EXPERIMENTAL STUDY

Effect of Jiaweiwumei decoction on regulatory T cells and interleukin-10 in a rat model of ulcerative colitis


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Abstract

OBJECTIVE: To investigate the effect of a decoction made from the Traditional Chinese Medicine Wumei pill, on regulatory T cells and interleukin-10 (IL-10) in a rat model of ulcerative colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS).

METHODS: Rat ulcerative colitis was induced with TNBS. All modeled rats were randomly divided into six groups: normal control group; model group; sulfasalazine suppositories treatment group; and high, moderate, and low dosage of Jiaweiwumei decoction groups (12 rats each). Colon injury index was evaluated after 14 days. After peripheral blood lymphocyte separation, CD4+ T cells and CD4+/CD25+ T cell percentage was detected by flow cytometry. The content of IL-10 in serum and intestinal mucosa tissue was detected by sandwich enzyme-linked immunosorbent assay.

RESULTS: Colon injury indices in the decoction groups were effectively reduced, compared with the model group (P < 0.05). Compared with that of the control group, the CD4+/CD25+ to CD4+ T lymphocyte ratio of the model group was significantly lower, while the decoction treatment improved the CD4+/CD25+ to CD4+ T lymphocyte ratio (P < 0.05). The serum and mucosal IL-10 content of the model group was significantly lower (P < 0.05) than that in the control group, while the decoction group had significantly higher serum and intestinal mucosal IL-10 content than that in the model group (P < 0.05). The regulatory T cell content was negatively correlated with the colonic injury index (r = 0.68, P < 0.05), and positively correlated with the content of serum IL-10 (r = 0.87, P < 0.05) and intestinal mucosal IL-10 (r = 0.79, P < 0.05).

CONCLUSION: Jiaweiwumei decoction had significant effects on regulatory T cells and IL-10 in rats with TNBS-induced ulcerative colitis.

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Key words: Colitis, ulcerative; T-lymphocytes, regulatory; Interleukin-10; Jiaweiwumei decoction

INTRODUCTION

Ulcerative colitis, a refractory chronic inflammatory bowel disease, has main clinical symptoms of abdominal pain, diarrhea, mucopurulent bloody stool, and tenesmus. Ulcerative colitis is characterized by a slow onset, prolonged course, and recurrence. Decades of research have shown that ulcerative colitis incidence has increased gradually since World War II in China. As ulcerative colitis commonly relapses and is intractable, meaning a poor quality of life for patients, it is necessary to find effective treatments. In recent years, special
attention has been paid to the content of CD4+ /CD25+ regulatory T cells (Tregs) and interleukin-10 (IL-10) in the pathogenesis of ulcerative colitis.\(^1\) Wu-mei pill in Traditional Chinese Medicine (TCM) is prescribed for the treatment of typhoid ascariasis of the biliary tract and dysentery in "Shanghan-Lun", which is a famous clinical textbook of TCM known in English as the Treatise on Cold Damage Disorders. However, Wumei pill’s treatment and immunologic mechanisms on ulcerative colitis are still not clear. This study aimed to investigate the effect of Jiaweiwumei decoction on this disease in terms of regulatory T cells and interleukin-10.

**MATERIALS AND METHODS**

**Materials and reagents**

Adult male Sprague Dawley rats with an initial body weight of 225-250 g were purchased from the laboratory animal center, The Fourth Military Medical University (license No. SCXK [Army] 2007-007, Xi’an, China). Rats were maintained in accordance with the guidelines for the care and use of laboratory animals in specific pathogen free conditions.\(^4\) Sulfasalazine suppositories (SASP, 250 mg/tablet) was purchased from Shanghai Sanwei pharmaceutical company (Authorized document number: H31020450, Shanghai, China). Fluorescein isothiocyanate (FITC) labeled CD4+, CD25+ antibody and IL-10 Enzyme-linked immuno sorbent assay (ELISA) Kit were purchased from Becton, Dickinson, Inc., (San Diego, CA, USA). The traditional Chinese herbal medicine was composed of: Wu-mei (Fructus Mume) 16 g, Xixin (Herba Asari Mandshurica) 6 g, Ganjiang (Rhizoma Zingiberis) 10 g, Huanglian (Rhizoma Coptidis) 16 g, Xixin (Radix Angelicae Sinensis) 4 g, Guizhi (Fructus Cinnamomi) 6 g, Renshen (Radix Ginseng) 6 g, Huajiao (Pericarpium Zanthoxyli Bungean) 4 g, Wu-mei pill’s treatment and immunologic mechanisms on ulcerative colitis are still not clear. This study aimed to investigate the effect of Jiaweiwumei decoction on this disease in terms of regulatory T cells and interleukin-10.

**Colon tissue score**

After day 14 of treatment, all rats were anesthetized with chloral hydrate. The mesentery was longitudinally opened, and intestinal contents were washed out with cold saline. The colon was flattened. Colon mucosa damage, e.g. congestion, edema, erosion, ulcers, pseudo membrane extent, and area of pathological changes were observed and recorded. The colon tissue morphology score was made according to the reference in Table 1, and the colon tissue was scored according to the standards of grading.\(^7\)

**Detection of Peripheral blood mononuclear cells**

According to the literature,\(^4\) a peripheral blood mononuclear cell (PBMC) suspension was isolated and prepared from rat peripheral blood. FITC labeled antibodies (Becton Dickinson Inc.), 20 μL, were added into 100 μL PBMC suspension, and then mixed before a dark incubation at room temperature for 20 min. Sam-

<table>
<thead>
<tr>
<th>Score</th>
<th>Colonic mucosa tissue characteristics observed by naked eye</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>No damage</td>
</tr>
<tr>
<td>1</td>
<td>Mucosal hyperemia, edema, no ulcer</td>
</tr>
<tr>
<td>2</td>
<td>Mucosal hyperemia, edema, mild erosion, no ulcer</td>
</tr>
<tr>
<td>3</td>
<td>Mucosal hyperemia, edema, moderate erosion, single ulcer</td>
</tr>
<tr>
<td>4</td>
<td>Mucosal hyperemia, edema, moderate erosion, more than one ulcer</td>
</tr>
<tr>
<td>5</td>
<td>Mucosal hyperemia, edema, severe erosion, ulcer size &gt;1 cm</td>
</tr>
</tbody>
</table>
samples were then centrifuged at 980 x g for 5 min. The supernatant was removed, and PBS was used to re-suspend cells. Flow cytometry was used to detect the percentages of CD4+ and CD4+/CD25+ T cells.

**IL-10 detection**

Serum preparation: Rats were anesthetized with 1.5% sodium pentobarbital (2 mL/100 g) via intraperitoneal injection. Blood was obtained via abdominal aorta. Blood was allowed to clot for 20 min, and then centrifuged 1600 x g for 15 min. Serum was separated and kept at -20 °C. Tissue homogenate preparation: 6-8 cm of colon from 2 cm upward was cut removed along the longitudinal axis of the mesentery lumen. Lesions (pathological tissues) 0.1 g were obtained and cut into pieces. Then, 500 μL of 0.9% sodium chloride solution was added to prepare 20% homogenate, centrifuged to get the supernatant, and kept at -20 °C. IL-10 was detected by double antibody sandwich ELISA (BD Inc., San Diego, CA, USA), in strict accordance with the kit instructions.

**Statistical analysis**

SPSS 13.0 (version 13, Chicago, IL, USA) software was used to process data, which were represented by mean ± standard deviation (x ± s). One-way analysis of variance was performed and Student-Newman-Keuls test was used to compare differences between two groups. Correlation analysis of regulatory T cell quantity and each index were analyzed using Spearman analysis. P ≤ 0.05 was considered significant.

**RESULTS**

**Comparison of colon injury index**

Colon injury index analysis showed statistically significant difference between the model group and normal control group (P < 0.05). There were significant differences between each treatment group and model group (P < 0.05). There was no statistically significant difference between each treatment group and the SASP treatment group (P > 0.05), as seen in Table 2. These results suggest that Jiaweiwumee decoction effectively reduced colon injury from ulcerative colitis in rats induced by TNBS, with equivalent efficacy to SASP.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Score</th>
<th>CD4+ T lymphocyte percentage (%)</th>
<th>Ratio of CD4+/CD25+ to CD4+ T</th>
<th>Serum IL-10 (ng/mL)</th>
<th>Intestinal mucosa IL-10 (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>12</td>
<td>0</td>
<td>35.87±6.89</td>
<td>10.70±1.64</td>
<td>35.78±7.09</td>
<td>69.1±8.9</td>
</tr>
<tr>
<td>Model</td>
<td>12</td>
<td>4.4±0.5</td>
<td>32.91±7.39</td>
<td>4.59±1.28</td>
<td>18.51±3.81</td>
<td>33.6±6.1</td>
</tr>
<tr>
<td>SASP</td>
<td>12</td>
<td>1.3±0.6</td>
<td>34.56±6.29</td>
<td>7.48±0.54</td>
<td>23.92±5.24</td>
<td>51.1±8.2</td>
</tr>
<tr>
<td>Low dose</td>
<td>12</td>
<td>1.6±0.2</td>
<td>35.21±7.23</td>
<td>7.01±0.62</td>
<td>25.32±6.41</td>
<td>42.1±9.1</td>
</tr>
<tr>
<td>Moderate dose</td>
<td>12</td>
<td>1.3±0.5</td>
<td>34.81±6.21</td>
<td>7.89±0.34</td>
<td>33.57±5.32</td>
<td>51.1±8.2</td>
</tr>
<tr>
<td>High dose</td>
<td>12</td>
<td>1.3±0.3</td>
<td>34.35±6.28</td>
<td>8.56±0.28</td>
<td>31.24±6.33</td>
<td>62.3±10.9</td>
</tr>
</tbody>
</table>

Notes: the normal control group received distilled water 2 mL orally, once daily. High, moderate, and low dose groups received 1.428, 0.71, and 0.357 g/mL decoction, respectively, 2 mL orally, once daily. The SASP group received SASP suspension (0.035 g/mL), 2 mL orally, once daily. All rats were treated for 14 days. CD: cluster of differentiation; IL: interleukin; SASP: sulfasalazine suppositories. Compared with the model group, *P < 0.05; compared with the Normal control group, †P < 0.05; compared with the high dose group, ‡P < 0.05.
DISCUSSION

The results of this study indicate that the percentage of CD4+/CD25+ T regulatory lymphocytes and the level of IL-10 in the peripheral blood of ulcerative colitis rats was lower than that of the normal rats, which is consistent with previous clinical and animal experimental results. The results of this study also indicate that the TCM Jiaweiwumei decoction at high, middle, and low doses and SASP all decreased the colon index to some extent. This result indicates that the decoction treatment could effectively reduce the colon injury induced by TNBS via increased IL-10 in serum and intestinal mucosa and an increased percentage of CD4+/CD25+ T regulatory cells in peripheral blood. In addition, correlation analysis of the experimental results shows that the percentage of CD4+/CD25+ T regulatory cells in the peripheral blood was negatively correlated with the scores of colon injury index, and was positively correlated to IL-10. Meanwhile, our results suggest that determination of CD4+/CD25+ T regulatory cells in the peripheral blood could reflect the severity of ulcerative colitis, and could be regarded as an indicator of efficacy evaluation. In TCM, the Wumei pill formula is prescribed for the treatment of typhoid ascariasis of the biliary tract and dysentery in Shang Han Lun. Clinical research has confirmed its curative effect in treatment of chronic ulcerative colitis. Our results indicate that the therapeutic effect of the decoction on experimental ulcerative colitis was significant, and its mechanism was potentially related to a reduction in the intestinal mucosal immune response by up regulating the expression of IL-10 and T regulatory cells, which plays a role in mucosal repair.

REFERENCES