Inhibition of Aloperine on Dextran Sulphate Sodium-induced Chronic Colitis in C57BL/6 Mice

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Abstract: Objective To investigate the effects of aloperine (ALO) on a model of dextran sulphate sodium (DSS)-induced chronic colitis in C57BL/6 mice. Methods Repeated colitis was induced by administration of four cycles of 4% DSS. The severity of colitis was assessed on the basis of clinical signs, ratio of colon weight and colon length, and histological grading scores. Moreover, secretory immunoglobulin A (S-IgA) and plasma haptoglobin (HP) were analyzed by enzyme-linked immunosorbent assay, and the changes of mRNA expression of ICAM-1 and MIF gene in colorectal tissue were detected by quantitative reverse transcriptase real-time polymerase chain reaction using SYBR Green I. Results ALO administration significantly attenuated the colon damage, caused substantial reductions of the rise in HP, and maintained the level of cecum S-IgA. ALO inhibited the ICAM-1 mRNA expression and had no effect on MIF mRNA expression. Conclusion The effect of ALO on DSS-induced chronic colitis in mice is investigated for the first time, which suggests that ALO could be an attractive therapeutic candidate in the treatment of inflammatory bowel disease.

Key words: aloperine; dextran sulphate sodium; haptoglobin; inflammatory bowel disease; secretory immunoglobulin A

Introduction

The inflammatory bowel disease (IBD), ulcerative colitis (UC), and Crohn’s disease are idiopathic, chronic, relapsing, and the inflammatory conditions that are immunologically mediated. The prevalence of IBD rapidly increased in Europe and North America in the second half of the 20th century and was becoming more common in the rest of the world as different countries adopt a Western lifestyle. Loss of tolerance to enteric environmental triggers in mucosal immune response associated with subsequent activation of these cells, such as lymphocytes and macrophages, causes a self-augmenting cycle of cytokine production, cell recruitment, and inflammation (Balfour, 2006; Daniei, 2002; Bouma and Strober, 2003; Xavier and Podolsky, 2007; MacDonald and Monteleone, 2005). Furthermore, one of the most serious complications of IBD is colorectal cancer, therefore, it is still a great challenge to develop new and specific therapies for IBD.

Aloperine (ALO, Fig. 1) is an alkaloid isolated from *Sophora alopecuroides* L. which is mainly used for the treatment of acute or chronic gastroenteritis in Chinese folk medicine for a long time with low side effect (Mou et al, 2005; Han, Zhou, and Liu, 2006; Cheng et al, 2006). With the consideration that ALO has the effects including antibacterial activity, anti-inflammation, inhibition of lymphocyte proliferation, macrophage activation by IL-1, and enhancement of IL-2 production (Cheng et al, 2006; Li et al, 1997; Wang et al, 2003), we hypothesize that ALO might exert its beneficial effect on colitis.

Therefore, the aim of the research was to investigate...
the effects of ALO on a model of dextran sulphate sodium (DSS)-induced chronic colitis in C57BL/6 mice. DSS could produce the inflammation in colonic mucosa, which is more closely related to human UC.

Materials and methods

Drugs and chemicals
Aloperine (98.5%, Batch No. 070903) was purchased from Yanchi Pharmaceutical Company (Ningxia, China). Dextran sulphate sodium (molecular weight 5000) was obtained from Amersham Biosciences (Amersham Biosciences Pharmacia Biotech, Sweden); Hematoxylin-eosin was purchased from Sigma Chemical Co. (Sigma, USA); RNAlate solution was from Ambion Company (Ambion, USA), total RNA extract reagent was purchased from Invitrogen Company (Invitrogen, USA); SYBR Prime Script™ RT-PCR Kit was purchased from Takara Biotechnology Co., Ltd. (Takara, China). Enzyme linked immunosorbent assay S-IgA and haptoglobin kit were purchased from Nanjing Jianchen Bioscientific Company (China), other regents were of analytical grade.

Animal care
Female C57BL/6 mice, six-week old, obtained from Animal Centre of Southern Medical University, were housed by five or six per cage and kept in the animal house facilities with room temperature of (20 ± 2) °C, 50% humidity, and 12:12 h light-dark cycle, fed with a standard pellet diet and administrated with tap water ad libitum. Animal Research Board Committee of Southern Medical University approved the studies. Mice were acclimatized for two weeks before the study. Body weights, water and food intake, and occult blood scores were measured every week during the experiment.

After a 14-day acclimation period, a total of 46 mice were randomly divided into four groups. Group 1 (n = 11) was administrated with water as control. Groups 2 – 4 received four cycles of DSS administration (each cycle: treated with 4% DSS dissolved in distilled water for 7 d and then with distilled water for 7 d). This schedule was used to simulate the cycle of acute flare-ups alternating with a period of disease inactivity observed in human UC patients (Melgar, Karlsson, and Michaëlsson, 2005; Borm and Bouma, 2004). Group 2 (model group, n = 15) was ig administrated with distilled water (0.2 mL/kg) once daily for the whole experimental period. Groups 3 and 4 (n = 10, respectively) were ig administrated with ALO once daily at the dose of 25 mL/kg (low dosage group of ALO, ALOL) and 50 mL/kg (high dosage group of ALO, ALOH), respectively. Mice were weighed every week for the determination of percent weight change. This was calculated as: weight change = (weight at week X—weight at week 0)/weight at week 0 × 100%. Occult and rectal bleeding and stool consistency were monitored everyday using the orthotolidine methods. Occult blood scores and stool consistency scores were determined according to a standard scoring system as previously described (Melgar, Karlsson, and Michaëlsson, 2005; Khoury, Jurjus, and Reimund, 2004).

Histological scoring
The mice were killed by dislocation 56 days after experiments. Some colon samples were stored at −80 °C for PCR. The colon was resected between the ileocaecal junction and the proximal rectum, close to its passage under the pelvisternum. The colon was placed onto a non-absorbent surface and measured with a ruler, taking care not to stretch the tissue. Colon length was measured as an indication of colonic inflammation. The colon was fixed with 10% neutral buffered Formalin, and prepared for routine processing. Serial tissue sections (3 µm) were made, mounted on glass slides, and routinely stained with hematoxylin-eosin for histopathological analysis.

Histopathological grading of chronic UC
Sections were coded with an accession number and reviewed by a pathologist without access to the code. Each section was scored for lesions based on severity, ulceration, hyperplasia, and Crypts alteration (Melgar, Karlsson, and Michaëlsson, 2005).

Production of ceicum S-IgA and plasma haptoglobin
Cecum content was collected rapidly and suspended in two-fold saline, and the supernatant by centrifugation (4 °C, 5 min, 5000 r/min) was collected. Content of ceicum S-IgA was determined by mouse S-IgA double antibody Sandwich ELISA Kit according to the manufacturer’s protocol. For the estimation of plasma haptoglobin (HP), blood was collected into heparin tube from mice via removing eyeball. Care was taken to minimize hemolysis, and the blood samples were stored at −20 °C. The HP concentration in the plasma was determined by mouse HP double antibody sandwich
ELISA Kit according to the manufacturer’s protocol. Using the results observed for the standards, a standard curve was constructed and correlation and regression were calculated.

RNA isolation and quantitative reverse transcriptase real-time polymerase chain reaction (qRT-PCR) using SYBR-Green I fluorescent dye

The RNA-later-preserved colon tissues were homogenized, and extracted using Trizol Reagent according to the manufacturer’s instructions. Extracted RNA was dissolved in 20 μL free RNA water. Total RNA (2 μg) from each sample was converted into cDNA. The cDNA synthesis was performed as described previously. Relative gene expression quantification for ICAM-1, with GAPDH as an internal reference gene, was carried out using Mx3005 RT-PCR Amplification Detection System in triplicates, based on the SYBR-Green method. Primer sequences were as follows: ICAM-1, sense 5'-CAACTGGAAGCTGTTTGAGCTGAG-3' and reverse 5'-AGGGTGAGGTCCCTTGCCTACTTG-3'; MIF, sense 5'-CTGCACACGATCGCAAGA-3' and reverse 5'-TTGGCAGCGTTCATGTCGTAA-3'; GAPDH, sense 5'-AAATGGTGAAGGTCGGTG-3' and reverse 5'-TGAAGGGGTGTTTGATGG-3'. The PCR reaction mixture consisted of 0.1 μmol/L of each primer, 1 × SYBR Premix EX Taq (Perfect Real Time) premix reagent, and 50 ng cDNA to a final volume of 20 μL. Cycling conditions were 95 ℃ for 10 min, followed by 40 cycles at premature 95 ℃ for 5 s, annealing temperature was 59 ℃ for 30 s. PCR specificity was confirmed by dissociation curve analysis and gel electrophoresis. The relative induction of gene mRNA expression, comparative Ct (ΔΔCt) was calculated using the following equation: 

\[ \Delta\Delta C_t = (C_{target} - C_{GAPDH})_{treatment} - (C_{target} - C_{GAPDH})_{nontreatment} \]

and final data were derived from 2⁻averageΔΔCt. GAPDH was used as internal control.

Statistical analysis

Data were expressed as \( \bar{x} \pm s \) and analyzed using SPSS 13.0 statistical software. Body weights were compared with repeated measure variance analysis and one way-ANOVA. Values were taken to be statistically significant (\( P < 0.05 \)).

Results

General observations

There was no death of mice during the experiment. First, the effects of ALO on symptomatic parameters including loss of body, water and food consumption, occult, and rectal bleeding and stool consistency caused by colitis were evaluated in this study. Typically, all the mice that had received 4% DSS exhibited severe diarrhea accompanied by rectal bleeding after DSS-exposed two weeks. As shown in Fig. 2, the mice receiving DSS were recorded a 2.73%–11.08% decrease in body weight compared with those in the control group (\( P < 0.01 \)) at different time points, whereas those receiving ALO at 25 and 50 mg/kg were recorded a gain of 32.29% in ALO group and 32.96% in ALO group at the end of week 8, respectively. As seen in Table 1, weight/length of the mice colon in model group (0.038 ± 0.003) was a significant augment compared with that of the control group (0.020 ± 0.003) (\( P < 0.01 \)). In sharp contrast, ALO treatment at doses of 25 and 50 mg/kg suppressed the increase in the weight/length of the mice colon [(0.023 ± 0.002) for ALO and (0.024 ± 0.003) for ALOH], respectively. No statistic significant difference in food and water intake was found in ALO groups compared with model group (\( P > 0.05 \)).

Histopathological examinations

On histological examination of colon from normal mice, the histological features of the colon were typical of a normal structure. In DSS-treated mice, the inflammation extended through the mucosa, muscularis mucosae, and submucosa. Extensive granulation tissue with presence of fibroblasts, lymphocytes, leukocytes, and diffuse inflammatory infiltrates was apparent. In some sections of ulcerated areas, necrotic tissue adjacent to surface cells could be observed. The mucosa adjacent to ulcers showed grossly elongated crypts. Furthermore, severe and extensive denudation of the surface epithelium (erosions) and mucodepletion of glands appeared. After administration of ALO, lesion of the colonic histopathology was dramatically reduced: There was an attenuation of morphological signs of cell damage; The colonic mucosa showed the healing process of the ulcers; And an evolution to a more chronic inflammatory infiltrate was carried out, with mononuclear predominance and initiation of a repair process, as shown in Figs. 3 and 4.

Changes in plasma HP and cecum S-IgA for ALO-treatment mice

As shown in Fig. 2, an important increase in plasma
Fig. 2  Effect of ALO on DSS-induced C57BL/6 colitis mice (\( \bar{X} \pm s \))
A: Time-course of water intake. Weekly changes in water intake
B: Time-course of food intake. Weekly changes in food intake
C: Time-course of percent body weight change
D: Weekly changes in occult blood scores
E: Weekly changes in stool consistency scores
F: Effect of ALO on the cecum S-IgA and plasma HP content
**\( P < 0.01 \) vs control group  ▲▲\( P < 0.01 \) vs model group

Table 1  Effect of ALO on quantified parameters of DSS-induced C57BL/6 colitis mice (\( \bar{X} \pm s \))

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Histological gradings</th>
<th>Spleen indexes</th>
<th>Ratios of colon weight and colon length / (g·cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>11</td>
<td>0.00 ± 0.000</td>
<td>0.0023 ± 0.000 32</td>
<td>0.020 ± 0.003</td>
</tr>
<tr>
<td>model</td>
<td>15</td>
<td>13.47 ± 0.834**</td>
<td>0.0090 ± 0.0032**</td>
<td>0.038 ± 0.003**</td>
</tr>
<tr>
<td>ALOL</td>
<td>10</td>
<td>8.23 ± 1.235▲▲</td>
<td>0.0030 ± 0.0003▲▲</td>
<td>0.023 ± 0.002▲▲</td>
</tr>
<tr>
<td>ALOH</td>
<td>10</td>
<td>7.56 ± 0.976▲▲</td>
<td>0.0030 ± 0.0003▲▲</td>
<td>0.024 ± 0.003▲▲</td>
</tr>
</tbody>
</table>

**\( P < 0.01 \) vs control group  ▲▲\( P < 0.01 \) vs model group

Fig. 3  Representative micrographs showing histopathology of DSS-induced colitis in C57BL/6 mice stained with haematoxylin
a: Noncolitic group showed normal histology of mice colon
b: Model group showed epithelial injury, focal loss of epithelial glands, extensive intestinal ulceration with abundant inflammatory infiltrate in lamina and submucosa, mucosa depletion, and oedema
c and d: ALOL (c) and ALOH (d) showed an attenuation of morphological signs of cell damage, epithelial regeneration, and decrease in inflammatory cell infiltrate, same as below
HP protein (0.65 ± 0.12) and decrease in cecum S-IgA (0.67 ± 0.13) characterized the colitis caused by DSS compared to control group [(0.45 ± 0.03) for HP and (1.43 ± 0.40) for S-IgA], which was consistent with the histological findings. Moreover, after treatment with ALO, data clearly indicated a significant reverse alteration.

**ALO inhibition on mRNA expression of colon tissue ICAM-1 for DSS-exposed mice**

The levels of mRNA expression measured by qRT-PCR were then analyzed. The amplification kinetics of the gene ICAM-1, migration inhibitory factor (MIF), and GAPDH assays were approximately equal, gel electrophoresis of PCR products was single band, therefore, ΔΔCt method for the determination of mRNA expression was chosen. As shown in Fig. 5, the ICAM-1 mRNA expression fold change of DSS-treated mice was 1.57 vs normal 0.32, exposure of colon to DSS-caused strong mRNA expression of ICAM-1 gene, in contrast, ALO induced the down-regulation of ICAM-1 mRNA expression in the treated groups [(0.50 ± 0.225) for ALOH group and (0.21 ± 0.15) for ALOL group] vs model (1.57 ± 0.555). No significant change of macrophage-MIF mRNA expression was observed.

**Discussion**

ALO, one of the quinolizidine alkaloids, is a new anti-inflammatory drug. Our results demonstrate an improvement of DSS-induced colitis in mice treated with ALO as reflected in the experimental data, and by means of a macroscopic and histological disease score. ALO significantly mitigated the appearance of diarrhoea and the disruption of colonic architecture. Moreover, there was an remission of morphological signs of cell damage, and an evolution to a more chronic inflammatory infiltrate, with mononuclear predominance and initiation of a repair process. The colonic mucosa showed the healing process of ulcers. As we know, the protective effect of mucus as an active barrier may be attributed largely to its viscous and gel-forming properties that are derived from mucin glycoprotein constituents (Mora et al., 2006). S-IgA is also a component of mucin. Colon epithelia and local plasma cells produce preferentially secretory dimers and larger polymers of immunoglobulin A, perform immune exclusion in a first-line defense, thereby counteract microbial colonization and mucosal penetration of soluble antigens. The experiment showed that ALO protected the crypt gland and mucous architecture, and maintained the cecum S-IgA level. All these observations may have significance on the beneficial effect of ALO on colitis.

Compelling evidence indicated that emigration of leucocytes to the site of inflammation might be related to ICAM-1 and the enhanced colonic mucosal endothelial cell ICAM-1 expression would be an early event in the inflammatory cascade of acute colitis (Danese et al., 2005; van Assche and Rutgeerts, 2005; Roebuck and Finnegan, 1999). Our results showed that mRNA expression of ICAM-1 was corrected with the development of colonic inflammation and moreover ALO administration was able to suppress mRNA expression of ICAM-1.
MIF is a ubiquitously expressed cytokine with a variety of mitogenic and pro-inflammatory functions (Morand, 2005; Ohkawara et al., 2005). Plasma concentration of MIF in patients with active Crohn’s disease was six-fold higher than that in healthy individuals and the development of chronic colitis is dependent on the cytokine (de Jong et al., 2001). The data indicated that ALO had no apparent effect on MIF mRNA expression in DSS-treated mice.

In conclusion, the present study demonstrated that ALO could attenuate the intestinal inflammation in experimental DSS-induced colitis model of mice, which was associated with the down-regulation of ICAM-1 mRNA expression. Therefore, ALO could be a potential therapeutic agent for the treatment of IBD, further investigations should be done in future.

Acknowledgments

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References


