Study on the therapeutic effects of salicylazosulfapyridine on ulcerative colitis in rats

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Abstract: Objective: To explore the therapeutic effects of salicylazosulfapyridine (SASP) on rats with ulcerative colitis (UC). Methods: The UC rat model was induced by colocolysis with TNBS (trinitrobenzene sulfonic acid)/alcohol. Thirty male SD rats were randomly divided into sham group, model group and SASP group with 10 rats in each group. During the two-week drug treatment, the weights, the intestinal mucosal injuries and the inflammation levels of rats in three different groups were recorded. Results: Compared with the model group, the body weight losses of rats (P<0.05) caused by UC were significantly improved and the incidences of loose stools (P<0.05) were alleviated in the SASP group. SASP could improve the injuries and morphological pathology changes of the colon tissue of rats, including the decrease of colon index, ulcer ratio and the number of aberrant crypt foci (ACF). SASP could significantly lower the inflammatory reactions in colon mucosa and effectively control the inflammation (P<0.05). Conclusion: SASP can effectively relieve intestinal inflammatory reactions and have good effects on UC in rats.

Keywords: Salicylazosulfapyridine, ulcerative colitis, inflammation, rat

Introduction

Ulcerative colitis (UC), also known as chronic non-specific ulcerative colitis, is a kind of chronic intestinal inflammation involving colon and rectum, which is one of the most common inflammatory bowel diseases [1-3]. The disease, with slow onset and long duration, poses a serious threat to human health and their life quality [4]. Since the 21st century, the incidence of UC in the world has presented a significant upward trend [5, 6].

UC is mainly characterized by abdominal pain, diarrhea and mucopurulent bloody stool, even fever and other constitutional symptoms [2]. At present, the pathogenesis of UC hasn’t been clearly illustrated yet. It is difficult to cure but easy to relapse; known as one of the modern refractory diseases, UC is a great burden to patients, families and even the whole society. In current clinical trials, the preferred drugs for the treatment of UC are aminosalicylic acids, which can reduce the synthesis of prostaglandins mainly by inhibiting cyclooxygenase and thereby mitigating the intestinal inflammatory reactions. But these drugs have great side effects, including easily lead to gastrointestinal perforation, gastrointestinal bleeding and other complications, so its application has been severely restricted [7, 8]. Therefore, it has become an urgent problem for clinicians, which needs to be solved by finding a drug which can achieve significant improvement in UC condition with fewer side effects and higher safety at the same time.

In intestine tract, salicylazosulfapyridine (SASP) can decompose into 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP) by microorganism. 5-ASA can be adsorbed on the intestinal connective tissue to inhibit the synthesis of prostaglandins and leukotrienes, thus reducing the intestinal inflammatory reactions. However, SP is a kind of sulfonamide antimicrobial with a certain bacteriostasis [9, 10]. Some studies have shown that SASP can decrease the frequency of diarrhea of rats and be conducive to intestinal sodium and water homeostasis, thereby improving the symptoms of rats with colitis, but not increasing the gastrointestinal side effects [11, 12]. Karr et al. found that SASP could relieve the symptoms of colonic mucosal hyperemia, decrease the level of TNFα (an inflammatory factor) in the experimental animals and then effectively control the inflam-
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Inflammatory reactions [13]. Therefore, it was speculated that SASP could effectively treat UC and reduce the inflammatory reactions. This experiment aimed to study the therapeutic effects of SASP on UC via UC rat model, so as to provide strong evidence for clinical medication and reduce medication errors.

Materials and methods

Materials

Experimental animals: Thirty male SD rats for experiment were in specified pathogen free (SPF) condition (aged 6-8 weeks; weighed 300-350 g).

Experiment reagents: SASP was purchased from American Sigma; Trizol reagent (Ambion, Life Technologies, USA), Reverse transcription kits (Prime Script 1stStr and cDNA Synthesis Kit) and Fluorescent Quantitative PCR reagents were purchased from Takara Corporation, Shanghai; the detection kits of TNF-α and IL-1β enzyme linked immunosorbent assay were purchased from R&D (USA) [14].

Methods

Modeling methods: The SD rats were fasted for 12 hours before operation. A silicone tube (diameter: 2 mm, length: 12 cm) was inserted into every rat of model group and SASP group through anus after the anesthesia with diethyl ether. While 8 cm of the tube was inserted, the solution (0.5 mL) which contains equal volume of TNBS (150 mg/kg) and ethanol, was injected into the colon to establish UC model. The rats of sham group were treated with the same method, but the 0.5 mL TNBS/ethanol solution was replaced with 0.5 mL normal saline. After modeling, the rats of SASP group were given 2 mL SASP (400 mg/kg) for intragastric administration, once a day; while the rats of sham group and model group were given 2 mL distilled water respectively for gastric perfusion after establishing model, once a day. Daily observation of rats in each group, including body weights, stools and other general situations, continued for two weeks.

Intestinal histopathologic examination: Two weeks after modeling, the rats were executed by cervical dislocation. The fresh colon tissues were collected by laparotomy and fixed with 10% methanal, stained with HE after being embedded with paraffin, and then observed under light microscope (100×).

Counting the numbers of aberrant crypt foci (ACF) in rats’ colon: The left fresh colons of rats were taken and the attached adipose tissues were removed. The length ways were cut along the mesentery and tiled on plastic plates after being rinsing with ice PBS, then they were fixed with 10% paraformaldehyde for 4 hours, then soaked with methylene blue (2 g/L) for 30 seconds, and finally the numbers of ACF were counted under light microscope (40×).

Visceral indexes: After executing cervical dislocation, the body weights (g) were weighed, and the thymuses, spleens and colons of rats were taken and weighed respectively after drying residual blood by filters.

1) Thymus index refers to the weight index of thymus, and the unit is mg/g. Thymus index = (thymus weight/rat weight) *10; 2) Spleen index refers to the weight index of spleen, and the unit is mg/g. Spleen index = (spleen weight/rat weight) *10; 3) Colon index refers to the weight index of colon and the unit is mg/g. Colon index = (colon weight/rat weight) *10.

Fluorescence quantitative PCR assay: The fresh colon tissues were removed and the total RNA of colon tissue was extracted by Trizol method. The cDNA was obtained by Takara reverse transcription kit. The actin gene (β-actin) was used as the internal control and fluorescence quantitative assay was performed by the ABI PRISM 7500 Fluorescence quantitative PCR (Applied Biosystems, USA). All the primers were synthesized by Shanghai Sangon Company, and the sequence of primers (5’→3’) is shown in Table 1.

The amplification conditions were as follows: forty cycles including 95°C pre-denaturation for 20 s, followed by 95°C for 30 s and 60°C for 30 s. We calculated the Ct value of each sample, and the relative quantification was calculated with $2^{-\Delta\Delta Ct}$ by comparing the Ct value of the internal reference gene.

Detection of serum IL-6, IL-1β with enzyme-linked immunosorbent assay: Blood from hearts of rats in each group were collected, standing for one hour until the serum was separated out, and then centrifugation (3000 rpm/min) was performed for 10 minutes. The
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Table 1. The sequence of primers (5’→3’)

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>IL-6</td>
<td>CTCGGAGATCTGGAAT</td>
<td>CCAGTTGCTAGCTCCAT</td>
</tr>
<tr>
<td>TNF-α</td>
<td>TCTTTACAGGACAGGCT</td>
<td>GCCAGAGGAGGTTCATT</td>
</tr>
<tr>
<td>β-actin</td>
<td>CGTGACACGCAATGGCA</td>
<td>AACAGTCCGCTAGAAC</td>
</tr>
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Statistical methods: The statistical analysis of data was performed by Graphpad Prism 6. The measurement data were expressed by mean ± standard deviation (X ± S), and the means of two groups were compared by one-way ANOVA analysis. P<0.05 showed that the differences were statistically significant.

Results

SASP could significantly relieve the weight losses of rats caused by UC and reduce the incidences of loose stools

After two-week treatment, the body weights of rats in UC model group reduced to 200 g or so, which was significantly lower than that of sham group (P<0.05) (Figure 1A). While the body weights of rats of SASP group increased significantly compared with the model group (P<0.05). The incidence of loose stools of UC model group was 8-fold higher than that of sham group (P<0.05), while the rate of loose stools of SASP groups significantly alleviated, with no significant difference from sham group (P>0.05) (Figure 1B).

SASP could improve the injuries of colon tissue and morphological pathology changes in rats

Pathological staining of rats' colon tissue in each group showed that, the glands of UC model group were disordered and uneven compared with those of sham group. There were many atypical hyperplasia, severe mucosal hyperemia and a large number of inflammatory cells infiltration. While in SASP group, a typical hyperplasia of glands, mucosal hyperemia and inflammatory cell infiltration were alleviated (Figure 1A-C). The colon index (4.90±2.02 mg/g vs 8.21±2.14 mg/g) and the proportion of colon ulcer (24.85±2.86 vs 38.67±8.42) were obviously reduced after SASP treatment compared with the model group (Table 2). The differences were statistically significant (P<0.05). At the same time, SASP treatment could significantly reduce the splenomegaly and thymic atrophy caused by colitis: the spleen index decreased to 3.57 mg/g and thymus index significantly increased to 2.04±0.66 mg/g compared with UC model group. The dif-
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The number of ACF in colon mucosa of rats in the model group increased to 32-34 (as a significant decline); the number of ACF decreased to 18-20 after SASP treatment compared with sham group (P<0.05) (Table 3).

SASP could significantly reduce inflammatory reactions of the colon mucosa and effectively control inflammation

The expression levels of inflammatory factor IL-6 and IL-1β in serum of rats in each group are shown in Figure 3A, 3B. The serum inflammation levels of rats in UC model group were significantly higher than those in sham group (P<0.05), while the levels of both IL-6 and IL-1β in SASP group significantly decreased (P<0.05). The expressions of mRNA of the inflammatory factor in the colon tissue are shown in Figure 3C, 3D. The expressions of IL-6 in the colon tissue of the model group increased by more than 10 times compared with that of sham group and the expressions of IL-1β increased by 4 times. After SASP treatment, the inflammation expressions of both IL-6 and IL-1β in the colon tissue reduced (P<0.05).

Discussion

UC, a chronic intestinal non-specific disease with a high incidence in colon tissue, is easy to relapse but difficult to cure. It can occur at any age, generally in young adults aged from 20 to 50 years old, whose occurrence, development mechanisms and drug treatment have always been hot spots in clinical research [15, 16]. Currently, some studies have found that the dysregulation of immune inflammation can play an important role in the course of UC. Generally, the interaction of pro-inflammatory cytokines and anti-inflammatory cytokines in normal intestinal tract formed a dynamic equilibrium, but when the equilibrium becomes broken, the over-activation of pro-inflammatory cytokines can easily lead to the immunologic injuries of intestinal tissue, then resulting in a series of symptoms such as abdominal pain, diarrhea and mucopurulent bloody stool [17]. IL-6 (a kind of pro-inflammatory cytokine) can be a chemotactic factor for neutrophils, produce a variety of inflammatory mediators and enhance the local inflammatory reactions [18-20]. The expression level of IL-1β (an important pro-inflammatory cytokine and a vital mediator for the regulation of immune system and inflammatory reactions) can reflect the condition of injuries of tissue cells [21, 22]. Therefore, this experiment indicated the degree of intestinal inflammatory reactions by detecting the mRNA and protein expression levels of IL-6 and IL-1β in the colon tissue.

In this experiment, the expression level of serum inflammatory factor IL-6 in rats in UC model group was 6 times significantly higher than that of sham group and the expression level of IL-1β increased twice (P<0.05), which implied that UC could cause systemic inflammatory response (Figure 3). However, the levels of IL-6 and IL-1β in SASP group significantly reduced (P<0.05, Figure 3). Secondly, the expression of IL-6 mRNA of colon tissue in UC model group was more than 10 times higher than that in sham group, and the expression of IL-1β mRNA increased by 4 times. The inflammation expression of IL-6 and IL-1β in colon tis-
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SASP could against the thymic atrophy and splenomegaly caused by UC and have an unparalleled superiority in the treatment of UC. However, its therapeutic effects in patients still lack adequate supporting clinical cases and need to be studied further.

In conclusion, this study found that SASP could relieve the inflammatory reactions in serum and intestinal tract of rats with UC, so as to protect the intestinal tract and reduce the adverse effects of UC, which are of positive clinical significance and practical value.

Disclosure of conflict of interest

None.

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References


Figure 3. Inflammation levels of serum and inflammatory reactions of the colon tissue of rats in each group (*P<0.05; **P>0.05). A: The expression levels of IL-6 in serum of rats in three groups; B: The expression levels of IL-1β in serum of rats in three groups; C: The expression levels of IL-6 mRNA in colon tissue of rats in three groups; D: The expression levels of IL-1β mRNA in colon tissue of rats in three groups.

Sue decreased after SASP treatment (P<0.05), which indicated that SASP could obviously reduce the intestinal inflammation of UC and systemic inflammatory reactions (Figure 3). This effect was very accurate.

On the other hand, it was very clear to see that there were colonic structure injuries, uneven glands and a large number of inflammatory cell infiltration of rats in UC model group according to HE staining results, while the intestinal mucosal lesion was lighter and the infiltration of inflammatory cell significantly alleviated in SASP group (Figure 2), and the following colon indexes and the ulcer proportion of colon also decreased (P<0.05, Table 2). The body weight and loose stools of three groups also reflect that rats with UC had frequent loose stools and apparent weight losses, while the rats in SASP group had slighter weight losses and its diarrhea was also relieved. All these findings suggested that SASP had clear therapeutic effects on the rats with UC, including the improvement of symptoms and the relief of intestinal inflammation. Meanwhile, changes of thymus index and spleen index implied that...
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