Effect of traditional Chinese medicinal enemas on ulcerative colitis of rats

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AIM: To investigate the effects of traditional Chinese medicinal enema (TCME) on inflammatory and immune response of colonic mucosa of rats with ulcerative colitis (UC), and to observe the pathogenic mechanism.

METHODS: Thirty UC rats, induced by intestinal enema together with 2.4-dinitrochlorobenzene (DNCB) and acetic acid, were randomly divided into 3 groups, i.e., GI, GII and GIII. Groups GI and GII were administered with TCME and salazosulfapyridine enema (SASPE), respectively. Group GIII was clusted with only normal saline (NSE), served as control. Group GIV was taken from normal rats as reference. Thirty UC rats, induced by intestinal enema together with 2.4-dinitrochlorobenzene (DNCB) and acetic acid, were randomly divided into 3 groups, i.e., GI, GII and GIII. Groups GI and GII were administered with TCME and salazosulfapyridine enema (SASPE), respectively. Group GIII was clusted with only normal saline (NSE), served as control. Group GIV was taken from normal rats as reference.

RESULTS: Before therapies, in model groups, GI, GII and GIII, levels of IL-6 (IL-6) in the colonic mucosa was assayed by H-Tdr incorporation assay. Colonic mucosal lymphocyte subpopulation adhesive molecules, $CD_{8}$, $CD_{11a}$, $CD_{11b}$ and $CD_{4}$ were significantly different (38.28±6.1 U/mL, 16.54±2.23 ng/L, 8.61±0.89 ng/L, 13.51±3.11% and 12.22±1.15%, respectively) compared to those in GIV group (31.56±2.47 U/mL, 12.81±3.8 ng/L, 5.28±0.56 ng/L, 16.68±4.11% and 16.79±1.11%, respectively). After therapeutic enemas, in GI group, the contents of IL-6 (32.48±2.53 U/m), TNF-α (13.42±5.7 ng/L) and IFN-γ (5.87±0.84 ng/L) were reduced; then, the contents of $CD_{8}$, $CD_{11a}$ (13.42±5.7 ng/L) and $CD_{4}$ were (16.28±0.19%) were raised. There was no significant difference between groups GI and GIV, but the difference between groups GI and GII was quite obvious (P<0.05). The expressions of TNF-α mRNA and IFN-γ mRNA in group GIIII were much higher than those of group GIV, but those in group GI were significantly suppressed by TCME therapy.

CONCLUSION: Ulcerative colitis is related to colonic regional mucosal inflammatory factors and immune imbalance. TCME can effectively inhibit regional mucosal inflammatory factors and improve their disorder of immunity.

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INTRODUCTION

Ulcerative colitis (UC) is a refractory, chronic and non-specific disease. Its pathogenesis is probably related to the deficiency of autoimmunity and imbalance of immunoregulation[1-5]. The relationship between pathogenesis and immunity of colonic mucosa remains a focus at present[6-11]. In view of poor curative effect and high recurrence rate, traditional Chinese medicinal formulae were used to treat the disease in recent years, and the therapeutic effectiveness is quite satisfactory[12]. In order to explore the mechanism of UC and pharmacological action of the traditional Chinese medicinal formulae, rat models were established to observe the effect of traditional Chinese medicinal enema on immunological and inflammatory factors in colonic mucosa of rats with UC.

MATERIALS AND METHODS

Materials

Six-week-old Wistar rats (n=56), half of male and half of female were purchased from Shanghai Laboratory Animal Center of Chinese Academy of sciences (SLAC. CAS), calf serum, ConA and PHA (Institute of Cell Research of CAS) and mRNA reagent kit and PCR reagent (Promega). IL-6, TNF-α, IFN-γ reagent kit and PE marked $CD_{8}$, $CD_{4}$ monoclonal antibody (Sigma). H-Tdr was from Shanghai Atomic Nuclear Institute of CAS. Cell factor primer-detoxon was purchased from Shanghai Biological Engineering Center of CAS. The formulae of traditional Chinese medicinal herbs for enema consisted of Huangqi (astragalus), Dahuang (caulis fibraureae), Huangbai (cortex phellodendri), Wubeizi (galla chinensis) and Baiji (rhizoma bletilae), mixed with 1g crude drug per milliliter by Medicament Section of Shanghai Tongji Hospital. Salazosulfapyridine (SASP, batch No.921101, Shanghai Sine Pharmaceutical Factory) was prepared as suspension (20 mg/mL) at the same section.

UC model establishment

The models were prepared in reference to literature[13]. Fifty six Wistar rats weighing 200±20 g, were fed for 1 week before experiment. Then 38 rats were randomly taken as subjects for experiments. After depilation on back and neck, the rats were administered 0.3 mL of DNCB acetone liquid (DNCB 2.0 g: acetone 100 mL) solution on their bare backs and necks once daily for total 14 d. At the 15th day, 0.25 mL of 0.04 mmol/L DNCB ethanol (500 mL/L) solution was infused through a nylon hose inserted into the colon of rat 8 cm depth. At the 16th day, 2 mL of 80 mL/L-1 acetic acid solution was infused at the same depth. Then, the colon was rinsed with 5 mL normal

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saline after 15 s of retention. The remaining 18 rats acted as controls after infusion of normal saline (NS) at 1 week since the preparation of the models. 8 rats were taken respectively from the group of model and group for reference to detect the levels of colonic mucosa lymphocytes: IL-6, TNF-α, IFN-γ and LSAM. The remaining 30 rats were randomly divided into 3 groups of 10 each: GI, GII and GIII were clustered daily with 2 mL of TC, SASP and NS for 28 d respectively. Another group (GIV) of 10 rats, administered 2 mL of NS daily for 28 d, was taken as reference of normality. At the end of 4 wk experimental period, the lymphocytes were again detected and also the expressions of TNF-α mRNA and IFN-γ mRNA were examined.

**Assay of cytokines and immunological factors**

Separation and culture of intestinal theca mucosa cells

Mucosa cells were separated and routinely cultured to prepare monolayer culture cells suspension of colonic mucosa, and the suspension was adjusted to 5×10^5 cells/mL. Then, 1.0 mL of suspension, was put in 24-well plate, then 1.0 mL of ConA (final density 5 mg/L) was added, and cultured at 37 °C for 24 h in a humidified atmosphere containing 50 mL/L CO₂ for 1 h and at 94 °C for detection of IL-6, TNF-α, IFN-γ and LSAM levels according to the manufacturer’s instructions.

**RNA extraction**

Total RNA from colonic mucosa tissue of rats was extracted as previously described[14-16]: using single-step method of RNA isolation (acid guanidinium thiocyanate-phenol-chloroform extraction). Formaldehyde denatured electrophoresis stained with ethidium bromide (EB) was used to examine infect RNA. Reverse transcription reaction was performed as previously described[14-16]: a total amount of 1 µg of RNA as template was mixed with RNasin (40 U/µL) 0.5 µL, 25 µmol/L oligo (dT) 1 µL, 5×RT buffer 5 µL, dNTP (10 mmol/L) 1 µL and AMV (9 U/µL) 1 uL to a total reaction volume of 25 µL, followed by incubation at 37 °C for 1 h and at 94 °C for 5 min. PCR reaction was carried out as described previously[20-22]. 10 µL cDNA was added with 0.1 µg of primer, 0.05 µg of β-action primer and 2.5 U of Taq enzyme (5 U/µL). Total PCR reaction volume was 100 µL. PCR condition as was followed: denaturation at 94 °C for 1 min; primer annealing at 54 °C for 40 s, and extension at 72 °C for 40 s, followed a further extension at 72 °C for 7 min. Other cycles were as follows: after 30 cycles of amplification, 20 µL of PCR product was electrophoresed on 20 g/L agarose gel, then recorded with camera under ultraviolet for density scanning and calculated the relative expression of the gene.

**Statistical analysis**

Experimental results were expressed as mean±SD. P<0.05 was considered statistically significant. All statistical calculations were performed using the SPSS for Windows version 9.0 software package.

**RESULTS**

**Expression of colonic mucosal cytokines and adhesion molecules in rats of model group (MG) and control group (CG)**

At 1 week after setting of models, the expression of cytokines in MG was found to be significantly higher than that in CG (P<0.01, Table 1). The expressions of lymphocyte T subpopulation surface adhesive molecules, CD_{4+}CD_{11a+} and CD_{4+}CD_{11a+}, were remarkably lower in MG than those in CG (P<0.01, Table 2).

**Colonic mucosal TNF-α mRNA and IFN-γ mRNA**

In Group GI clustered with TCME, the levels of IL-6, TNF-α and IFN-γ were obviously reduced. Levels of CD_{4+}CD_{11a+} and CD_{4+}CD_{11a+} were remarkably raised, which were no obvious differences compared with those in GIV group (i.e., control group) (P>0.05, Tables 3 and 4, but, there were significant differences between groups GI and GII (P<0.05, Tables 3 and 4).

**Table 1 Expression of colonic mucosal cytokines in MG and RG before therapy (mean±SD)**

| Group   | IL-6 (U/mL) | TNF-α (ng/L) | IFN-γ (ng/L) |
|---------|-------------|--------------|--------------|
| Model   |             |              |              |
| Control | 38.29±2.61  | 16.54±1.23   | 8.61±0.89    |
| Control | 31.56±2.47  | 12.81±1.38   | 5.28±0.56    |

**P<0.01 vs control group.**

**Table 2 Comparison between expressions of colonic lymphocyte surface adhesive molecules (mean±SD, %)**

| Group   | CD_{4+}CD_{11a+} | CD_{4+}CD_{11a+} | CD_{4+}CD_{11a+} | CD_{4+}CD_{11a+} |
|---------|------------------|------------------|------------------|------------------|
| Model   |                  |                  |                  |                  |
| Control | 32.48±2.53       | 13.42±1.57       | 5.67±0.84        |
| Control | 34.56±3.21       | 14.81±1.48       | 6.36±0.62        |

**P<0.05 vs control group.**

**Table 3 Changes of colonic mucosal cytokines after therapy (n=10, mean±SD)**

| Group   | IL-6 (U/mL) | TNF-α (ng/L) | IFN-γ (ng/L) |
|---------|-------------|--------------|--------------|
| GI      |             |              |              |
| GII     |             |              |              |
| GIII    |             |              |              |
| GIV     |             |              |              |

**Table 4 Expression of colonic mucosal lymphoid T surface adhesive molecules (n=10, mean±SD)**

| Group   | CD_{4+}CD_{11a+} | CD_{4+}CD_{11a+} | CD_{4+}CD_{11a+} | CD_{4+}CD_{11a+} |
|---------|------------------|------------------|------------------|------------------|
| GI      |                  |                  |                  |                  |
| GII     |                  |                  |                  |                  |
| GIII    |                  |                  |                  |                  |
| GIV     |                  |                  |                  |                  |

**P>0.05, P<0.05 vs GIV; P<0.05 vs GII.**

**Expression of TNF-α mRNA and IFN-γ mRNA in colonic mucosal tissue**

The expression of TNF-α mRNA and IFN-γ mRNA in colonic mucosal tissue, was higher in GII group than those in GIV group (Figures 1, 2). After treatment, TNF-α mRNA and IFN-γ
mRNA levels were obviously suppressed in groups GI and GII, especially in group GI.

**Figure 2** Analysis of IFN-γ mRNA expression in UC mice after a 4-week treatment. A 430-bp fragment was amplified by PCR. M: Marker; Lane 1: Group GIV (control); Lane 2: Group GI (TCME); Lane 3: Group GII (SASPE); Lane 4: Group GIII (NSE). The primers used were: upstream, 5′-TTTGGGTCTCTTGGCT GTT-3′; and the downstream, 5′-CTCTTTTCGCTTCCTGT-3′ [29].

**DISCUSSION**

The pathogenesis of colonic colitis remains unclear up to now. It is believed that colonic colitis results from the interaction of many factors, such as environment, immunity and heredity, etc. Probably, susceptible population in heredity is affected by environmental factors, such as water, food and infection, which trigger excessive reaction of intestinal immunity. The reaction can cause an inflammatory stimulation to intestinal mucosa and damage [26,27]. More and more attention has been paid to autoimmunity. An opinion suggests that there exists an inevitable correlation between the immunity system of intestinal mucosa and its integrity [26-28]. It has been reported that IL-6, a mononuclear macrophage, is a cytokine secreted by T and B cell. IL-6 can promote proliferation of T cell and enhance T cell reaction to cell toxicity. Our experimental result showed that IL-6 was high active in colonic mucosal tissue of UC models, the higher level of IL-6 can further aggravate the injury on colonic tissue by antigen-antibody reaction and complement activation [29,30]. More endogenous IFN-γ and TNF-α produced by colonic epithelia of UC models cause the changes in epithelia, which may lead directly to scathes of inflammatory epithelia [31,32]. Disorder of autoimmunity is closely related to the imbalance of Th1 and Th2 cells. For IFN-γ being on Th2 cell, abnormal expressions of IFN-γ and IFN-γ mRNA cause inevitably the imbalance of Th1/Th2 and result in immune disorder [33,34]. CD40+ /CD8α are cell surface adhesive albumin, also known as lymphocyte function antigen-1 (LFA-1), a representative of β2 integrants. It possesses a series of functions, such as introduction of adhesion, chemotaxis of cell and homing of lymphocytes, etc. It expresses on surfaces of white cells, phagocytes and large granular lymphocytes, acting as a key channel in interaction and information between cells. A few of cellular factors, affected by them, such as IL-6, IFN-γ, TNF-α and endotoxin, can affect their expressions [35-37]. CD40+/CD8α and CD40+/CD8α (on T, cell) were found in sufferers with moderate and severe ulcerative colitis, and significantly lower than those in normal control rats [38]. This is in conformity to our findings.

Our study showed that expressions of IL-6, TNF-α, TNF-α mRNA, IFN-γ, IFN-γ mRNA were found to be obviously higher and the levels of lymphocyte T surface adhesive molecules, CD40+CD8α and CD40+CD8α were lower than those in normal rats in colonic mucosal tissue of UC models. The results were in agreement with the findings of others [39]. The results indicated that ulcerative colitis was correlated with dysfunction and disorder of autoimmunity. The abnormal expressions of local inflammatory factors and adhesive albumin of cell surface in colonic tissue play a critical role in regulation of immunity. The mutual affects of these factors and abnormality of regulation of immune system are regarded as the core of pathogenesis. The imbalance of regulation between pro-inflammatory cytokines and anti-inflammatory cytokines is considered an important mechanism of intestine inflammation, including ulcerative colitis.

At present, ulcerative colitis lacks more effective therapy for radical cure. Principally, steroid hormones and salicylic acid preparation are used to control and suppress the inflammation. In recent years, along with the development of immunology and molecular biology, the accumulation of knowledge about the disease and further understanding of the mechanism of drug action, new methods of treatment have come up into being one after another. But the ordinary UC sufferers can not afford remedies due to the exorbitant price of drugs, and the safety of the remedies needs to be further identified. Our study demonstrated that salazosulfapyridine (SASP) is still the principal remedy for ulcerative colitis. It can improve the conditions of patients in mild and moderate states. But the side-effect and high relapse rate after the remedy are unsatisfactory. Traditional Chinese medicine is popular in China. It is characteristic of TCM to treat intestinal diseases with TCM herbs enemas [21]. The study showed that the treatment of TCME was better on UC than SASPE. Our TCME could effectively inhibit the activity of granulocyte, macrophage and monocyte. Also it could reduce immune response and formation of inflammation in colonic mucosal tissue, which might be due to that our TCME could reduce the expressions of IL-6, TNF-α, IFN-γ and raise the levels of surface adhesive molecules (CD40+/CD8α and CD40+/CD8α), could also suppress the abnormal expressions of IFN-α and IFN-γ mRNA, improve the disorder of immunity in UC and the Baiji could protect colonic mucosa, Bahuang and Huangbai could promote cruro. SASP suspension has more side-effects than the formulae of TCM herbs, although it has a certain effect in treatment of ulcerative colitis clinically. Therefore, the retention enema prepared from TCM herbs may be an ideal choice to manage the disease.

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