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• *BRIEF REPORTS* •

Anti-inflammatory mechanism of oxymatrine in dextran sulfate sodium-induced colitis of rats

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Abstract

AIM: To investigate the anti-inflammatory mechanism of oxymatrine in dextran sulfate sodium (DSS)-induced colitis of rats.

METHODS: Acute colitis was induced by giving 2% DSS orally in drinking water for 8 d. Twenty-six male rats were randomized into oxymatrine-treated group (group A, 10 rats), DSS control (group B, 10 rats) and normal control (group C, 6 rats). The rats in group A were injected muscularly with oxymatrine at the dosage of 63 mg/(kg·d) from d 1 to 11 and drank 2% DSS solution from d 4 to 11. The rats in group B were treated with 0.9% saline in an equal volume as group A and drank 2% DSS solution from d 4 to 11. The rats in group C were treated with 0.9% saline as group B from d 1 to 11 and drank water normally. Diarrhea and bloody stool as well as colonic histology were observed. The levels of serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were determined by ELISA, and nuclear factor-κB (NF-κB) activity and the expression of inter-cellular adhesion molecule-1 (ICAM-1) in colonic mucosa were detected by immunohistochemistry method.

RESULTS: Compared with DSS control group, the inflammatory symptoms and histological damages of colonic mucosa in oxymatrine-treated group were significantly improved, the serum levels of TNF-α, IL-6, and the expression of NF-κB, ICAM-1 in colonic mucosa were significantly reduced.

CONCLUSION: The fact that oxymatrine can reduce the serum levels of TNF-α, IL-6, and the expression of NF-κB and ICAM-1 in colonic mucosa in DSS-induced colitis of rats indicates that oxymatrine may ameliorate the colonic inflammation and thus alleviate diarrhea and bloody stool.

Key words: Oxymatrine; Colitis; Colonic mucosa

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INTRODUCTION

The symptoms and colonic histopathology of rodent colitis model induced by dextran sulfate sodium salt (DSS) resemble more the human ulcerative colitis (UC) than other chemically induced colitis, which has become a research tool for the pathogenesis of UC and the development of new drugs in our country and abroad. Sophora flavescens Ait has distinct anti-inflammatory effect and, several mixture formulas with Sophora flavescens Ait are effectively used to treat UC in the clinic[1]. Oxymatrine, as the main efficacy component of light yellow sophora root, has been reported to be effective in the treatment of viral hepatitis and bronchial asthma. Our previous experiments suggested that oxymatrine might significantly improve the colonic inflamed damage in rat colitis induced by DSS, but its exact mechanism is unclear[2]. The present experiment aimed to elicit the anti-inflammatory mechanism of oxymatrine in terms of the regulation of nuclear factor-κB (NF-κB) to tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and inter-cellular adhesion molecule-1 (ICAM-1)[3-5]. The inflamed damage of DSS colitis in rats appears early, so many researches reported to administer studied drugs along with or ahead of DSS administration to obtain predicated efficacy[6-10]. As diarrhea and bloody stool alleviate rapidly after the withdrawal of DSS, which may affect the observation of drug effect, the present study administered oxymatrine 3 d earlier than DSS and terminated oxymatrine and DSS at the same time according to the literature mentioned above and preliminary experiments.

MATERIALS AND METHODS

Materials

Healthy male SD rats (n = 26, 4-5 wk) were obtained from Shanghai Experiment Center of Chinese Academy of Science, Shanghai, China. DSS (Sigma) was prepared as 2% solution with distilled water. Injection oxymatrine (kurorinone) was obtained from Ningxia Pharmaceutical Factory, Ningxia, China, with purity of more than 98%. TNF-α and IL-6 ELISA kits were purchased from R&D Corporation (Cat. R6000 and RTA00). NF-κBp65 (F-6) monoclonal antibody

was from Santa Cruz Co., Ltd (Cat. Sc-8008). Mouse CD54 monoclonal antibody was from Cedarlane Laboratory Co., Ltd (Cat. 01010603). Pepsin (Cat. DIG3009) and SP immunohistological staining hypersensitive kit (Cat. 9702) were obtained from Fuzhou Maixin Biotechnological Co., Ltd, Fujian, China.

Methods

Experimental design Rats (n = 26) were randomized into three groups: oxymatrine treatment group (group A, n = 10), DSS control (group B, n = 10) and normal control (group C, n = 6). In group A, oxymatrine was injected intramuscularly at the dose of 63 mg/(kg·d) on d 1-11 and 2% DSS solution was given from d 4-11; in group B, equal volume of 0.9% saline was used to take oxymatrine and other procedures referred to group A; in group C, only an equal volume of 0.9% saline was given intramuscularly on d 1-11 and drinking water was obtainable freely. Drinking volume of DSS in group A was controlled to near group B. The symptoms of rats were assessed by disease activity index (DAI) based on stool consistency and incidence of stool hemorrhage[11]. After blood sampling from inferior vena cava to obtain serum stored in -20 ℃ for detection on d 12, all rats were killed and the intact colons were taken and excised longitudinally to be fixed in 40 g/L formaldehyde. On d 13, colonic segments (8 cm) up from the anus were removed, embedded in paraffin and placed on lysine slices[2]. Sections were stained with hematoxylin and eosin for assessment of histological changes and scoring[6].

Detection of serum TNF-α and IL-6 ABC-ELISA assay of double antibodies sandwich were adopted.

Immunohistochemical detection of ICAM-1 SP immunohistological staining hypersensitive kit was used. The sections were deparaffinized and hydrated as routine and digested with pepsin for 15 min at 37 ℃. A solution (50 μL) was added and kept for 15 min at room temperature followed by a B solution (50 μL) for 30 min at room temperature; 50 μL of the primary antibody (diluted in 1∶75) was added and kept at 37 ℃ for 1 h, then placed at room temperature for 15 min and kept at 4 ℃ overnight (no primary antibody added samples were used for negative control); C solution (50 μL) was added, and kept for 1.5 h at room temperature followed by a D solution (50 μL) for another 1 h. Finally, the sections were processed with DAB, stained with hematoxylin followed by routine dehydration and slice-sealing.

Assessment criteria of the results: positive cells presented as buffy, located on cell membrane. Positive vessel was defined as one more positive vascular endothelial cells that were observed in the vessels of mucosa and submucosa. Then the positive vessels of distal colon (about 4 cm up from the anus) and the number of positive vessels of single colonic segment were counted (positive vessels/cm).

Immunohistochemical detection of NF-κB activity After digestion with pepsin, the sections were rinsed thrice with 0.1% PBS Triton X-100 for 5 min respectively, primary antibody diluted in 1∶100.

Assessment criteria of the results: positive cells presented as buffy, located on nuclei and cytoplasm. The counting method of positive endothelial cells refers to procedures above. The counting criteria of positive epithelia cells are as follows: 50 crypts were randomly observed in the distal colon (about 4 cm up from anus) and the percent of positive cells per crypt was counted, then the scores were counted according to the standards: 0 score for ≤5%; 1 score for 6-25%, presented as +; 2 scores for 26-50%, presented as ++; 3 scores for 51-75%, presented as +++; 4 scores for 76-100%, presented as ++++. Finally, the mean score of each rat was obtained.

Statistical analysis

Statistical analysis was performed according to one factor analysis of variance and Wilcoxon rank sum test. P<0.05 was considered to be statistically significant.

RESULTS

Comparison of DAI and histological scores

Eight days after drinking 2% DSS, SD rats appeared to have diarrhea, bloody stool; erosion and superficial ulcer of colonic mucosa; epithelial damage; inflammatory cell infiltration and proliferation of lymph follicles in mucosa and submucosa; dilatation and proliferation of capillaries and small vessels, which resembled human UC[11]. Each colon existed with mucosal damage that was located in distal colon. Compared with B group, both DAI and histological scores in group A decreased significantly (P<0.05, Table 1).

Table 1 Scores of DAI and mucosal damage in group A and B (n = 10) (mean±SD)

Group	DAI	Scores of mucosal damage
A	6.0±1.2[a]	5.9±1.1[a]
B	7.3±1.1	7.1±0.7

[a]P<0.05 *vs* group B.

Comparison of serum levels of TNF-α and IL-6

The serum levels of TNF-α and IL-6 in rat DSS-colitis increased distinctly than normal rats and declined obviously after treatment with oxymatrine. The serum levels of TNF-α and IL-6 in groups A-C were as follows: (9.49±1.01) and (55.50±12.13) ng/L, (13.70±1.33) and (77.80±14.03) ng/L, (8.32±1.15) and (40.57±4.79) ng/L. Compared with B group, the levels of serum TNF-α and IL-6 in group A were significantly reduced (P<0.01, P<0.05, Table 2).

Table 2 Serum levels of TNF-α and IL-6 in three groups (ng/L) (mean±SD)

Group	TNF-α	IL-6
A (n = 10)	9.49±1.01[b]	55.50±12.13[a]
B (n = 10)	13.70±1.33[d]	77.80±14.03[f]
C (n = 6)	8.32±1.15	40.57±4.79

[a]P<0.05 *vs* group C; [b]P<0.01 *vs* group B; [d]P<0.01, [f]P<0.01 *vs* group C.

Expression of colonic ICAM-1

In rat DSS-colitis, ICAM-1 expressed on vascular endothelial

cells and macrophages and it's expressions in capillaries and venules were higher than arterioles and most frequently appeared in mucosa and submucosa. The positive rates were high in sites and nearby of colonic erosion and superficial ulcer, and became more and more low following the alleviation of inflammatory damage up from 3 cm distance to the anus. Both the stainings of crypt epithelia in mucosa and lymphocytes were negative.

After administration of oxymatrine, the amount of positive vessels in rat DSS-colitis reduced obviously; only small amount of positive vessels were observed in colons of group C. The mean amount of positive cells in groups A-C were as follows: (82.75±19.46), (137.27±23.31), (12.97±1.53)/cm with significant differences by comparison between any two groups ($P<0.01$). The amount in group A decreased obviously compared to group B.

Expression or activation of NF-κB

In rat DSS-colitis, NF-κB expressed on vascular endothelial cells and mucosal epithelial cells located in nuclei and/or cytoplasm. Its expressions in capillaries and venules were higher than arterioles, and most frequently appeared in mucosa and submucosa. The positive rates were high in sites and nearby of colonic erosion and superficial ulcer, and became more and more low following the alleviation of inflammatory damage from 3 cm distance to the anus.

After administration of oxymatrine, the amount of positive vessels and positive rates of epithelial cells in rat DSS-colitis reduced obviously; the mean amount of positive cells of endothelial and epithelial cells in distal distant colon were (24.09±4.39) and (0.86±0.17)/cm in group A, (30.49±6.07) and (1.19±0.36)/cm in group B. The amount in group A decreased obviously compared to group B. None or only little amount of positive cells were observed in colons of group C with amount of (3.83±1.00)/cm of positive endothelial cells. The amount of positive cells of vessels in distal colon in group A decreased obviously compared to group B ($P<0.05$).

DISCUSSION

The present experiment indicates that the serum levels of TNF-α and IL-6 in rat DSS colitis increased distinctly than normal rats and declined obviously after treatment with oxymatrine, which suggests that oxymatrine may inhibit the expression of the above pro-inflammatory cytokines and therefore ameliorate the colonic damage related to them. TNF-α can recruit leukocytes in the inflammatory sites, stimulate monocytes, and vascular endothelial cells to express cytokines, induce the cascade effects for other cytokines and finally result in inflamed lesion of tissues. So it is necessary to inhibit the expression of TNF-α in the early stage of DSS colitis, prevent and alleviate the development of colitis[12,13]. IL-6 is capable of promoting lymphocyte proliferation and leading to the production of acute phase proteins in liver[9], also plays an important role in the development of colonic inflammation. The anti-inflammatory effect of oxymatrine may be associated with its inhibitory role to the expression of TNF-α and IL-6[14,15].

The experiment also demonstrated that expression of ICAM-1 by vascular endothelial cells and macrophages were enhanced greatly than normal in DSS-induced colonic sites; prophylactic treatment with oxymatrine reduced the inflamed, infiltration and ICAM-1 expression in rat colons, which indicates that oxymatrine may ameliorate DSS colitis by inhibiting ICAM-1 production. Bendjelloul *et al*.[16], reported that expression of ICAM-1 in ICAM-1 defected mice appears as negative or mild positive, its interaction with leukocytes and inflammatory activity were alleviated. ICAM-1 plays a key role in the trans-endothelial migration and immunological cell activation of leukocytes and prophylactic administration of ICAM-1 mAb could lighten the inflamed damage[7,17].

The present study also showed that no expression of NF-κB was observed in non-inflammatory colonic epithelial cells in rat and only mild positive was observed among vascular endothelial cells. However, NF-κB activation presented in both colonic epithelial cells and vascular endothelial cells; prophylactic treatment with oxymatrine reduced the colonic inflammation and NF-κB activation, which indicates that oxymatrine may ameliorate DSS colitis by downregulating NF-κB activation. Marrero *et al*.[18], also reported, that DSS colitis was related to the high activation of NF-κB. Inducers of NF-κB include TNF-α, oxidative stress and so on; NF-κB is capable of activating many genes such as adhesion molecule ICAM-1 and cytokine TNF-α, IL-1, IL-6, etc.[3-5,19,20] and therefore it is possible to block the key initial step of inflammation and its secondary effect by inhibiting NF-κB activity[21].

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