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Short Communication

Protective effects of glycyrrhizic acid by rectal treatment on a TNBS-induced rat colitis model

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Abstract

Objectives The research compared rectal and oral treatments with glycyrrhizic acid for trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats.

Methods Wistar rats were randomly divided into seven groups: one normal and six with colitis, including TNBS, glycyrrhizic acid (2, 10 and 50 mg/kg, rectally treated and 10 mg/kg, orally treated) and sulfasalazine (positive control, 225 mg/kg rectally treated) groups. Colitis was induced by colonic administration of TNBS in 30% ethanol.

Key findings There were significant pathological changes in colon in TNBS-treated groups, and rectal glycyrrhizic acid significantly attenuated colitis. Myeloperoxidase, tumour necrosis factor-α and interleukin-1β of colon tissue or serum in the rectal glycyrrhizic acid groups were markedly reduced when compared with the TNBS group, and lower than in the orally treated glycyrrhizic acid group. It was further noted that, *in vitro*, glycyrrhizic acid (up to 100 μg/ml) inhibited interleukin-6 and elevated interleukin-10 production in lipopolysaccharide-activated macrophages, and significantly inhibited proliferation of spleen lymphocytes, suggesting the immunoregulatory function of glycyrrhizic acid.

Conclusions Rectally administered glycyrrhizic acid has significant protective effects against TNBS-induced colitis in rats, and the rectal route may be a complementary treatment for inflammatory bowel disease.

Keywords colitis; glycyrrhizic acid; immunoregulation; rectal administration

Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing and nonspecific intestinal inflammatory disorder, and among the most challenging human diseases.[1] More than 1.4 million people in the USA and 2.2 million in Europe currently suffer from IBD, and several studies have shown increasing incidence and prevalence of IBD in the Asia-Pacific region.[2,3] Although the aetiology of IBD remains elusive, significant research has been carried out and successfully identified some of the pathological and clinical profiles unclear in IBD patients. There is evidence for intense local immune response associated with transmural infiltration of macrophages, lymphocytes, neutrophils and mast cells, followed by sustained massive production of cytokines, including tumour necrosis factor (TNF-α), interleukins (such as IL-1β, IL-6) and other inflammatory mediators.[4–8]

Glucocorticosteroids, sulfasalazine, 5-aminosalicylic acid and immunosuppressive drugs, such as 6-mercaptopurine and ciclosporin, along with various types of therapeutic treatments are clinically used for treatment of IBD. However, additional therapies are still required, as many patients either have no response to the currently available options or suffer from significant complications or toxic side effects.[9,10]

Glycyrrhizic acid, a component of *Glycyrrhiza glabra*, is the major active constituent of the traditional Chinese medicinal herb *Glycyrrhiza glabra*. Glycyrrhizic acid is known to have anti-inflammatory, anti-ulcer, anti-hepatotoxic, anti-cancer and anti-virus properties and has been used to heal patients with hepatitis C and upper respiratory tract infections.[11–13] Yuan *et al*.[14] found that diammonium glycyrrhizinate protected against rat colitis induced by acetic acid and inhibited over-production of NF-κB, ICAM-1 and pro-inflammatory cytokines TNF-α. Diammonium glycyrrhizinate is the disodium salt formulation of glycyrrhizic acid, to which it converts inside the digestive tract. Acetic acid-induced colitis is a chemical damage model, while murine TNBS colitis model, induced by hapten, is a well-

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characterized model with which to study the early or initiating events in the development of mucosal inflammation, resembling many of the clinical, histopathological and immune characteristics of IBD in humans.[15,16] In TNBS-induced colitis, intestinal inflammation develops as a result of the covalent binding of the haptenizing agent to autologous host proteins, with subsequent stimulation of a delayed-type hypersensitivity to TNBS-modified self-antigens.[16–18]

In this study, we investigated the protective effects of glycyrrhizic acid, rectally administered, against colitis induced by TNBS in rats, and compared these effects with oral treatment. In addition, we tested the effects of glycyrrhizic acid on the pro-inflammatory cytokine production of mouse peritoneal macrophages (PMΦ) and spleen lymphocytes proliferation *in vitro*.

Materials and Methods

Reagents

Glycyrrhizic acid was obtained from Xi'an Fujie Pharmaceutical Co., Ltd (Xi'an, China). Sulfasalazine was purchased from Shanghai Dafu Pharmaceutical Co., Ltd (Shanghai, China). 2,4,6-Trinitrobenzene sulfonic acid (TNBS, 5% solution), concanavalin A (ConA) and lipopolysaccharide (LPS) (*Escherichia coli*, serotype O55 : B5) were purchased from Sigma (St Louis, USA). RPMI-1640 and fetal calf serum were supplied by Hyclone (UT, USA). Lymphocyte separation media (for mice) was purchased from Tianjin Haoyang Biological Manufacture Co., Ltd (Tianjin, China).

Mouse IL-6 and IL-10, rat TNF-α and IL-1β ELISA kits were purchased from R&D Systems (Minneapolis, USA). Blood urea nitrogen (BUN) and creatinine (Cr) kits were obtained from Nanjing Jiancheng Biological Engineering Materials Co., Ltd. All other chemicals used were of analytical grade.

Experimental animals

Female Wistar rats, 230–250 g, and BALB/c mice, 18–22 g, of SPF grade were obtained from Laboratory Animal Center of Wuhan University (Wuhan, China). All animals were kept in conventional cages at constant temperature (23 ± 2°C) and humidity (50–70%) with a 12-h light–dark cycle. After they acclimatized for one week, 42 rats were randomly assigned to seven groups: Normal, TNBS, TNBS+GL2, TNBS+GL10, TNBS+GL50 (treated rectally with glycyrrhizic acid 2, 10 or 50 mg/kg), TNBS+GLO (treated orally with 10 mg/kg glycyrrhizic acid) and TNBS+SASP (treated rectally with 225 mg/kg sulfasalazine). The experimental protocols were approved by the local institution's Animal Care and Use Committee of Laboratory Animal Center of Wuhan University.

Induction of colitis in rats and treatments

Colitis was induced according to Chevalier *et al*.[19] with modifications. Firstly, rats were slightly anaesthetized with ether, and then 100 mg/kg TNBS dissolved in 30% ethanol (v/v) was instilled into the colon 1 ml/kg. Then the animals were maintained in a head-down position for 1 min to prevent leakage. Rats in Normal group received physiological saline instead of TNBS solution in a comparable volume rectally.

Glycyrrhizic acid (2, 10, 50 mg/kg, rectal administration) and sulfasalazine (225 mg/kg, rectal administration), as a positive control medicine, were dissolved in 0.9% saline solution and administered daily at a dose of 1 ml/kg, and GLO (glycyrrhizic acid 10 mg/kg, oral administration) group was set as an administration route control group. Rats in Normal group received physiological saline in a comparable volume rectally once a day. The individual dose of glycyrrhizic acid and sulfasalazine was adjusted based on body weight change during the experimental process. Rats in all groups were checked daily for behaviour and body weight.

Blood and tissue collection

Animals were sacrificed on the eighth day after induction with TNBS. Blood and colon were obtained for further analysis.

Blood was drawn by puncture from the inferior vena cava. Serum was separated by centrifugation at 3000 rev/min for 10 min at 4°C, and then stored at −80°C. Serum content of TNF-α and IL-1β were assessed by ELISA with kits; BUN and Cr levels were determined with commercial kits; serum K^+ and Na^+ concentrations were analysed using HITACHI-7060 automatic biochemistry analyzer (Hitachi, Japan). After colon tissues were rinsed with ice-cold saline solution, weight and length were recorded. The distal 2 cm colon of each rat was cut out for histopathological examination, and the rest were homogenized in 1 : 5 volumes of phosphate-buffered saline (PBS). Then the homogenate was centrifuged at 3000 rev/min for 10 min. The supernatant was used for myeloperoxidase (MPO), superoxide dismutase (SOD) and malondialdehyde (MDA) tests.[20–24] Results were presented as U/mg protein (or nmol/mg protein).

H&E staining and electron microscopy

Colon samples were fixed in 4% buffered paraformaldehyde (PBS, pH 7.4) for HE staining and in 2.5% glutaraldehyde for electron microscopy, then performed according to the standard protocols. Tissue sections were examined under Caikon XSP-11CD microscope (Caikon Optical Instrument Co., Ltd, China), H-600 transmission electron microscope (Hitachi Co. Ltd, Japan) or S-570 scanning electron microscope (Hitachi Co. Ltd, Japan), respectively.

Isolation, treatment of peritoneal macrophages and assessment of IL-6 and IL-10

Macrophages were isolated from BALB/c mice according to Ha *et al*.[25] Macrophages were cultured on 24-well plastic plates at 2×10^6/well for 4 h at 37°C in a humidified 5% CO_2 incubator, and were divided into Control (PMΦ) and LPS (LPS+PMΦ), and glycyrrhizic acid groups GL1 (LPS+PMΦ+GL 1 μg/ml), GL10 (LPS+PMΦ+GL 10 μg/ml) and GL100 (LPS+PMΦ+GL 100 μg/ml). Non-adherent cells were removed by rinsing the plates three times with RPMI-1640 medium, then treated with 1 ml RPMI-1640 with 1 μg/ml LPS, 10% fetal calf serum and glycyrrhizic acid 1, 10 and 100 μg/ml. Supernatants were collected after 24 h of LPS treatment for IL-6 and IL-10 determination, using commercially available kits by ELISA.

Separation of spleen lymphocyte cells and proliferation tests

Spleens were aseptically removed from BALB/c mice and single cell suspension was prepared by pressing the organs through a plastic screen. Primary lymphocytes were separated with lymphocyte separation media by centrifugation at 2000 rev/min for 10 min at 4°C. The cells were washed twice with Hanks' medium, and resuspended in the culture medium at a density of 2×10^6/ml, then placed in 96-well culture plates (100 μl/well). The cells were divided into Control (LYM), ConA (ConA+LYM), glycyrrhizic acid (GL) wells with concentrations from 1 ng/ml–1 mg/ml (ConA+LYM+GL), and were then treated with 100 μl RPMI-1640 with 8 μg/ml ConA, 10% fetal calf serum and glycyrrhizic acid at different concentrations. After a 2-day incubation, the degree of cell proliferation was determined via the MTT colorimetric method.[26,27]

Statistical analysis

Results were expressed as means ± SEM. The statistical significance of any difference in each parameter among the groups was evaluated by one-way analysis of variance followed by least significant difference (LSD) or Dunnett' test. *P*-values of <0.05 were considered statistically significant.

Results

Effects of rectal glycyrrhizic acid on thymus, spleen index and colon weight of unit length in colitic rats induced with TNBS

Intracolonic administration of TNBS and ethanol produced severe inflammation in rat colon, with haemorrhagic spots and extensive disruption (Figure 1). Rectal glycyrrhizic acid treatment reduced colonic lesion and colon weight of unit length when compared with the TNBS group. These parameters of the rectal glycyrrhizic acid group were lower than the sulfasalazine and oral glycyrrhizic acid groups. Rectal glycyrrhizic acid treatment also protected the thymus and spleen after TNBS administration (Table 1).

Histological results

Through HE staining, SEM and TEM (HE and TEM photomicrographs not shown) of colon, the TNBS treated group showed advanced lesions as necrosis, oedema and infiltration by polymorphonuclear leucocytes and lymphocytes. There were desquamated areas and loss of the epithelium with mucin depletion. Architecture of crypts was distorted and the lamina propria was thickened in peripheral areas of distorted crypts, especially in basal areas (Figure 2b). Treatment with glycyrrhizic acid or sulfasalazine significantly attenuated the extent and severity of gross lesion and histological signs of cell damage. Exfoliation of epithelial cells, dilated crypts and inflammatory cells were not found in the glycyrrhizic acid treated groups, and these were observed in some areas of sulfasalazine group.

Effect of rectal glycyrrhizic acid on serum TNF-α and IL-1β levels in colitic rats induced with TNBS

As illustrated in Figure 3, serum TNF-α and IL-1β levels were significantly elevated following TNBS instillation relative to those of Normal group. Glycyrrhizic acid and sulfasalazine rectal treatments reduced the serum TNF-α and IL-1β levels, but they remained higher than normal values.

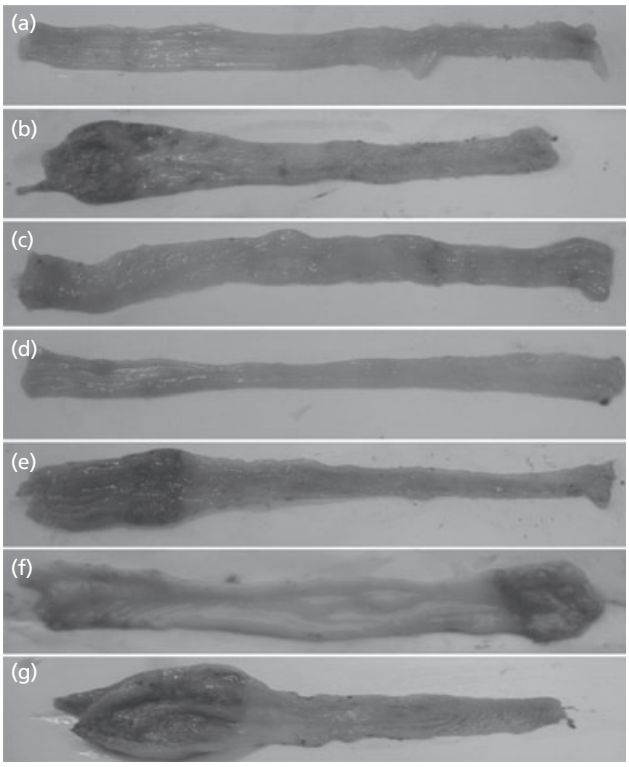

Figure 1 Effect of glycyrrhizic acid on colon in colitic rats induced with TNBS. (a) Normal rat colon; (b–d) colitic rats treated with glycyrrhizic acid (2, 10 and 50 mg/kg, respectively) rectally; (e) colitic rats treated with glycyrrhizic acid (10 mg/kg) orally; (f) colitic rats treated with sulfasalazine (225 mg/kg) rectally; (g) colitic rats induced with TNBS treated with physiological saline.

Effect of rectal glycyrrhizic acid on serum K^+, Na^+ and BUN levels and Cr levels in colitic rats induced with TNBS

BUN and Cr levels – markers of kidney damage – showed no significant increase in rats treated with rectal glycyrrhizic acid, and K^+ and Na^+ levels remained at normal values (figures were not shown).

Effect of rectal glycyrrhizic acid on colon myeloperoxidase and superoxide dismutase activity and malondialdehyde levels in colitic rats induced with TNBS

As assessed by elevated MPO activity in the colonic tissues, TNBS caused a significant increase in neutrophil infiltration when compared with Normal group, while rectal glycyrrhizic acid showed better inhibiting effects on MPO than oral glycyrrhizic acid (as depicted in Table 2). Colon SOD activity in TNBS group was significantly lower than the corresponding value in Normal group, and induction of colitis produced a significant increase in colonic MDA content. Rectal glycyrrhizic acid treatment protected against SOD depletion and

Table 1 Effect of GL on thymus, spleen index and colon weight of unit length in colitic rats induced with TNBS

Group	Dose (mg/kg)	Thymus index (mg/g)	Spleen index (mg/g)	Colon weight of unit length (g/cm)
Normal	–	0.92 ± 0.07	2.26 ± 0.10	0.10 ± 0.01
TNBS	–	0.31 ± 0.11$^{\#\#}$	3.73 ± 0.44$^{\#\#}$	0.40 ± 0.11$^{\#\#}$
TNBS+GL2	2	0.45 ± 0.28	3.70 ± 0.52	0.32 ± 0.16
TNBS+GL10	10	0.48 ± 0.26	2.82 ± 0.37**	0.21 ± 0.06**
TNBS+GL50	50	0.51 ± 0.23	2.68 ± 0.57**	0.15 ± 0.05**$^{\Delta\Delta}$
TNBS+GLO	10	0.33 ± 0.17	3.76 ± 1.03	0.24 ± 0.07*
TNBS+SASP	225	0.26 ± 0.11	3.42 ± 0.38	0.35 ± 0.09

Normal, non-immunized normal rats; TNBS, colitic rats induced with TNBS and not treated with test agents; TNBS+GL2, colitic rats treated with glycyrrhizic acid (2 mg/kg) rectally; TNBS+GL10, colitic rats treated with glycyrrhizic acid (10 mg/kg) rectally; TNBS+GL50, colitic rats treated with glycyrrhizic acid (50 mg/kg) rectally; TNBS+GLO, colitic rats treated with glycyrrhizic acid (10 mg/kg) orally; TNBS+SASP, colitic rats treated with sulfasalazine (225 mg/kg) rectally. Results are expressed as means ± SEM, $n = 6$/group. $^{\#\#}P < 0.01$ vs Normal group; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs TNBS group; $^{\Delta\Delta}P < 0.01$ vs GLO group.

MDA over-production in TNBS-induced colitis, though there was no significant difference between rectal groups and the oral group.

Effect of glycyrrhizic acid on IL-6 and IL-10 production of mice PMΦ and spleen lymphocyte cell proliferation

Table 3 demonstrates a marked increased IL-6 level and a slight decreased IL-10 level in mice PMΦ stimulated with LPS, and glycyrrhizic acid treatment produced a significant reduction in IL-6 concentration and up-regulated IL-10 levels, though there was no significant difference of IL-10 levels between Control and LPS groups. Figure 4 shows that there was significant lymphocyte proliferation in the presence of ConA, which was inhibited in the presence of glycyrrhizic acid at various concentrations (1–100 μg/ml).

Discussion

Induction of colitis by TNBS in rats, a hapten-induced colitis model, together with a barrier breaker, ethanol, results in extensive mucosal injury and inflammation of colon. This animal model mimics some characteristics of human IBD.[28–30] Several major causative factors in the initiation of human colitis, such as enhanced vasopermeability, prolonged neutrophil infiltration and increased production of inflammatory mediators, are involved in this model. The study focused on the comparison between rectal and oral routes of glycyrrhizic acid administration against TNBS-induced colitis. We found that glycyrrhizic acid administered by the rectal route effectively attenuated colitis at different dosages. Higher dosage of rectal glycyrrhizic acid better protected against colitis induced by TNBS, though there were no significant differences when compared with oral treatment.

Although the aetiology of IBD remains unknown, it is mostly the consequence of a defective response of the adaptive/innate system to luminal antigens, encompassing several inflammatory conditions. Numerous researchers have identified a prominent role for macrophages and lymphocyte cells in the initiation and maintenance of IBD.[31–34] This research investigated the effects of glycyrrhizic acid on macrophages, lymphocytes and cytokines secreted by these cells. Though normal macrophages and T cells are important for host defence, they might also lead to effector cell populations with substantial autoreactivity and the capacity to cause mucosal inflammation.

Studies showed that in active IBD, activated macrophages accumulate in the colon mucosa and secrete many pro-inflammatory cytokines, such as IL-6, IL-1β and TNF-α, mediating the chronic mucosal inflammation in IBD patients.[32] IL-10 is an anti-inflammatory cytokine that has a broad spectrum of immunosuppressive/anti-inflammatory actions and can efficiently inhibit T-cell proliferation and cytokine responses.[35,36] Experiments *in vitro* investigated the effects of glycyrrhizic acid on lymphocyte proliferation and production of IL-6, IL-10 in activated macrophages. Ha *et al*.[25] found that LPS (1 μg/ml) treatment of macrophages *in vitro* caused a considerable increase in IL-6. Our research showed that glycyrrhizic acid markedly inhibited the elevation of IL-6 and up-regulated the level of anti-inflammatory IL-10, suggesting the immunoregulatory function of glycyrrhizic acid.

Apoptosis of T cells appears to be critical in inhibiting the mucosal immune response, and defective apoptosis may be a key factor for inappropriate T-cell accumulation and perpetuation of chronic mucosal inflammation in IBD.[37] In this study, spleen lymphocyte cell proliferation tests were carried out to identify the effects of glycyrrhizic acid on ConA-induced T-lymphocyte proliferation. We found that glycyrrhizic acid inhibited the proliferation of lymphocytes at concentrations of 1–100 μg/ml. This result showed that glycyrrhizic acid may inhibit the mucosal inflammatory response caused by T lymphocytes, and thus exhibits its protective effects in IBD.

The activated macrophages, lymphocytes and pro-inflammatory cytokines secreted by these cells appear to initiate and perpetuate chronic intestinal inflammation. Reactive oxygen species (ROS) that degrade the extracellular matrix are also produced by these macrophages. Excessive production of ROS or the impairment of antioxidant defence mechanisms may induce inflammatory and immune responses, which could directly or indirectly lead to lesion of intestinal epithelial cells and, subsequently, severe impairment in experimental colitis.[38] The current investigation *in vivo* showed that pro-inflammatory cytokine TNF-α and IL-1β levels were increased in serum after TNBS instillation.

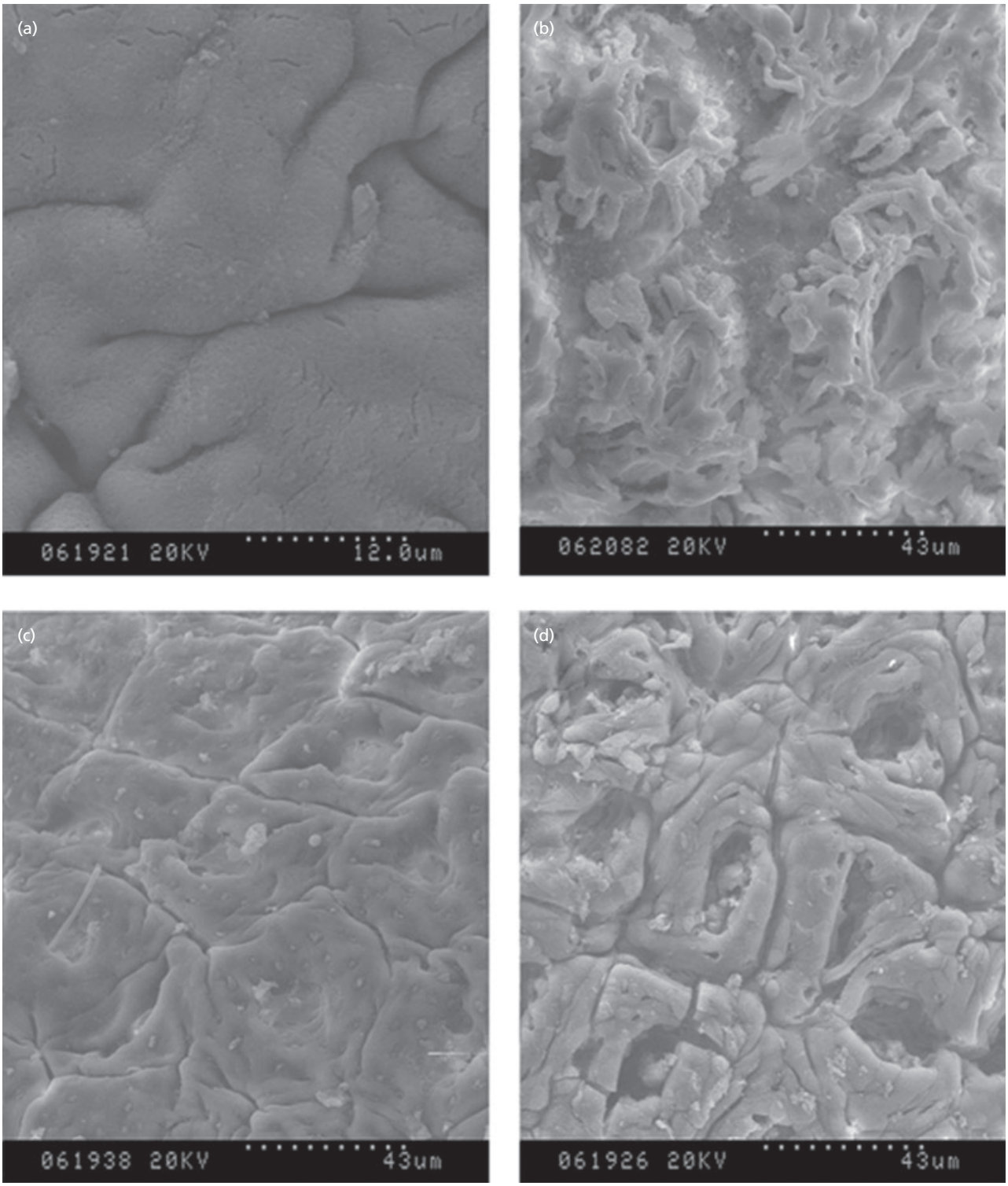

Figure 2 SEM photomicrograph of colon tissues in rats. (a) Normal rat colon shows intact epithelial surface. (b) TNBS-induced colitis showing obvious fibrous connective tissue, abnormal crypt structure in epithelium. (c) Colitic rats treated with glycyrrhizic acid (10 mg/kg) rectally, showing microvilli reconstruction and relieved pathological changes. (d) Colitic rats treated with sulfasalazine (225 mg/kg) rectally, showing microvilli reconstruction and fibrous connective tissue (Magnification ×20 000).

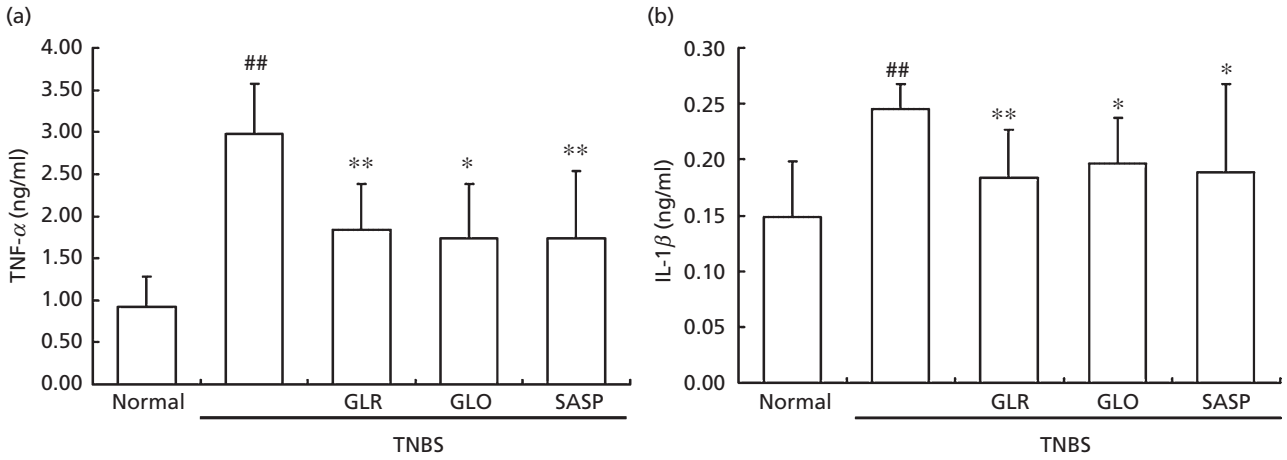

Figure 3 Effect of glycyrrhizic acid on serum TNF-α (a) and IL-1β (b) in colitic rats induced with TNBS. GLR, rectal glycyrrhizic acid; GLO, oral glycyrrhizic acid; SASP, rectal sulfasalazine. Rats in Normal and TNBS group were daily administered with saline solution as the vehicle. Results are expressed as the means ± SEM, $n = 6$/group. $^{\#\#}P < 0.01$ vs Normal group; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs TNBS group.

Table 2 Effect of glycyrrhizic acid on colon myeloperoxidase and superoxide dismutase activity and malondialdehyde level in colitic rats induced with TNBS

Group	Dose (mg/kg)	MPO (U/g)	SOD (U/ml)	MDA (nmol/mg)
Normal	–	22.1 ± 2.5	61.0 ± 17.6	26.8 ± 11.6
TNBS	–	138.7 ± 8.9##	22.7 ± 7.0##	102.2 ± 30.1##
TNBS+GL2	2	108.2 ± 20.1**	32.6 ± 7.4*	70.5 ± 16.2*
TNBS+GL10	10	85.3 ± 16.4**	45.8 ± 7.5**	42.5 ± 18.4**
TNBS+GL50	50	79.2 ± 16.5**	52.1 ± 15.4**	38.7 ± 10.7**
TNBS+GLO	10	92.1 ± 42.2*	44.2 ± 22.2*	46.1 ± 15.4**
TNBS+SASP	225	67.2 ± 18.9**	45.7 ± 11.7*	58.8 ± 20.5*

Rats in Normal and TNBS group were administered daily with saline solution as the vehicle control. Results are expressed as the means ± SEM, $n = 6$/group. $^{\#\#}P < 0.01$ vs Normal group; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs TNBS group.

Table 3 Effect of GL on IL-6 and IL-10 of mice PMΦ stimulated by LPS (1 μg/ml)

Group	Dose (μg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)
Normal	–	22.0 ± 12.2	348.4 ± 33.2
LPS	–	362.3 ± 131.2##	257.1 ± 22.5
TNBS + GL1	1	303.1 ± 83.3	391.2 ± 28.1**
TNBS ± GL10	10	171.5 ± 17.7*	401.0 ± 42.5**
TNBS ± GL100	100	41.2 ± 17.3**	382.7 ± 21.3**

Results are expressed as means ± SEM, $n = 6$/group. $^{\#\#}P < 0.01$ vs Control group; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs LPS group.

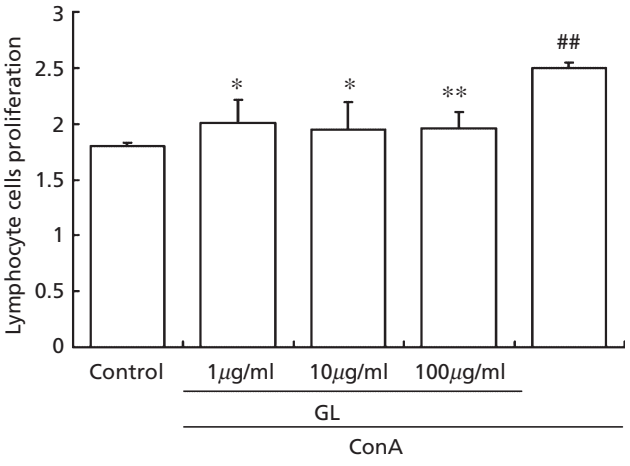

Figure 4 Inhibitory effect of glycyrrhizic acid on mouse spleen lymphocyte proliferation stimulated by ConA.

Cytokine overproduction maintains the highly chronic inflamed state of the bowel and motility disorders.[39,40] Above all, IL-1β appears to be a primary stimulator of diarrhoea, the major symptom of intestinal inflammation, and helps to propagate local systemic inflammatory process by activating a cascade of immune cells.[41–43] TNF-α is one of the most significant factors participating in the inflammatory process in IBD patients. It induces the production of adhesion molecules and activation of immune and non-immune cells. In this study, rectal glycyrrhizic acid significantly reduced TNF-α and IL-1β, indicating that its anti-inflammatory effects are probably due to its powerful immunoregulatory properties. Treatment with rectal glycyrrhizic acid in this study inhibited colonic MPO and MDA elevation and restored the SOD activity toward the normal value. The inflammatory response

caused by TNBS is characterized by a substantial increase of the neutrophil infiltration into the colonic tissue, and activated neutrophils produce the superoxide anion, through MPO, leading to intestinal injury.[44] MPO has been assessed as one of the most important parameters in valuating the injury of colon tissue in animal experiments. In our study, we found that MPO activity and MDA levels were significantly decreased while the activity of SOD in colon tissue was markedly increased in GL50 (rectal glycyrrhizic acid 50 mg/kg).

Our study indicated that TNBS-induced colitis was associated with macroscopic, microscopic and biochemical changes. Treatment with glycyrrhizic acid, rectally or orally, attenuated colitis as shown by decreased serum levels of TNF-α and IL-1β, reduced colon MPO activity and MDA concentration, and elevated SOD activity. With the same dosage, glycyrrhizic acid rectal treatment showed similar protective effects against colitis when compared with oral treatment. On the other hand, glycyrrhizic acid is hydrolysed in the intestine to glycyrrhetic acid, which inhibits the enzyme 11β-hydroxysteroid dehydrogenase and causes pseudoaldosteronism. These would lead to hypokalaemia, water-sodium retention or even renal failure.[45–47] Our research showed that glycyrrhizic acid treatment had no significant side effects on kidney function and serum K^+ and Na^+ concentrations. All this suggests that glycyrrhizic acid by rectal administration would be a safe complementary treatment.

Conclusions

The research investigated the protective effects of glycyrrhizic acid against rat colitis induced by TNBS. We found that glycyrrhizic acid significantly reduced a wide range of inflammatory mediators relevant in intestinal inflammation and prevented the development of TNBS-induced colitis. The effects of administration by the oral and rectal routes were compared, and local administration showed similar protective effects to the oral route, suggesting that rectal administration of glycyrrhizic acid may be a complementary treatment in the future.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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