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

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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 intraperitoneally with oxymatrine at the dosage of 63 mg/(kg·d) from d 1 to 11 and drank water 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 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 inflammatory symptoms and histological damages of colonic mucosa were observed. The levels of serum tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), 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.

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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 given intramuscularly on d 1-11 and drinking volume of DSS in saline was given intramuscularly on d 1-11; in group C, only an 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 stool consistency and incidence of stool hemorrhage rats were assessed by disease activity index (DAI) based on group A was controlled to near group B. The symptoms of water was obtainable freely. Drinking volume of DSS in saline was given intramuscularly on d 1-11 and drinking

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).

**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).

**Expression of colonic ICAM-1**

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

| Group | DAI | Scores of mucosal damage |
|-------|-----|--------------------------|
| A     | 6.0±1.2<sup>a</sup> | 5.9±1.1<sup>b</sup> |
| B     | 7.3±1.1 | 7.1±0.7 |

<sup>a</sup>*P* < 0.05 vs group B.

| Group | TNF-α | IL-6   |
|-------|-------|--------|
| A (*n* = 10) | 9.49±1.01<sup>c</sup> | 55.50±12.13<sup>c</sup> |
| B (*n* = 10) | 13.70±1.33<sup>d</sup> | 77.80±14.03<sup>d</sup> |
| C (*n* = 6)  | 8.32±1.15 | 40.57±4.79 |

<sup>c</sup>*P* < 0.05 vs group C; <sup>d</sup>*P* < 0.01 vs group B; <sup>e</sup>*P* < 0.01, <sup>f</sup>*P* < 0.05 vs group C.

MNIST digit images are provided as supplementary material.
cells and macrophages and 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 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\(^{[12]}\), 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\(^{[14,13]}\). 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\(^{[17]}\).

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\(^{[19]}\), 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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