Glycyrrhizic acid prevents enteritis through reduction of NF-κB p65 and p38MAPK expression in rat

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Abstract. Glycyrrhizic acid has a variety of biological properties, including a protective function in the liver, and anti-inflammatory, anti-ulcer, anti-anaphylaxis, anti-oxidant, immunoregulatory, antiviral and anticancer activities. The efficacy of glycyrrhizic acid can be increased when combined with other medicines. In the present study, the potential protective effects of glycyrrhizic acid against enteritis in rats, and its role in regulating anti-inflammation, anti-oxidation, angiogenic and apoptotic mechanisms were investigated using enzyme-linked immunosorbent and bicinchoninic acid assays, and reverse transcription-quantitative polymerase chain reaction and western blotting analyses. Adult male Sprague-Dawley rats were injected with 20 mg/kg methotrexate (MTX) to establish enteritis. Additionally, rats with MTX-induced enteritis were peritoneally injected with 200 mg glycyrrhizic acid for 9 weeks. The current study demonstrated that glycyrrhizic acid could alleviate MTX-induced increases of tumor necrosis factor-α, interleukin (IL)-1β and IL-6 levels, and raise IL-10 levels, in rats with enteritis. Treatment with glycyrrhizic acid significantly reduced D-lactate and intercellular adhesion molecule-1 gene expression ($P<0.01$), but did not inhibit diamine oxidase activity in MTX-induced enteritis. Pretreatment with glycyrrhizic acid significantly suppressed the promotion of p38 mitogen-activated protein kinase (p38MAPK), nuclear factor-κB p65 (NF-κB p65) protein expression, interferon-γ protein concentration, and caspase-3 and cycloxygenase-2 activity in MTX-induced enteritis ($P<0.01$). The findings of the current study suggest that glycyrrhizic acid may prevent enteritis by reducing NF-κB p65 and p38MAPK expression levels, which may inform future therapeutic strategies for the treatment of enteritis.

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Introduction

The intestinal mucosa is the major location of nutrient digestion and absorption, as well as the primary gateway for invasion by pathogenic microorganisms and their toxins (1). Under physiological conditions, extra-intestinal tissues and organs are effectively protected from pathogenic microorganisms and their toxins by the intestinal mucosa barrier (IMB) (2). The aforementioned mechanism involves the IMB, intestinal mucosa immune barrier, micropopulation barrier, chemical barrier, slime layer and aqueous layer (3). As a result, when the IMB is damaged, microorganisms and their toxins can move through the IMB, and enter the portal vein and lymphatic system, leading to bacterial translocation, potentially causing systemic inflammatory response syndrome and multiple organ dysfunction syndrome (4).

Dysfunction of the IMB is closely associated with diseases of the digestive system. Numerous factors, including intestinal infection, inflammation and mechanical damage, can induce abnormal IMB function (5). IMB dysfunction increases intestinal mucosa permeability, causing bacteria and antigens to be translocated from the enteric cavity to the lamina propria mucosae, resulting in the activation of immune cells and an abnormal mucosal immune response (6). Inflammatory bowel diseases, such as enteritis, can also damage the IMB by increasing the release of inflammatory cytokines, further exacerbating the abnormal mucosal immune response (7). It is widely understood that IMB dysfunction causes the molecular pathogenesis of enteritis (7,8).

Glycyrrhiza, a group of perennial leguminous grasses, have been used in traditional medicine in Asian countries, including China, India and Japan, for thousands of years, and are now widely used in Europe and the Middle East (9). Glycyrrhizic acid is water-soluble compound, consisting of two molecules of glucuronic acid and one molecule of glycyrrhetinic acid (10). Glycyrrhizic acid, which exhibits a series of effects, such as liver protection and membrane stabilization, is one of the major constituents of *Glycyrrhiza* root extract. Its biological effects include anti-inflammatory, anti-ulcer, anti-anaphylaxis, anti-oxidant, immunoregulatory, membrane stabilization, antiviral and anticancer activities, as well as liver protective properties (9,10). The pentacyclic triterpene structure in glycyrrhetinic acid gives glycyrrhizic acid a similar structure to glucocorticoids and, as such, similar anti-inflammatory effects (11). The anti-inflammatory effect of glycyrrhizic

acid does not cause severe adverse reactions, therefore, it is widely used in the treatment of acute and chronic hepatitis, bronchitis, acquired immune deficiency syndrome and other diseases (12,13).

Nuclear factor (NF)-κB p65 is a common transcription factor which is activated by inflammatory cytokines, growth factor or chemokines in order to regulate transcription (14). Following stimulation by an inflammatory cytokine or various cytokines, a trimer of NF-κB and IκBa dissociates in the cytoplasm, and phosphorylation of IκBa facilitates nuclear localization to induce NF-κB expression, which is a classical NF-κB pathway (15). Following nuclear entry, NF-κB can regulate gene expression. Positive and negative feedback loops and subsequent inflammatory reactions further affect the inflammatory reaction of the body (16).

Mitogen activated protein kinase (MAPK) signal transduction pathways are associated with cell proliferation, differentiation, cell apoptosis and angiogenesis (17). In particular, the p38MAPK signal transduction pathway regulates stress responses, such as inflammation and cell apoptosis (18). Following the activation of MAPK pathways by lipopolysaccharides and other factors, numerous inflammatory mediators are generated via complex signal conduction pathways, which promote inflammation. p38MAPK can be activated by various stimulus along diversified transduction pathways which activate numerous transcription factors and mediate a range of biological effects (19).

The present study aimed to investigate the mechanism by which glycyrrhizic acid prevents enteritis in rats and whether its effects are mediated via reduction of NF-κB p65 and p38MAPK expression levels.

Materials and methods

Chemicals. Methotrexate (MTX) was purchased from Shanghai Zhongxi Sunve Pharmaceutical Co., Ltd. (Shanghai, China). Tumor necrosis factor-α (TNF-α; MTA00), interleukin-1β (IL-1β; DLB50), IL-6 (D6050), IL-10 (D1000B), cycloxygenase-2 (COX-2; KCB4198) and interferon-γ (IFN-γ; RIF00) enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems Europe, Ltd. (Abingdon, UK). Bicinchoninic acid (BCA) assay kit (P0010S) was purchased from Beyotime Institute of Biotechnology (Jiangsu, China).

MTX-induced enteritis rat model and grouping. A total of 24 adult male Sprague-Dawley rats, aged 6-7 weeks and weighing 220-250 g, were obtained from the Animal Resource Center of the Linyi People's Hospital (Linyi, China). Rats were housed at an ambient temperature of 22±1˚C, with a 12-h light-dark cycle, and free access to food and water. The current study was conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (20) and the protocol was approved by the Ethics Committee of Linyi People's Hospital. The experimental rats were randomly allocated into three equal groups: Control, MTX and glycyrrhizic acid groups (n=8 per group). In the control group, normal rats were peritoneally injected with 1 ml normal saline. In the MTX and glycyrrhizic acid groups, enteritis was induced in rats by injection with 20 mg/kg MTX. In the MTX group, MTX-induced rats were peritoneally injected with 1 ml normal saline. In the glycyrrhizic acid group, MTX-induced rats were peritoneally injected with 200 mg glycyrrhizic acid for 9 weeks (21). After 9 weeks, rats was sacrificed via decollation and blood samples and intestinal tissue samples were subsequently harvested. Blood samples were centrifuged at 13,200 x g for 10 min at 4˚C to obtain the serum. Serum and intestinal tissue samples were stored at -80˚C prior to analysis.

Collection of rat blood samples and measurement of inflammatory mediators by ELISA. Peripheral blood was collected after glycyrrhizic acid treatment and centrifuged at 13,200 x g for 10 min at 4˚C. The supernatant was collected, and the levels of TNF-α, IL-1β, IL-6, IL-10, IFN-γ and COX-2 were measured using the described ELISA kits, according to the manufacturer's protocol.

Measurement of plasma D-lactate concentration and diamine oxidase (DAO) activity. Peripheral blood supernatant was obtained as described for the ELISAs. The D-lactate concentration levels and DAO activity were measured using spectrophotometry (Multiskan MS plate reader; Thermo Fisher Scientific, Inc., Waltham, MA, USA) , as previously described (22,23).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) of intercellular adhesion molecule-1 (ICAM-1). Total RNA was extracted from rat intestinal tissue samples using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The extracted RNA (1 μg) was reverse transcribed into cDNA using a SuperScript III First-Strand Synthesis system (Invitrogen; Thermo Fisher Scientific, Inc.). qPCR was performed on a Gene Amp 2400 PCR system (PerkinElmer, Inc., Waltham, MA, USA) using a Premix *Ex Taq* kit (Takara Bio, Inc., Kyoto, Japan), according to the manufacturer's protocol as follows: 94˚C for 5 min followed by 40 cycles of 95˚C for 30 sec, 60˚C for 45 sec and 72˚C for 30 sec, and 72˚C for 10 min. Primer sequences were as follows: ICAM-1 forward 5'-AACGACGCTTCTTTTGCTC-3' and reverse 5'-CTCTGGCGGTAATAGGTGTAA-3'; and GAPDH (reference) forward 5'-CGTGTTCCTACCCCCAATGT-3' and reverse 5'-TGTCATACTTGGCAGGTTTCT-3'. Relative expression levels were normalized against GAPDH, using the $2^{-\Delta\Delta Cq}$ method (24).

Western blotting. Intestinal tissue samples were collected and incubated with 100 μl tissue lysis buffer (Beyotime Institute of Biotechnology) for 30 min on ice. Homogenates were centrifuged at 13,200 x g for 10 min at 4˚C, the supernatant was collected and the protein concentration measured using a the BCA kit, according to the manufacturer's protocol. Equal concentrations of protein sample were separated by running on 10% SDS-PAGE gels (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) at 100 V for 75 min and transferred onto polyvinylidene difluoride membranes (0.22 mm; Bio-Rad Laboratories, Inc., Munich, Germany). The membrane was incubated with anti-NF-κB-p65 (sc-372; 1,2,000), rabbit polyclonal anti-phosphorylated (p)-p38MAPK (sc-101758), anti-p38MAPK (sc-728), rabbit polyclonal anti-iNOS (sc-651; all 1:1,000) or β-actin (1:2,000; sc-130656; all Santa Cruz Biotechnology, Inc., Dallas, TX,

USA) primary antibodies overnight at 4˚C. Membranes were then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (sc-2370; 1:2,000; Santa Cruz Biotechnology, Inc.) and enhanced chemiluminescence kit (GE Healthcare Life Sciences, Chalfont, UK). The relative protein expression was determined using a Gel Dox 1000 fluorescent image analysis system (Bio-Rad Laboratories, Inc.).

Caspase-3 activity. Protein was extracted from intestinal tissue samples, as described for western blotting, and quantified using the BCA assay kit, according to the manufacturer's protocol. Equal concentrations of protein were incubated with Ac-DEVD-pNA (Beyotime Institute of Biotechnology) at 37˚C for 2 h in the dark, as previously described (25), and the absorbance at 405 nm was measured using a Varioskan Flash spectral scanning multimode reader.

Statistical analysis. Data from the individual groups were presented as the mean ± standard deviation and were analyzed using analysis of variance followed by Tukey-Kramer multiple comparisons test. Statistical analyses were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL< USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Glycyrrhizic acid alters the expression of inflammatory factors in rat enteritis. The chemical structure of glycyrrhizic acid (95.0%; Sigma-Aldrich, St. Louis, MO, USA) is presented in Fig. 1. The current study examined the effect of glycyrrhizic acid on the levels of several inflammatory factors in a rat model of enteritis. The protein concentration levels of TNF-α, IL-1β and IL-6 were significantly increased in rats with MTX-induced enteritis compared with the control group (P<0.01; Fig. 2A-C). Pretreatment with glycyrrhizic acid significantly alleviated the increase of TNF-α, IL-1β and IL-6 resulting from MTX-induced enteritis (P<0.01; Fig. 2A-C). Conversely, IL-10 levels were significantly reduced in rats with MTX-induced enteritis compared with the control group (P<0.01; Fig. 2D), and glycyrrhizic acid treatment significantly raised the IL-10 levels compared with the MTX group (P<0.01; Fig. 2D).

D-lactate concentration levels and DAO activity are increased by glycyrrhizic acid in rat enteritis. The current study probed whether glycyrrhizic acid prevents D-lactate and DAO changes that occur in rat enteritis. The D-lactate concentration and DAO activity were significantly increased in MTX-induced enteritis compared with control group (P<0.01; Fig. 3). Furthermore, treatment with glycyrrhizic acid significantly reduced the D-lactate concentration levels compared with the MTX group (P<0.01), however, glycyrrhizic acid did not inhibit the increased DAO activity in MTX-induced enteritis (Fig. 3B).

ICAM-1 expression is reduced by glycyrrhizic acid in rat enteritis. As presented in Fig. 4, ICAM-1 mRNA expression was significantly higher in MTX-induced enteritis compared with expression in control rats (P<0.01). However, administration of glycyrrhizic acid significantly reduced the ICAM-1 gene expression compared with MTX-induced enteritis rats (P<0.01; Fig. 4).

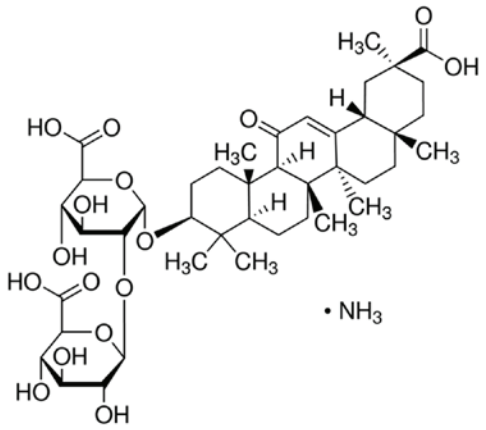

Figure 1. Chemical structure of glycyrrhizic acid.

Increase of NF-κB-p65 protein expression in enteritis is prevented by glycyrrhizic acid in rat enteritis. As shown in Fig. 5, MTX-induced enteritis resulted in significantly increased NF-κB-p65 expression compared with the control group (P<0.01). As expected, glycyrrhizic acid significantly suppressed the MTX-induced increase in NF-κB-p65 expression (P<0.01; Fig. 5).

Glycyrrhizic acid prevents increase in IFN-γ concentration levels in rat enteritis. There was a significant increase IFN-γ levels in rats with MTX-induced enteritis compared with the control rats (P<0.01; Fig. 6). As presented in Fig. 6, glycyrrhizic acid treatment prevented the promotion of IFN-γ levels observed in the MTX group (P<0.01).

Glycyrrhizic acid reduces caspase-3 activity in rat enteritis. To understand the underlying mechanism of glycyrrhizic-mediated anti-apoptosis, the present study examined whether glycyrrhizic acid could inhibit caspase-3 activity in an MTX-induced enteritis model. As presented in Fig. 7, rats in the MTX group exhibited significantly increased caspase-3 activity compared with control group (P<0.01). However, the elevation of caspase-3 activity was significantly inhibited by treatment with glycyrrhizic acid compared with the MTX group (P<0.01; Fig. 7).

Glycyrrhizic acid prevents iNOS protein expression in rat enteritis. To examine the underlying mechanisms of glycyrrhizic-mediated changes in nitric oxide (NO) levels, the current study examined whether glycyrrhizic acid inhibits iNOS protein expression in the MTX-induced enteritis rat model. As presented in Fig. 8, the iNOS protein expression was significantly elevated in MTX-induced enteritis compared with the control group (P<0.01). However, treatment with glycyrrhizic acid reduced the increase of iNOS protein expression, as compared with the MTX-induced enteritis rats (Fig. 8).

Glycyrrhizic acid inhibits COX-2 activity in rat enteritis. To investigate the mechanism by which the anti-oxidant effects of glycyrrhizic acid are mediated, the present study examined the influence of glycyrrhizic acid on COX-2 activity. Compared

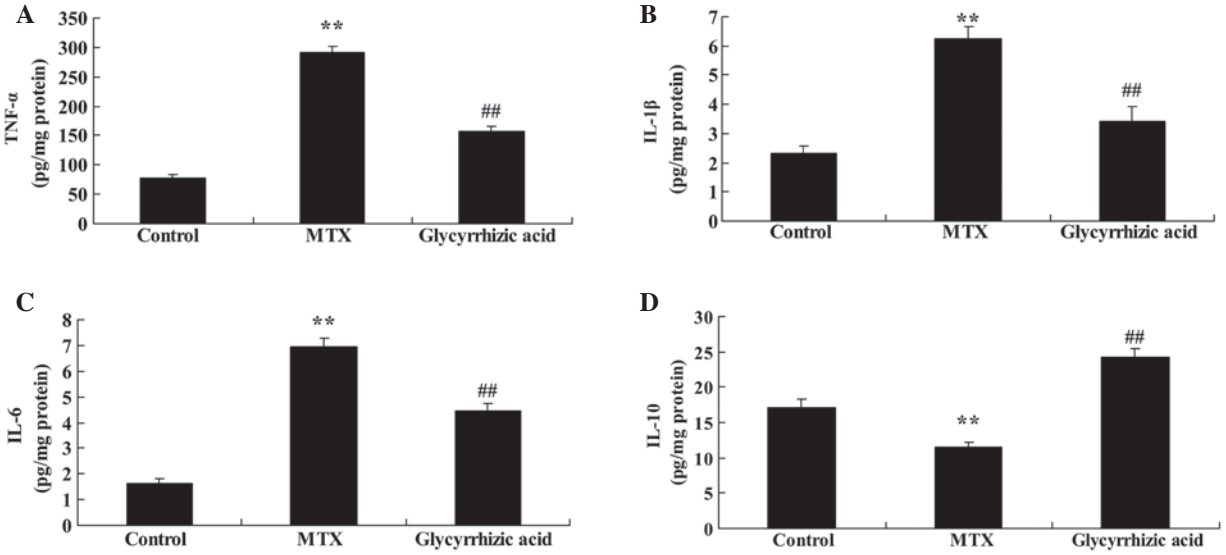

Figure 2. Effect of glycyrrhizic acid on expression of inflammatory factors in enteritis. (A) TNF-α, (B) IL-1β, (C) IL-6 and (D) IL-10 levels were measured using enzyme-linked immunosorbent assay in control, MTX-induced model and glycyrrhizic acid groups. **P<0.01 vs. control group; $^{\#\#}$P<0.01 vs. the MTX group. TNF-α, tumor necrosis factor-α; IL, interleukin; MTX, methotrexate.

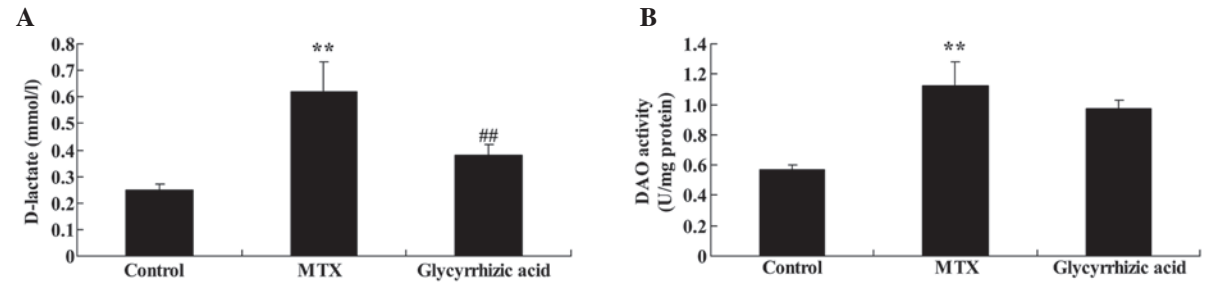

Figure 3. Effect of glycyrrhizic acid on D-lactate and DAO in rat enteritis. (A) D-lactate and (B) DAO were measured in control, MTX-induced model and glycyrrhizic acid groups. **P<0.01 vs. control group; $^{\#\#}$P<0.01 vs. the MTX group. MTX, methotrexate; DOA, diamine oxidase.

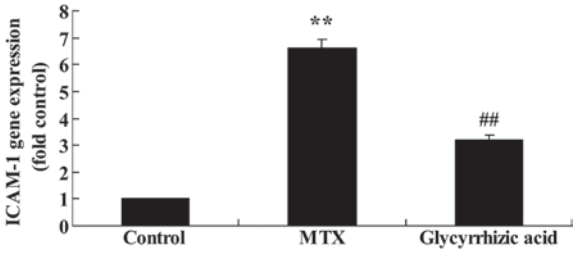

Figure 4. Glycyrrhizic acid prevents ICAM-1 expression in MTX-induced enteritis. ICAM-1 expression was measured in control, MTX-induced model and glycyrrhizic acid groups using reverse transcription-quantiative polymerase chain reaction analysis. **P<0.01 vs. the control group; $^{\#\#}$P<0.01 vs. the MTX group. ICAM-1, intercellular adhesion molecule 1; MTX, methotrexate.

with the control group, the COX-2 activity was significantly increased in rats with MTX-induced enteritis (P<0.01; Fig. 9). This increase was significantly attenuated by glycyrrhizic acid treatment compared with the MTX group (P<0.01; Fig. 9).

Glycyrrhizic acid prevents p38MAPK expression in rat enteritis. To examine the effects of glycyrrhizic acid on the MAPK signaling pathway, the protein expression levels of total and p-p38MAPK, a key mediator of MAPK signaling, were measured using western blotting. The present study observed that the p38MAPK protein expression was significantly increased in MTX-induced enteritis compared with the control group (P<0.01; Fig. 10). However, treatment with glycyrrhizic acid significantly attenuated the increase in p38MAPK protein expression observed in the MTX group (P<0.01; Fig. 10).

Discussion

IMB dysfunction is closely associated with the paroxysm of enteritis. IMB dysfunction, induced by psychological stress, intestinal infection, mechanical injury and other factors, can increase intestinal mucosa permeability (1,2). Increased mucosa permeability contributes to the translocation of bacteria and antigens from the enteric cavity to the lamina propria mucosae, resulting in activation of immune cells and an abnormal mucosa immune response (26). Additionally, damaging factors are present during the enteritis paroxysm, which can further injure the IMB and aggravate the abnormal mucosa immune response (26). Drugs for the treatment of enteritis can reduce intestinal mucosal inflammation and regulate IMB function (27). IMB function is one of the therapeutic targets in the treatment of enteritis and is an important area of investigation within enteritis pathophysiology research (26,27).

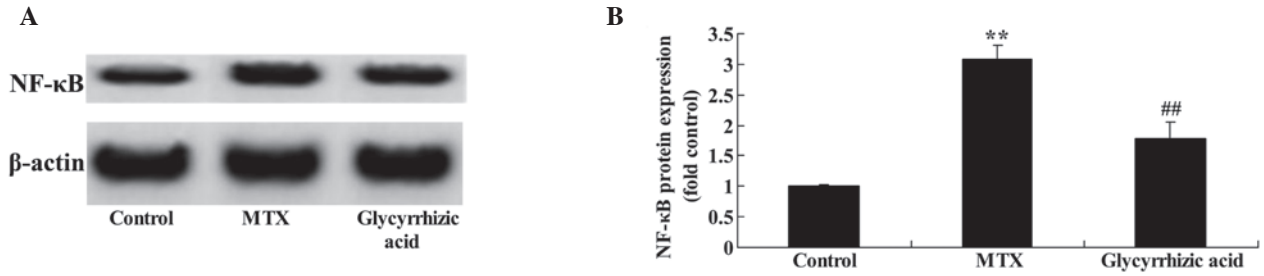

Figure 5. Glycyrrhizic acid prevents NF-κB-p65 expression in rat enteritis. (A) NF-κB-p65 expression was measured by western blotting assays and (B) statistical analysis of NF-κB-p65 protein expression was performed using protein from control, MTX-induced model and glycyrrhizic acid groups. **P<0.01 vs. control group; ##P<0.01 vs. the MTX group. NF-κB, nuclear factor-κB; MTX, methotrexate.

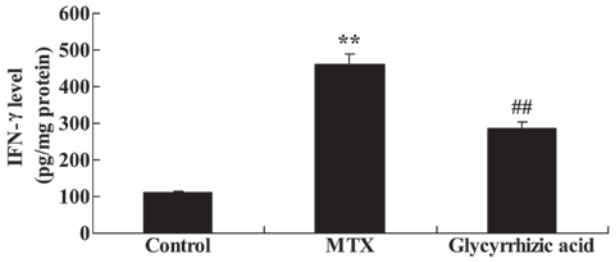

Figure 6. Glycyrrhizic acid reduces IFN-γ levels in enteritis rat. IFN-γ was measured in control, MTX-induced model and glycyrrhizic acid groups. **P<0.01 vs. control group; ##P<0.01 vs. the MTX group. IFN-γ, interferon-γ; MTX, methotrexate.

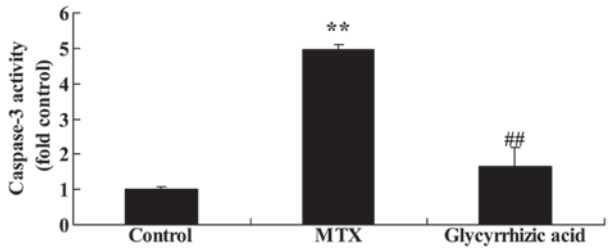

Figure 7. Glycyrrhizic acid prevents caspase-3 activity in enteritis rat. Caspase-3 activity was measured in tissue sample from control, MTX-induced model and glycyrrhizic acid groups. **P<0.01 vs. control group; ##P<0.01 vs. the MTX group. MTX, methotrexate.

The current study observed that pretreatment with glycyrrhizic acid significantly suppressed the concentration levels of various inflammatory mediators that had been increased in MTX-induced enteritis, including TNF-α, IL-1β, IL-6 and D-lactate levels, whereas, IL-10 levels were reduced in enteritis and increased by glycyrrhizic acid. Bhattacharjee *et al* (28) demonstrated that glycyrrhizic acid suppresses inflammatory responses and protects against *Leishmania* parasites within the host. Orazizadeh *et al* (29) observed that glycyrrhizic acid protects against nanoparticle-induced hepatotoxicity through anti-inflammatory mechanisms, and suggested that glycyrrhizic acid may be a novel candidate for research and development of enteritis therapeutics.

ICAM-1 is expressed on the membrane of endothelial cells, white blood cells (WBC) and enterocytes of intestinal tissues, acting as a receptor for β2-integrin present on WBCs. Therefore, ICAM-1 is important in leukocyte movement and aggregation during enteritis (30). Enteritis also induces the expression of adhesion molecules, including ICAM-1 (31). During the acute phase of enteritis, expression of ICAM-1 on the surface of activated vascular endothelial cells is markedly increased, while the mortality of ICAM-1-deficient mice is markedly reduced (30). Lower mortality, and reduced inflammatory cell infiltration and injury of the intestinal tract in ICAM-1-deficient mice indicates that ICAM-1 is important in the development of enteritis (31). The present data demonstrated that administration of glycyrrhizic acid significantly reduces ICAM-1 mRNA expression in MTX-induced enteritis rat. In addition, Wang *et al* (21) suggested that glycyrrhizic acid attenuates glycative stress in the kidneys of diabetic mice through inhibition of monocyte chemotactic protein-1 and ICAM-1. Together, these results suggest that the anti-inflammatory activity of glycyrrhizic acid during enteritis may be partially mediated through inhibition of ICAM-1.

IFNs are a group of broad-spectrum anti-viral glycoproteins secreted by cells upon attack by viruses. IFN-γ, which is secreted by lymphocytes, is involved in the expression of histocompatibility antigen and immune adjustment (32). It is understood that the interaction between IFN-γ and TNF-α can change the structure of intestinal epithelial cells and the barrier function, leading to increased intestine permeability. Increased expression of IFN-γ is commonly observed in enteritis (4,32). In agreement with this, the present study demonstrated that glycyrrhizic acid could reduced MTX-induced NF-κB-p65 and IFN-γ protein expression in rat enteritis. Feng *et al* (33) demonstrated that glycyrrhizic acid protects against advanced glycation end-product (AGE)-induced endothelial dysfunction via inhibition of the receptor for AGE/NF-κB signaling pathway. Wu *et al* (34) reported that glycyrrhizic acid significantly reduced inflammatory via IFN-γ. This indicates that inhibition of the IFN-γ signaling pathway may be associated with the anti-inflammatory effects of glycyrrhizic acid in enteritis.

Enteritis affects intestinal mucosa microcirculation, inflammatory cell infiltration, blood hypercoagulability, microthrombus and inflammatory polyp formation, leading to hypoxia and ischemia of the intestinal mucosa microcirculation, and a severe imbalance of cell factors, including the IL and TNF-α superfamilies, colony stimulating factor, chemokines and growth factors (35). iNOS levels are increased during enteritis and are positively associated with the disease activity index, which reflects the degree of inflammation. iNOS catalyzes the production of NO, which is associated with the pathophysiology

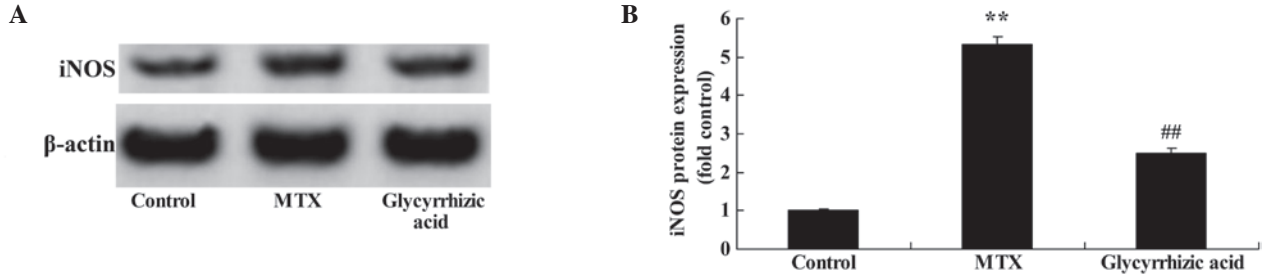

Figure 8. Glycyrrhizic acid prevents iNOS expression in rat enteritis. (A) Western blotting was performed to measure iNOS protein expression and (B) statistically analyzed in samples from control, MTX-induced model and glycyrrhizic acid groups. **P<0.01 vs. control group; ##P<0.01 vs. the MTX group. iNOS, nitric oxide synthase; MTX, methotrexate.

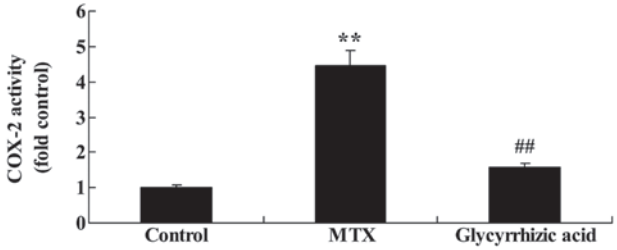

Figure 9. Glycyrrhizic acid prevents COX-2 activity in rat enteritis. COX-2 activity was measured in samples from control, MTX-induced model and glycyrrhizic acid groups. **P<0.01 vs. control group; ##P<0.01 vs. the MTX group. COX-2, cyclooxygenase-2; MTX, methotrexate.

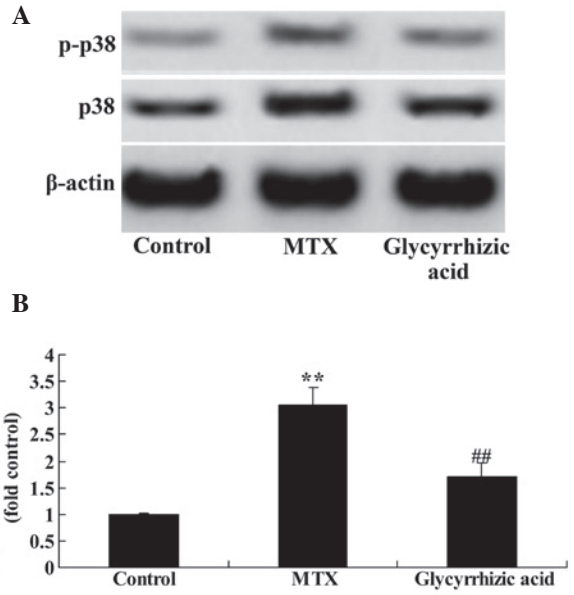

Figure 10. Expression and phosphorylation of p38 in enteritis rat. (A) p- and total p38 expression were measured in protein samples from control, MTX-induced model and glycyrrhizic acid groups by western blotting and (B) quantified using statistical analysis. **P<0.01 vs. control group; ##P<0.01 vs. the MTX group. p-p38, phosphorylated-p38 mitogen-activated protein kinase; MTX, methotrexate.

of inflammatory diseases and cancer (5,32). The results of the current study demonstrated that glycyrrhizic acid significantly inhibits MTX-induced caspase-3 activity and iNOS expression in enteritis. This suggests that glycyrrhizic acid has anti-apoptotic effects and suppresses iNOS expression during enteritis.

COX-2 is an inducible enzyme that is expressed at low levels in the majority of tissues under normal conditions (36). When induced by pro-infammatory cytokines, several cell types, including endothelial cells, vascular smooth muscle cells, mononuclear macrophages and fibroblasts, rapidly increase the expression of COX-2 to 8 to 10-fold the normal level (37). Increased COX-2 leads to the production and accumulation of prostaglandin inflammatory factors, promoting inflammatory responses and tissue damage (36). Overexpression of COX-2 promotes cell proliferation, inhibits apoptosis and inhibits the immune response, leading to dysregulation of the balance between proliferation and apoptosis (36,37). The present study demonstrated that glycyrrhizic acid significantly reduces MTX-induced COX-2 activity in enteritis. Bhattacharjee *et al* (28) also demonstrated that glycyrrhizic acid suppressed COX-2 during *L. donovani* infection. Additionally, Cherng *et al* (38) reported that glycyrrhizic acid inhibited NF-κB and COX-2 expression, prevented DNA damage and facilitated DNA repair.

As an important member of the MAPK signaling pathway, p38MAPK is widely expressed in a variety of tissues (39). Under normal physiological conditions, p38MAPK typically exhibits low activity and is activated upon stimulation by growth factors, lipopolysaccharides and stress (40). Previous research has demonstrated that when cells are stimulated by the aforementioned factors, p38MAPK regulates the inflammatory response via the production of pro-infammatory cytokines, including TNF-α, IL-1, IL-6 and IL-8, as well as anti-inflammatory cytokines, such as IL-10 (40,41). As a result, p38MAPK influences the balance between pro- and anti-inflammatory cytokines, thereby influencing processes that cause enteritis (42). The current study demonstrated that glycyrrhizic acid significantly downregulates the protein expression levels of p38MAPK in MTX-induced enteritis. Additionally, several other previous studies demonstrated that glycyrrhizic acid attenuates glycative stress in the kidneys of diabetic mice through suppression of NF-κB and p-p38MAPK (21,43). These data support the hypothesis that the anti-inflammatory effects of glycyrrhizic acid may be associated with p38MAPK signaling.

In conclusion, the results of the current study demonstrate that glycyrrhizic acid exerts a protective effect during MTX-induced enteritis and may be useful as a therapeutic agent for digestive tract diseases. Furthermore, the protective effect of glycyrrhizic acid is associated with anti-inflammatory and anti-apoptotic pathways, suppression of ICAM-1,

IFN-γ, iNOS and COX-2 expression, and downregulation of the p38MAPK pathway. Therefore, glycyrrhizic acid may be a novel therapeutic approach for the treatment of enteritis.

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