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• *BASIC RESEARCH* •

Effects of Changtai granules, a traditional compound Chinese medicine, on chronic trinitrobenzene sulfonic acid-induced colitis in rats

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Abstract

AIM: To study the effects of Changtai granules (CTG), a traditional compound Chinese medicine, on chronic trinitrobenzene sulfonic acid-induced colitis in rats.

METHODS: Healthy adult Sprague-Dawley (SD) rats of both sexes, weighing 250-300 g, were employed in the present study. The rat colitis models were induced by 2, 4,6-trinitrobenzene sulfonic acid (TNBS) enemas at a concentration of 100 mg/kg in 50% ethanol. The experimental animals were randomly divided into dexamethasone (DX) treatment, CTG treatment, and model control groups, which were intracolicly treated daily with DX (0.2 mg/kg), CTG at doses of 2.9, 5.7 and 11.4 g crude drug/kg, and the equal amount of saline respectively from 6 h following induction of the colitis in rats inflicted with TNBS to the end of study. A normal control group of rats treated without TNBS but saline enema was also included in the study. After 3 wk of treatment, the animals were assessed for colonal inflammatory and ulcerative responses with respect to mortality, frequency of diarrhea, histology and myeloperoxidase activity (MPO).

RESULTS: The therapeutic effect of CTG on ulcerative colitis (UC) was better than DX. CTG effectively inhibited the activity of granulocytes, macrophages and monocytes in a dose-dependent manner. Also it reduced MPO and formation of inflammation in colonic mucosal tissue. Furthermore, administration of CTG significantly prevented body mass loss and death, and decreased frequency of diarrhea in UC rats, when compared with the model control group rats.

CONCLUSION: CTG would prove to be an ideal drug for chronic UC, and is warranted to be studied further.

INTRODUCTION

Ulcerative colitis (UC) is a common digestive disease in the Western countries[1-4]. It was believed that the occurrence of UC was rare in China. But recent reports reveal that UC cases have increased substantially. According to Jiang, UC cases increased 2.7 times from 1991 to 2000 *vs* from 1981 to 1990. UC is a refractory, chronic and non-specific disease[5-9]. As the etiology and mechanism of UC remain elusive and no specific treatment is available, the disease usually becomes chronic with refractory relapses, thus seriously endangering health of patients[10-14].

Salicylazosulfapyridine (SASP) and corticosteroids, despite their widespread use in the treatment of UC, do not offer an ideal cure because of prolonged treatment, various adverse reactions as well as tendency to relapse when medication is discontinued[15-19]. The diagnosis and treatment of UC remain to be a clinical challenge[20-24]. The emphasis laid on the study of UC, therefore, is to find an effective drug with fewer adverse reactions.

In view of a poor curative effect and a high recurrence rate, traditional Chinese medicinal (TCM) formulae have been attempted to treat the disease in recent years, and the therapeutic effectiveness is quite satisfactory[25-29]. Changtai granule (CTG) is a traditional compound Chinese medicinal formula. In order to explore the effect and pharmacological action of CTG on UC, rat models were established to observe the effect of CTG on mortality, frequency of diarrhea, histology and myeloperoxidase activity in the colon of rats with UC.

MATERIALS AND METHODS

Materials

Sprague-Dawley (SD) rats (250-300 g) obtained from the Animal Center of the Second Military Medical University

(Shanghai, China) were fed on a standard laboratory chow and free access to tap water. The rats were kept in a room at controlled temperature (22±1 ℃), humidity (65-70%), and 12:12-h light-dark cycles. One hundred and twenty rats were randomly divided into six groups: namely normal, model control, dexamethasone (DX, 200 mg/kg, i.g.) and CTG (2.9, 5.7, and 11.4 g crude drug/kg, i.g.) groups. DX and 2, 4,6-trinitrobenzene sulfonic acid (TNBS) were purchased from Sigma (St. Louis, USA). Hexadecyltrimethyl-ammonium was a product of Hengye Zhongyuan Chemical (Beijing, China).

Induction of the UC model in rats

The rats were fasted for 24 h before experimentation but with free access to drinking tap water. The colitis model was produced as before[30]. In brief, the rats (except those in the normal group) were lightly anesthetized with ether. A rubber catheter (12 cm long, external diameter 2 mm) was inserted rectally into the colon so that the tip was 8 cm proximal to the anus, approximately at the splenic flexure. TNBS (100 mg/kg) dissolved in 500 mL/L ethanol was instilled into the colon lumen through the rubber catheter, and saline was instilled as control. Saline, DX and CTG were administered 6 h after induction of the colitis model. Body weight change was recorded during the experimental period.

Morphological and pathological observation

The rats were anesthetized with ether and killed by cervical dislocation after 3 wk. The distal colon (10 cm) was removed, opened by a longitudinal incision and washed to remove the lumen contents; colon wet weight was weighed, and mucosal damage was assessed by measurement of ulcerative areas in colon mucosae. The excised colon was pinned out on a wax block washed with 0.9% saline and assigned a code number. The colon was immediately examined under a stereomicroscope, and any visible damage was scored on a 0-5 scale (Table 1). Small sections were taken from each colon and placed in 40 g/L formaldehyde for histological examination. The colon was fixed, cut longitudinally into 5-μm sections, and stained with hematoxylin and eosin. The second segment (200-400 mg) was then excised, immediately frozen in liquid nitrogen, and stored at -80 ℃ for subsequent assay of myeloperoxidase (MPO) activity.

Table 1 Criteria for scoring the gross morphologic damage

Score	Gross morphology
0	No damage
1	Localized hyperemia with no ulcers
2	Liner ulcers with no significant inflammation
3	Liner ulcers with inflammation at one site
4	More ulcerative and inflammatory sites, the size of ulcers <1 cm
5	Multiple inflammations and ulcers, the size of ulcers ⩾1 cm

Determination of biochemical markers

The activity of MPO was determined by chromatometry. In brief, the colon tissue samples taken for MPO detection were homogenized (50 g/L) for 30 s by using a polytron generator in ice-cold potassium phosphate buffer 50 mmol/L (pH 6.0) containing 0.5% hexadecyltrimethyl-ammonium bromide. The homogenates were frozen and thawed thrice, and then centrifuged at 4 000 r/min for 15 min at 4 ℃. MPO level in the supernatant was measured by using the technique as described before[31].

Statistical analysis

Experimental results were analyzed by SPSS and *t*-test for multiple comparisons between the groups. Data were finally expressed as mean±SD. *t*-test and Wilcoxon signed ranks test were used to analyze the data of diarrhea frequency and colon damage score respectively. Data of colon damage score were presented as medians (25^{th}-75^{th} percentiles). *P* value less than 0.05 was considered statistically significant.

RESULTS

Effects of CTG on rats with TNBS-induced colitis

During the inflammatory period, the rats with colitis had a high frequency of diarrhea (Table 1) with marked body mass loss in the 1^{st} wk (Table 2 A and B). Death was also found in the animals that received TNBS in 500 mL/L ethanol, especially in the model control group (Table 1). Administration of CTG (2.9, 5.7, and 11.4 g crude drug/kg) significantly decreased the diarrhea frequency in a dose-dependent manner. Furthermore, administration of CTG also prevented the rats from death. But the effects of DX showed further body mass loss and death emergence compared with the model control and CTG groups, suggesting that severe immunosuppression occurred. Compared with the CTG group, the diarrhea frequency in the DX group did not decrease significantly, although it decreased compared with the model control group.

Table 2A Effects of CTG on diarrhea frequency and mortality of UC rats

Group	Dose (g crude drug/kg)	*n*	Diarrhea				Mortality
			D 1	D 8	D 15	D 22	
Normal	–	20	0	0	0	0	0
Model	–	20	20[b]	10[b]	7[b]	2	2
DX	0.2 (mg/kg)	20	20	3[a]	5	1	4
CTG	2.9	20	20	2[d]	2	0	0
CTG	5.7	20	20	3[a]	1[a]	0	0
CTG	11.4	20	20	1[d]	0[d]	0	0

[b]*P*<0.01 *vs* normal, [a]*P*<0.05, [d]*P*<0.01 *vs* model.

Table 2B Effects of CTG on the weight of UC rats (*n* = 20)

Group	Dose (g crude drug/kg)	Weight (g)			
		D 1	D 8	D 15	D 22
Normal	–	188±13	228±30	253±39	283±50
Model	–	188±10	204±24[b]	236±26	275±37
DX	0.2 (mg/kg)	189±10[d]	183±12[d]	185±8[d]	191±12[d]
CTG	2.9	184±16	199±28	227±35	264±41
CTG	5.7	191±11	204±13	238±23	272±34
CTG	11.4	182±9	201±23	232±35	264±42

[b]*P*<0.01 *vs* normal, [d]*P*<0.01 *vs* model.

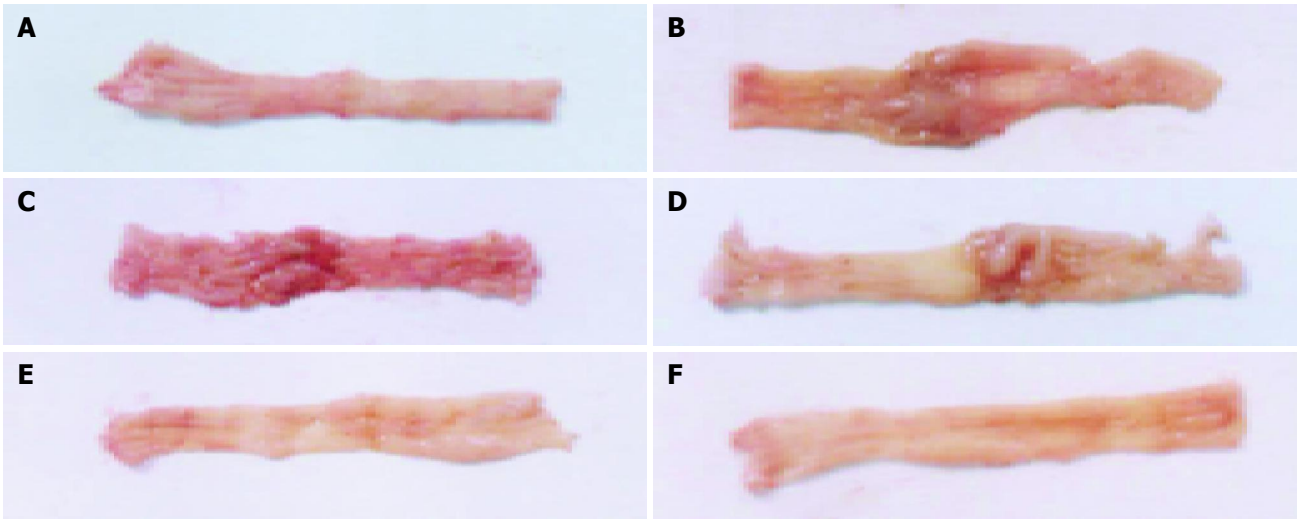

Figure 1 Photos of distal colons from normal (**A**), control (**B**), DX (**C**), and CTG at dose of 2.9, 5.7, and 11.4 g crude drug/kg (**D-F**) groups. At model control PIC, histological inflammatory features including mucosal hemorrhage, submucosal edema and inflammatory infiltration in the lamina propria and submucosa were observed. At CTG PIC, there was no remarkable inflammatory feature. At DX PIC, ulcer was recovered, but inflammatory feature was still remarkable.

Effects of CTG on colon mass and ulcer

Grossly visible colon wall incrassation, inflammation, and ulceration were found in the model control group animals. The levels of colonic tissue damage score of the control group increased significantly compared with those of the normal group ($P<0.01$), while in comparison with those of the control group, they decreased significantly in the DX and CTG groups ($P<0.01$). The colonic tissue damage scores of the CTG group animals decreased significantly compared with those of the DX group animals (Figure 1 and Table 3). In comparison with those of the control group, colon masses of the CTG group animals reduced significantly, but not those of the DX group animals (Table 3).

Table 3 Effects of CTG on colon mass, ulcer, MPO of colon mucosa of UC rats (n = 10)

Group	Dose (g crude drug/kg)	Colon mass index (mg/g)	Colon damage score	MPO (U/g)
Normal	–	3.2±0.6	0 (0–0)	0.17±0.11
Model	–	5.2±1.4[d]	5 (3–5)[d]	2.57±1.75[d]
DX	0.2 (mg/kg)	6.1±4.0	5 (0–5)[b]	0.80±1.00[a]
CTG	2.9	4.5±2.4	3 (2–5)[b]	0.60±0.61[b]
CTG	5.7	4.0±1.1[a]	3 (2–4)[b]	0.48±0.46[b]
CTG	11.4	3.8±0.5[b]	2 (0–3)[b]	0.47±0.36[b]

[a]$P<0.05$, [b]$P<0.01$ *vs* model, [d]$P<0.01$ *vs* normal.

Effects of CTG on MPO of colon mucosa

MPO activity was regarded as the main parameter reflecting the degree of colon injury and inflammation in inflammatory gut tissue. Compared with the normal group, this parameter was significantly increased in the model group ($P<0.01$). Both DX and CTG remarkably decreased this elevated parameter ($P<0.05$-0.01). Furthermore, the therapeutic effects of CTG at different doses (2.9, 5.7, and 11.4 g crude drug/kg) were better than that of DX (0.2 mg/kg) (Table 3).

Effects of CTG on pathology of colon

The bowel wall was normal by gross and microscopic examinations in the normal group. Diffuse hemorrhage, edema, congestion, superficial ulceration in the mucosa with infiltration of lymphocytes, plasma cells and polymorphonuclear cells, cryptitis and crypt abscess were observed in the control groups. The bowel wall was recovered to normal gradually in a dose-dependent manner when administered with CTG, while in the DX group, it was less recovered compared with that in the CTG group (Figure 2).

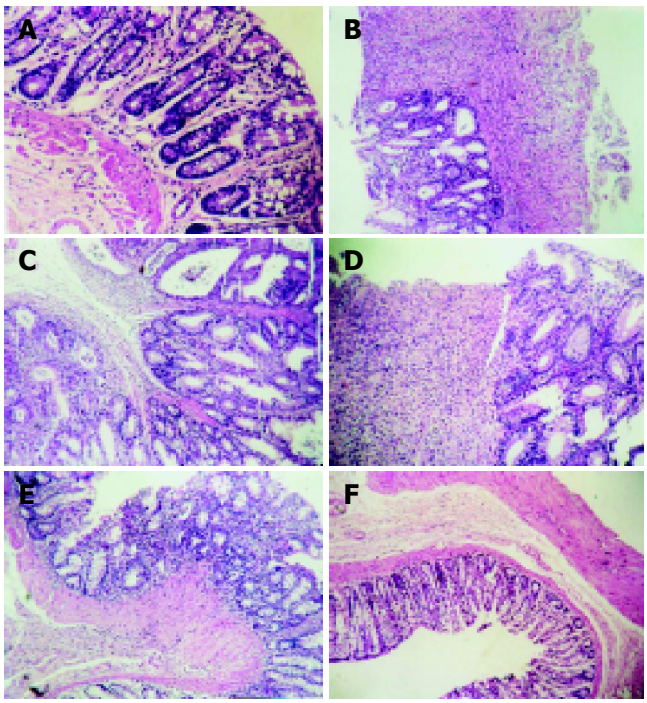

Figure 2 Histological findings in UC. **A**: Normal group, the mucosa is normal (HE, ×200); **B**: control group, gross ulcers, epithelial necrosis at mucosal surface and inflammatory cell infiltration (HE, ×200); **C**: DX group, gross ulcers and proliferous granuloma, inflammatory cell infiltration (HE, ×200); **D**: CTG group at dose of 2.9 g crude drug/kg, small mucosal ulcers and crypto abscess formation (HE, ×200); **E**: CTG group at dose of 5.7 g crude drug/kg, inflammatory cell infiltration (HE, ×200); **F**: CTG group at dose of 11.4 g crude drug/kg, the mucosa recovered (HE, ×100).

DISCUSSION

The pathogenesis of UC remains unclear up to now. It is generally accepted that UC is the result of interaction of multiple factors, including the environment, immunity and heredity. Probably, in hereditarily susceptible population, environmental factors such as water, food and infection trigger excessive reaction of intestinal immunity. The reaction may cause an inflammatory stimulation to the intestinal mucosa and damage it[32,33]. At present, there is no radical cure for UC. Steroids and salicylic acid preparations are usually used to control and suppress the inflammation. Salazosulfapyridine (SASP) is also a recommended remedy for UC. All of these remedies can improve the conditions of patients to varying degrees. But adverse reaction and high relapse rate are problems arising from these therapies.

With the development of immunology and molecular biology, an increasing knowledge about the condition and a further understanding of the mechanism of drug actions, in recent years, new therapies are emerging one after another. Nevertheless, some UC sufferers cannot afford these new therapies due to the exorbitant price of the drugs. In addition, the safety of these new drugs needs to be further identified.

Many studies showed that the treatment of TCM enemas was better on UC than SASP or DX with fewer adverse reactions. To minimize adverse reactions, poorly absorbable glucocorticosteroids have also been used for enema therapy including hydrocortisone foam and prednisolone metasulfobenzoate or molecules with increased first pass metabolization in the liver, e.g., betamethasone dipropionate and tixocortol pivalate. But rectal treatment with mesalazine enemas is often ineffective for extensive UC, although it is the first line therapy for distal UC at present. Meanwhile, it is inconvenient for patients to maintain the treatment for several months.

TCM therapy is more promising and acceptable for patients. CTG is prepared from TCM herbs including Huangbai and other three TCM herbs. According to our study, CTG possesses a variety of pharmacological effects including analgesic-antipyretic, anti-inflammatory, antibacterial and anti-diarrhea actions, as well as the effect of adjusting gastrointestinal function[34]. This study showed that CTG was better than DX for UC. CTG used in this study could effectively inhibit MPO activity and formation of inflammation and ulceration in colonic mucosal tissue in a dose-dependent manner. Furthermore, administration of CTG can significantly decrease the frequency of diarrhea in UC rats and prevent the animals from death. Therefore, CTG may be an ideal choice for curing the disease.

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