Effects of curcumin plus Soy oligosaccharides on intestinal flora of rats with ulcerative colitis

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Abstract: To explore the therapeutic effect of curcumin (Cur) and soybean oligosaccharides (SBOS) on ulcerative colitis (UC) through testing the intestinal flora and ulcerative colitis (UC). 80 male SD rats were selected divided into four groups with 20 rats in each group: normal group, sulfasalazine (SASP) group, model group and group of curcumin plus soy oligosaccharide. All animals were treated for 4 weeks. In the fifth week rats were decapitated. Macroscopic damage scores of colonic mucosa were calculated. A 4ml blood sample was taken to detect the contents of serum tumor necrosis factor -α (TNF-α) and interleukin 8 (IL-8) by the double antibody sandwich ABC-ELISA method (enzyme-linked immunosorbent assay). Colonic tissues with the most obvious lesions were obtained using a surgical scissor. A routine hematoxylin-eosin (HE) staining method was used to stain pathological specimens and images of staining results were obtained. Histological injury scores of colonic mucosa were calculated. Ulcerative colitis model rats had the highest macroscopic damage scores and histological injury scores of colonic mucosa. After treatment the contents of TNF-α and IL-8 decreased significantly in the group of curcumin plus soy oligosaccharide compared with the model group with statistical significance (P<0.01) while the contents were close to those in the SASP group. There was no statistical significance (P> 0.05). The treatment could decrease TNF-α and IL-8 expression and reduce colonic mucosa inflammation and tissue damage.

Key words: Curcumin; Soybean oligosaccharides (SBOS); Intestinal flora; Ulcerative colitis.

Introduction

Now an increasing number of studies suggest that the intestinal flora is an important motivating factor in immune injury process of UC. The immune injury process manifests itself in the proliferation of pathogenic bacteria and lack of probiotics. The tolerance of immune system of intestinal mucosa to intestinal flora is broken. As a result, excessive and persistent inflammation and gut barrier dysfunction occur, the permeability of gut barrier increases, and antigens, endotoxins, inflammatory cytokines and other pro-inflammatory substances in the intestine lumen enter intestinal lamina propria, inducing an immune response (1).

We detected the relationship between Bifidobacterium, Lactobacillus, enterococci and E.coli, four representatives of the intestinal flora and UC in order to explore the therapeutic effects of curcumin and soybean oligosaccharides (SBOS) on UC.

Materials and Methods

Eighty Sprague-Dawley rats (SD rats, clean grade), weighing 180-200g, were randomly divided into four groups with 20 rats in each group: normal group, sulfasalazine (SASP) group, model group and group of curcumin plus soy oligosaccharide. 2, 4-dinitro-chlorobenzene (2, 4-dinitro-chlorobenzene (DNCB) was used to establish rat models of ulcerative colitis (8). Afterwards, the rats were regularly fed. Typical lesions appeared 1-2 weeks later.

Drug treatment

After the successful modeling, rats in the treatment...
group were given intragastric infusion of curcumin (Cur) 100mg / kg.d-1, soy oligosaccharides (SBOS) 3.33ml / (kg.bw) body weight once a day. Rats in the control group were given intragastric infusion of sulfasalazine 180mg / kg • d-1 body weight once a day. Rats in the normal group and model group were given intragastric infusion of 1% sodium carboxymethyl cellulose solution once a day. All rats were treated for 4 weeks.

Specimen collection and index detection

All rats were treated for 4 weeks. After treatment, all rats were decapitated by cervical dislocation. 4ml blood samples of femoral vein were collected. Colonic tissues 8cm proximal to the anus were obtained using a surgical scissor to use for measuring the indicators. The length of gastric mucosal damage area≤0.5mm was scored 1 point; ≤1mm 2 points; ≤2mm 3 points and so on. When the width of damage area was no less than 2mm, scores were doubled. The expressions of TNF-α and IL-8 in serum were detected by ELISA method.

Quantitative PCR analysis of fecal flora

Fluorescence quantitative PCR method was used to quantitatively analyze the contents of E. coli, enterococci, Bifidobacterium, Lactobacillus in the feces of rats before and after treatment. Intestinal flora differences among those groups were compared. After referring to studies of Rinttila etc. (11-13), we designed specific PCR primers according to 16SrDNA sequences of different strains. Primer sequences came from Genbank. Compatibility of primers was verified by the Primer software and primers which could lead to mismatching were excluded. Using 16SrDNA sequences of different strains as templates, the primers were designed as follows: Primer sequence (5'-3 '): Bifidobacterium (442) (Upstream: 5'-GGGGTTGGAATGCGG-GATG-3'; Downstream: 5'-TAAGCGATGGACCTT-TCACACC-3'), Lactobacillus (341) (Upstream: 5'-AG-CAGTAGGGAATCTTCCA-3'; Downstream: 5'-CAC-GCTACACATCGAG-3'), E. coli (340) (Upstream: 5'-GTTAATACCTTGGCTCATGA-3'; Downstream: 5'-ACCCAGGTATCTTAAATCTTGT-3'), Enterococcus (144) (Upstream: 5'-CCCTTATGGATGGC-CCAATTTGA-3'; Downstream: 5'-ACTGTTG-TACTTCCCATTGT-3').

Statistical analysis

SPSS10.0 software package was used for statistical data analysis. Measurement data was expressed as mean ± standard deviation (± SD). Data before and after treatment were compared using paired t test. After homogeneity of variance analysis, the comparison between groups was tested by paired-samples t test. Cure rats were compared using x2 test. The bivariate two-tailed test was used for correlation analysis. The Pearson coefficient was calculated.

Results

Pathological specimens

Colonic biopsy specimens were stained with HE. Histological injury scores of colonic mucosa were calculated according to standards cited by Dieleman etc. (10). The situations were listed as follows: Colonic tissues in the rats from the normal group had a regular structure, and glandular organs were arranged in order with a small amount of inflammatory cell infiltration. Colon mucosa of rats from the model group was severely damaged, part of which had a defect. Mucosal hyperemia and edema in the colon tissues occurred. Infiltration of a large number of neutrophils, lymphocytes and plasma cells could be seen in the epithelium mucosa. Neutrophil infiltration accounted for the largest proportion. Disorders and deformations occurred in the intramucosal glandular organs. Pathological changes in rats from the group of curcumin plus soy oligosaccharide and SASP group were reduced. Mucosal integrity and the healing of ulcers could be seen. The newborn epithelial cells covered the ulcer lesions from the surface. Inflammatory cell infiltration decreased significantly compared with the model group. The group of curcumin plus soy oligosaccharide had the best healing results. (Figure 1).

Macroscopic damage scores of colonic mucosa in each group

The morphology of the colonic tissues in the normal group was normal, mucous membrane congestion did not occur, and neither of edema and ulceration occurred. After the successful modeling in the model group, the colon which was 8 cm proximal to the anus was cut open. Mucosal congestion, edema, erosion and ulceration occurred. The ulcer area was large, its boundaries were clear, inflammation was significant, and some intestinal walls were thickening. Parts of rat colons adhered to the surrounding tissue. After 4 weeks of treatment, pathological changes in rats from the group of curcumin plus soy oligosaccharide and SASP group were significantly lighter than those in the model group. Mild inflammation and congestion occurred, with no clear erosion or ulceration seen. Scoring results are shown (Table 1): Mucosal epithelial in the rats from the normal group showed no erosion and no ulcers. Macroscopic damage scores were not obtained. Ulcer formation was seen in the rats from the group of curcumin plus soy oligosaccharide and SASP group. Ulcerative colitis model rats had the highest macroscopic damage scores.
Table 1. Macroscopic damage scores and histological injury scores of colonic mucosa in each group ($\overline{x}$ ± s, n=20).

<table>
<thead>
<tr>
<th>Group</th>
<th>Macroscopic damage scores</th>
<th>Histological injury scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>6.48 ± 1.16</td>
<td>11.59 ± 1.2</td>
</tr>
<tr>
<td>Model group (n=20)</td>
<td>2.43 ± 1.12$^{a}a$</td>
<td>5.27 ± 1.18$^{a}a$</td>
</tr>
<tr>
<td>SASP group (n=20)</td>
<td>1.86 ± 1.16$^{a}a$</td>
<td>5.19 ± 1.08$^{a}a$</td>
</tr>
<tr>
<td>Cur+SBOS group (n=20)</td>
<td>36.8 ± 2.34$^{b}a$</td>
<td>35.83 ± 9.15$^{b}a$</td>
</tr>
</tbody>
</table>

Note: $^a$ P <0.05, $^{a}a$ P <0.01 as compared with model group.

After 4 weeks of treatment, macroscopic damage scores and histological injury scores of colonic mucosa in the group of curcumin plus soy oligosaccharide and SASP group decreased significantly. There was a statistical significance, compared with the SASP group (P<0.01). The therapeutic effects of the group of curcumin plus soy oligosaccharide and SASP group were close (P>0.05). There was no statistical significance in this group, compared with the SASP group. The above results suggest that curcumin plus soy oligosaccharides can treat ulcerative colitis effectively and its therapeutic effects are equal to those of SASP (Figure 2).

Changes of IL-1β, IL-4 and IL-6 levels in serum

The contents of IL-1β in serum were detected by the double antibody sandwich ABC-ELISA method (Table 2): IL-1β levels in the model group were highest while those in the normal group were lowest. IL-1β levels in the model group, the SASP group and the group of curcumin plus soy oligosaccharide increased compared with the normal group. Those comparisons had statistical significance (P<0.01). After treatment, IL-1β levels in the group of curcumin plus soy oligosaccharide decreased significantly compared with the model group. There was statistical significance (P<0.01). The IL-4 levels in the group of curcumin plus soy oligosaccharide were close to those in the SASP group. There was no statistical significance (P>0.05).

The contents of IL-4 in serum were detected by the double antibody sandwich ABC-ELISA method (see Table 2): IL-4 levels in the model group were lowest while those in the normal group were highest. IL-4 levels in the model group, the SASP group and the group of curcumin plus soy oligosaccharide decreased significantly compared with the normal group. Those comparisons had statistical significance (model group and SASP group P <0.01, the group of curcumin plus soy oligosaccharide P <0.05). After treatment, IL-4 levels in the group of curcumin plus soy oligosaccharide decreased significantly compared with the model group. There was statistical significance (P<0.01). The IL-4 levels in the group of curcumin plus soy oligosaccharide were close to those in the SASP group. There was no statistical significance (P>0.05).

In summary, curcumin plus soy oligosaccharides can decrease IL-1β and IL-6 levels (proinflammatory cytokines) in serum significantly, and increase anti-inflammatory cytokines IL-4 levels. Therapy effects of curcumin plus soy oligosaccharides are equal to those of SASP.

Changes of NF-κB expression in colonic mucosa of rats after treatment

NF-κB expressions in colonic tissues were detected using immunohistochemical methods. Under a high magnification microscope which has a total magnification of 10χ40, we observed that a small amount of brown NF-κB expression in the cytoplasm of epithelial cells in colonic mucosa of rats from the normal group. Its staining was pale (Fig.3A). In contrast, we observed that a large amount of NF-κB expression in the cytoplasm and nucleus of epithelial cells in colonic mucosa of rats from the model group. Dark brown particles and of curcumin plus soy oligosaccharide increased compared with the normal group. Those comparisons had statistical significance (model group P <0.01, the group of curcumin plus soy oligosaccharide and SASP group P <0.05). After treatment, IL-6 levels in the group of curcumin plus soy oligosaccharide decreased significantly compared with the model group. There was statistical significance (P<0.01). The IL-6 levels in the group of curcumin plus soy oligosaccharide were close to those in the SASP group. There was no statistical significance (P>0.05).

The contents of IL-6 in serum were detected by the double antibody sandwich ABC-ELISA method (Table 2): IL-6 levels in the model group were highest while those in the normal group were lowest. IL-6 levels in the model group, the SASP group and the group of curcumin plus soy oligosaccharide were close to those in the SASP group. Those comparisons had statistical significance (P<0.01). The IL-6 levels in the group of curcumin plus soy oligosaccharide decreased significantly compared with the model group. There was statistical significance (P<0.01). The IL-4 levels in the group of curcumin plus soy oligosaccharide were close to those in the SASP group. There was no statistical significance (P>0.05).

In summary, curcumin plus soy oligosaccharides can decrease IL-1β and IL-6 levels (proinflammatory cytokines) in serum significantly, and increase anti-inflammatory cytokines IL-4 levels. Therapy effects of curcumin plus soy oligosaccharides are equal to those of SASP.

Table 2. Changes of IL-1β, IL-4 and IL-6 levels in serum ( ± s, n = 20, pg / ml).

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group (n=20)</td>
<td>25.66 ± 2.87$^{aa}$</td>
<td>30.3 ± 7.69$^{aa}$</td>
<td>94.67 ± 8.48$^{aa}$</td>
</tr>
<tr>
<td>Model group (n=20)</td>
<td>68.63 ± 2.62$^{ab}$</td>
<td>77.67 ± 6.48$^{ab}$</td>
<td>48.37 ± 7.86$^{ab}$</td>
</tr>
<tr>
<td>SASP group (n=20)</td>
<td>37.69 ± 1.73$^{bb}$</td>
<td>36.79 ± 7.98$^{ab}$</td>
<td>84.82 ± 6.28$^{bb}$</td>
</tr>
<tr>
<td>Cur+SBOS group (n=20)</td>
<td>36.8 ± 2.34$^{b}a$</td>
<td>35.83 ± 9.15$^{ab}$</td>
<td>89.38 ± 7.54$^{ab}$</td>
</tr>
</tbody>
</table>

Note: $^a$ P <0.05, $^{a}a$ P <0.01 as compared with model group; $^b$ P <0.05, $^{b}a$ P <0.01 as compared with normal group.
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Deep staining can be seen (Figure 1C-D). The results are shown (Figure 3D): The cytoplasm of epithelial cells in colonic mucosa of rats from the normal group had a small amount of NF-κB expression with a relatively high average gray value; NF-κB expression in colonic tissue of rats in the model group was upregulated with a reduced average gray value; there was a significant difference compared with the normal group (P <0.01); After treatment, compared with the model group (Figure 3B), NF-κBp65 expression in colonic tissue of rats in the group of curcumin plus soy oligosaccharide was downregulated; there was a statistical significance (P<0.01); NF-κBp65 expression in the group of curcumin plus soy oligosaccharide was close to that in the SASP group; there was no statistical significance (P>0.05). In summary, curcumin plus soy oligosaccharides can reduce the expression of NF-κB colonic tissue in model rats. Therapy effects of curcumin plus soy oligosaccharides are equal to those of SASP (Table 3).

Changes of intestinal flora in each group before and after treatment

Detection results of the specificity of primers

PCR products of standard strains were analyzed by 2% agarose gel electrophoresis and the specificity of primers was tested. The results were shown as follows: Bifidobacterium had a specific product band at 442bp while other bacteria showed no specific amplification in this region (Figure 4A); Lactobacillus had a specific product band at 341bp while other bacteria showed no specific amplification in this region (Figure 4B); E. coli and enterococci had a specific product band at 340bp and 144bp, respectively (Figure 4C, 4D). The results showed that primers of each bacterium had a good specificity and that they could be used for real-time PCR.

Standard curves

The abscissa is the copy numbers of each standard while the vertical axis is the initial real-time PCR cycle numbers at which fluorescence threshold was reached. Standard curves were obtained, providing a reference standard for the quantitative analysis of samples (Figure 5).

Quantitative test results of fecal bacteria before and after treatment

The copy number of bacteria in each fecal sample was obtained by a CT value and the standard curve. Before treatment, the contents of E. coli and enterococci in the feces of rats from the group of curcumin plus soy oligosaccharide, the SASP group and the model group increased significantly, compared with the normal group while the contents of Bifidobacterium and Lactobacillus were significantly less than those of the normal group. The differences were statistically significant (P <0.01) (Table 4). After treatment (Table 5, 6) : The contents of

Table 3. Average gray value changes in NF-κB expression (± s, n = 20).

<table>
<thead>
<tr>
<th>Group</th>
<th>Average gray value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group (n=20)</td>
<td>140.66 ± 5.93 **</td>
</tr>
<tr>
<td>Model group (n=20)</td>
<td>64.94 ± 6.86 bb</td>
</tr>
<tr>
<td>SASP group (n=20)</td>
<td>131.15 ± 21.77 aab</td>
</tr>
<tr>
<td>Cur+SBOS group (n=20)</td>
<td>130.66 ± 13.46 aab</td>
</tr>
</tbody>
</table>

Note: * P <0.05, ** P <0.01 as compared with model group; * P <0.05, bb P <0.01 as compared with normal group.

![Figure 3. NF-KB expression in colonic mucosa of rats. A: Normal group (× 400): a small amount of NF-κB expression and a pale staining; B: Group of curcumin plus soy oligosaccharides illustrates the cytoplasmic staining, a small amount of stained nuclei and brownish yellow particles; C: SASP group (× 400): It illustrates the cytoplasmic staining, a small amount of stained nuclei and brownish yellow particles; D: Model group (× 400): It illustrates the cytoplasm and nucleus staining - a large amount and deep staining.]

![Figure 4. A: Bifidobacterium primer; B: Verification of the Lactobacillus primer; C: E. coli primer; D: enterococci primer.]

![Figure 5. A: The E. coli standard curve; B: The enterococci standard curve; C: The Bifidobacterium standard curve; D: The Lactobacillus standard curve.]

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E. coli and enterococci in the feces of rats from the group of curcumin plus soy oligosaccharide decreased significantly compared with those before treatment. There was a statistical difference (P <0.05). The contents of Bifidobacterium and Lactobacillus increased significantly compared with those before treatment. The differences were statistically significant (P <0.01). The contents of E. coli and enterococci in the feces of rats from the group of curcumin plus soy oligosaccharide decreased compared with the model group and SASP group. There was statistical significance (P <0.05). The contents of Bifidobacterium and Lactobacillus increased significantly compared with those before treatment. There was statistical significance (P <0.05). The contents of Bifidobacterium and Lactobacillus were close to those in the SASP group. There was no statistical significance (P >0.05). The contents of enterococci in the normal group those in the group of curcumin plus soy oligosaccharide were close to those in the SASP group. There was no statistical significance (P >0.05). Therefore, curcumin plus soy oligosaccharides can significantly decrease the contents of E. coli and enterococci in the feces of rats and meanwhile, increase the contents of Bifidobacterium and Lactobacillus (friendly intestinal bacteria). They can regulate intestinal flora effectively, making rats’ intestinal environment close to that of normal rats.

Changes of TNF-α and IL-8 contents in serum before and after treatment

The contents of TNF-α and IL-8 in serum were detected by the double antibody sandwich ABC-ELISA method (Table 7): The contents of TNF-α and IL-8 in serum of rats from the model groups were highest while those of rats from the normal groups were lowest. The contents of TNF-α and IL-8 in serum of rats from the model group decreased significantly compared with the normal group. Those comparisons had statistical significance (P <0.01). After treatment, the contents of TNF-α and IL-8 in the group of curcumin plus soy oligosaccharide decreased significantly compared with the normal group. There was statistical significance (P <0.01). The contents of TNF-α and IL-8 in the group of curcumin plus soy oligosaccharide were close to those in the SASP group. There was no statistical significance (P >0.05). In summary, curcumin plus soy oligosaccharides can decrease TNF-α and IL-8 expression significantly, whose therapy effects are equal to those of SASP.

Discussion

Traditional therapy for patients with UC gives first place to Western medicine, assisted by Chinese herbal medicine. Western medicine therapy usually uses amino
show that the contents of E. coli and enterococci in the pathogenesis of UC was explored. The results (SASP), the role of curcumin plus soy oligosaccharides in the serum. Through the comparison with sulfasalazine treatment of ulcerative colitis. The quantitative analyses on E. coli, enterococci, Bifidobacterium, Lactobacillus in the feces of rats. Meanwhile, the double analyses on E. coli, enterococci, Bifidobacterium, Lactobacillus (4-5). On one hand, as a probiotic bacterium in the intestine, Bifidobacterium can adhere to the intestinal epithelial cells, forming a biofilm barrier which has a protective effect and prevent the invasion of pathogenic bacteria. On the other hand, in the metabolic process Bifidobacterium is able to produce a large amount of formic acid and acetic acid, reducing the pH values in the intestinal environment and inhibiting the growth of harmful bacteria (6). Therefore, we used curcumin plus soy oligosaccharide in the experimental study of the treatment of ulcerative colitis. The quantitative real-time PCR method was use to carry out quantitative analyses on E. coli, enterococci, Bifidobacterium, Lactobacillus in the feces of rats. Meanwhile, the double antibody sandwich ABC-ELISA method was used to detect TNF-α and interleukin 8 (IL-8) expressions in serum. Through the comparison with sulfasalazine (SASP), the role of curcumin plus soy oligosaccharides in the pathogenesis of UC was explored. The results show that the contents of E. coli and enterococci in the feces of rats from the UC model group were higher than those of the normal group while Bifidobacterium and Lactobacillus reduced significantly, which indicates that there existed an intestinal flora imbalance in the model group and that the situation was greatly different from that in the normal group. The lack of Bifidobacterium and Lactobacillus weakens the role of mucosal defense against a variety of pathogenic factors. The proliferation of E. coli and enterococci leads to an increase in internal and external release of toxins, increasing local UC intestinal inflammation and immune disorders. The results illustrate that intestinal bacteria play an important role in the pathogenesis of UC. After treatment, the contents of TNF-α and IL-8 decreased significantly in the group of curcumin plus soy oligosaccharide compared with the model group with statistical significance (P <0.01) while the contents were close to those in the SASP group. There was no statistical significance (P>0.05). One of the mechanisms of how curcumin plus soy oligosaccharides resist against ulcer colitis may be associated with their promotion of friendly intestinal bacteria, especially Bifidobacterium, their reduction of the growth of pathogenic bacteria and the fact that curcumin plus soy oligosaccharides, meanwhile, could decrease TNF-α and IL-8 expression and reduce colonic mucosa inflammation and tissue damage.

References