by the Institutional Research Ethics Committee CEUA-HCPA (Porto Alegre, RS, Brazil) and is registered under the number 11-0244,

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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