Anti-inflammatory effect of Diammonium Glycyrrhizinate in a rat model of ulcerative colitis

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Abstract

AIM: To explore the anti-inflammatory mechanism of Diammonium Glycyrrhizinate in a rat model of ulcerative colitis induced by acetic acid.

METHODS: Sprague-Dawley female rats were divided into four groups: Diammonium Glycyrrhizinate group, dexamethasone group, acetic acid control and normal control group. Colonic inflammation was evaluated by disease activity index, gross morphologic damage, histological injury and colonic myeloperoxidase activity. Immunohistochemistry was used to detect the expression of NF-κB, TNF-α and ICAM-1 in colonic mucosa.

RESULTS: Compared to the acetic acid control, both Diammonium Glycyrrhizinate and dexamethasone showed a significant anti-inflammatory effect (P < 0.01). The expression of NF-κB, TNF-α and ICAM-1 in colonic mucosa was significantly lower in the Diammonium Glycyrrhizinate group and dexamethasone group than in the acetic acid group.

CONCLUSION: Diammonium Glycyrrhizinate could reduce inflammatory injury in a rat model of ulcerative colitis. This may occur via suppression of NF-κB, TNF-α and ICAM-1 in colonic mucosa.

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Key words: Ulcerative colitis; Diammonium Glycyrrhizinate; Mechanism

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INTRODUCTION

Traditional drugs for ulcerative colitis, though quite effective, may exert severe side-effects. New therapeutic agents with less side-effects would be very useful. Diammonium Glycyrrhizinate (DG) is a substance that is extracted and purified from a traditional Chinese medicinal herb. The herb has been used traditionally for the treatment of hepatitis due to its anti-inflammatory effect, resistance to biologic oxidation, membranous protection and a weak steroidal action. However, it was not known whether DG might also be effective in other inflammatory conditions, such as ulcerative colitis. To explore a possible anti-inflammatory effect of DG in ulcerative colitis, the expression of NF-κB, TNF-α and ICAM-1 in colonic mucosa was detected by immunohistochemistry in a rat model.

MATERIALS AND METHODS

Materials

Female SD rats were purchased from the Center of Experimental Animals in the 89th Hospital of PLA. NF-κB, TNF-α, and ICAM-1 antibodies and immunohistochemistry kits were purchased from Beijing Zhongshan Biotech Company. MPO kit was purchased from the Institute of Nanjing Jiancheng Biotechnology. DG was purchased from Jiangsu Chia Tai Tianqing Pharmaceutical Co., LTD (X20010756, DG0267).

Methods

Preparation of animal model: Forty SD rats were divided into four groups: DG group, dexamethasone group, acetic acid control, and normal control group. All rats were fasted for twenty-four hours. Before the colonic infusion of acetic acid, 0.3 mL (30 mg/kg) Natrium pentobarbital was injected peritoneally. A polyethylene catheter was put into the colon extending a distance of eight centimeters beyond the anus. For DG, dexamethasone, and the acetic acid control groups, 1 mL of 10% (v/v) acetic acid was...
infused into the colon through this catheter\cite{1}, held in place for 30 s, and then flushed with 5 mL normal saline. Only normal saline was infused into the colon in the normal control group. In the DG group, 40 mg/kg DG was injected intraabdominally every day for one week; in dexamethasone group, 0.2 mg/kg dexamethasone was injected intraabdominally daily for one week; in the acetic acid control and normal control groups, equal volumes of normal saline were injected into the abdominal cavity daily for one week.

**Preparation of colonic tissue samples:** Seven days after acetic acid infusion, the body weight of each rat was measured and recorded. The distal colon was removed at the level of 8 cm from the anus. Samples were evaluated by morphological scores, and then cut into two parts—one for H&E staining and immunohistochemistry, the other for the detection of MPO activity.

**Evaluation of colonic inflammation:** Colonic inflammation was evaluated seven days after acetic acid infusion by disease activity index (DAI), morphologic injury, histological changes, and MPO activity. DAI was calculated as the sum of scores assigned as follows: percentage of body weight reduction (0: no change, 1: 1%-5%, 2: 6%-10%, 3: 11%-15%, 4: > 15%), stool consistency (0: normal, 2: loose, 4: diarrhea) and the presence of fecal blood (0: normal, 2: positive occult blood test, 4: visible bleeding)\cite{2}. Morphological injury was evaluated using the scoring system reported by Dieleman, with slight modifications\cite{3} (Table 1). Ten fields were selected randomly to estimate histological injury according to the scoring system listed in Table 2\cite{4}. A kit was used to detect MPO activity in the colonic mucosa.

**Immunohistochemical detection of NF-κB p65, TNF-α and ICAM-1:** The expression of NF-κB p65, TNF-α and ICAM-1 in colonic mucosa was detected by a SP immunohistochemistry kit. On each slide, five fields (× 400) were selected randomly and the percentage of positive cells was counted among 1000 cells. The average of positive cells in five fields was the determined.

**Statistical analysis**

Data are presented as mean ± SD, and statistical differences among groups were tested using the SPSS statistical software package. Based on the result of a test of homogeneity of variances, one-way ANOVA and SNK-\( q \) test were applied to test differences between different groups. A \( P \) value of 0.05 was set as the level of statistical significance.

### RESULTS

**DAI, morphologic injury, histological changes, and MPO activity**

1-2 d after colon infusion of acetic acid, rats displayed diarrhea, pyemic stool, and reduced body weight. Morphologically, a dilated lumen, thickened wall, and brown or black color was observed continuously in the injured bowel. Edema, erosions, necrosis, superficial ulcerations, crypt abscesses, and inflammatory infiltration into the lamina propria were observed in the injured segment by light microscopy. In Table 3, according to DAI, scores of morphological and histological changes, and MPO activity, the colon showed significant pathogenic changes in the DG, dexamethasone, and acetic acid control groups compared to the normal control group that received saline alone \((P < 0.01)\), which demonstrated that acetic acid infusion results in injuries that are comparable to those seen in humans with ulcerative colitis. These inflammatory indices were significantly improved by DG and dexamethasone \((P < 0.01)\). The anti-inflammatory effect of DG was significantly lower than that of dexamethasone \((P < 0.01)\).

**Expression of NF-κB p65, TNF-α and ICAM-1 in injured colon**

In rats that received acetic acid, NF-κB p65 was positive mainly in nuclei of most endothelial cells, epithelial cells and mononuclear cells, especially in the mucosa and submucosa. TNF-α and ICAM-1 were positive mainly in the cytoplasm, membrane and rarely in nuclei. ICAM-1 was positive in most endothelial cells and macrophages. TNF-α positive cells, including mononuclear cells, macrophages and neutrophils, were located densely in lamina propria and in proximity to the muscularis. The percentage of cells positive for these three molecules was significantly correle-
ted with the degree of inflammatory injury (Table 4), and these markers were rarely expressed in samples taken from the normal control group. The positive percentage and density of NF-κB p65, TNF-α and ICAM-1 in injured colon was significantly higher than that in normal control. After DG or dexamethasone treatment, the positive percentage and density of these molecules were reduced significantly, which indicates that both DG and dexamethasone may inhibit the expression of these molecules. Also, the expression of these molecules was significantly lower in DG treated samples than in dexamethasone treated samples (P < 0.01).

**DISCUSSION**

DALI, morphologic injury and histological changes are usually applied to evaluate the severity of inflammatory injuries. MPO reflects the activity of neutrophils, which is also a good indicator of the acute inflammatory reaction [8,9]. According to the indices mentioned above, a rat model of ulcerative colitis (acute phase) was induced by colonic infusion of acetic acid in these experiments. Compared with the acetic acid control group, these inflammatory indices were significantly improved by treatment with DG or dexamethasone.

To understand the molecular mechanism of the effect of DG in this experimental model, the expression of NF-κB, ICAM-1 and TNF-α was detected by immunohistochemistry. All of these molecules are known to be upregulated in the acute inflammatory cascade, which plays an important role in the pathogenesis of ulcerative colitis [9,10]. Activation of NF-κB is key to the expression of many proinflammatory cytokines and adhesive molecules including ICAM-1 and TNF-α [8-11]. ICAM-1 induces the migration and infiltration of inflammatory cells into the lesion [12,13], while TNF-α causes apoptosis of the colonic mucosa [14,15]. Both ICAM-1 and TNF-α represent different key points in the progress of inflammatory injuries [16,17]. TNF-α, ICAM-1 and NF-κB were upregulated in the acetic acid control group, but reduced significantly after DG or dexamethasone treatment, which means that inhibition of these molecules was likely important for the protective effect of DG and dexamethasone.

As reported previously, NF-κB is a key molecule in both the initiation and progression phase of the inflammatory reaction [18,19]. Activated NF-κB translocates into the nucleus and induces the expression of proinflammatory cytokines, adhesive molecules and chemokines. In this rat model, the expression of NF-κB was inhibited by both DG and dexamethasone, which means that the anti-inflammatory mechanism of DG may be similar to that of dexamethasone. Though the efficacy of DG was less than that of dexamethasone, its side-effects are expected to be less severe.

In summary, DG was efficacious in experimental ulcerative colitis induced in rats, and associated with insignificant side effects. This result suggests that DG may be a promising drug candidate for the treatment of ulcerative colitis.

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**Table 4** Expression of TNF-α, ICAM-1 and NF-κB p65 in injured colon

| Group            | TNF-α  | Percentage | Density | ICAM-1  | Percentage | Density | NF-κB p65  | Percentage | Density |
|------------------|--------|------------|---------|---------|------------|---------|------------|------------|---------|
| DG               | 32.2 ± 8.2 | 19.0 ± 5.2 | 17.9 ± 5.0 | 34.3 ± 7.0 | 18.7 ± 5.7 | 19.0 ± 5.2 | 23.3 ± 5.2 | 15.5 ± 4.3 | 17.6 ± 4.9 |
| Dexamethasone    |        |            |         |         |            |         |            |            |         |
| Acetic acid control | 52.5 ± 9.1 | 74.1 ± 9.5 | 17.9 ± 5.0 | 60.2 ± 8.3 | 70.7 ± 9.7 | 70.7 ± 9.7 | 44.5 ± 8.9 | 51.5 ± 9.8 |         |
| Normal control   | 7.6 ± 1.1  | 9.0 ± 4.8  | 32.2 ± 8.2 | 9.1 ± 5.2  | 9.4 ± 4.9  | 9.4 ± 4.9  | 7.6 ± 1.1  | 8.1 ± 4.2  |         |

*p < 0.01, vs Normal control group; **p < 0.01, vs Acetic acid control group; ***p < 0.01, vs Dexamethasone group.*
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