Effect of acupuncture intervention on the intestinal mucosal inflammatory response and immune response balance in animals with ulcerative colitis

Meng-Fan Yang, Li-Qin Jiang

1. Introduction

Ulcerative colitis (UC) is a clinical common chronic inflammatory bowel disease, and immune response disorder and abnormal inflammatory responses are thought to be closely related to the occurrence and development of the disease[1,2], but the exact pathogenesis is not clear. Patients with UC are mainly characterized by recurrent attack, protracted abdominal pain, bloody purulent stool, tenesmus and so on, and the clinical treatment is quite difficult. In recent years, Chinese medicine treatment has been increasingly used in the treatment of gastrointestinal diseases, and acupuncture is considered to have certain regulation effect on the immune response, inflammation and other pathological physiological processes. The Zhongwan, Tianshu, Zusanli and so on are the acupuncture points closely related to deficiency of spleen and kidney as well as gastrointestinal dysfunction, and acupuncture stimulation in above acupuncture points can be used to regulate the viscera functions[3]. In the following study, the effect of acupuncture intervention on the intestinal mucosal inflammatory response and immune response balance in animals with UC was specifically analyzed.
2. Experimental animals, materials and methods

2.1 Experimental animals

The adult, male and SPF SD rats were selected as the experimental animals in this study and were purchased at the experimental animal center of Jiangsu University. A total of 36 rats with the body mass 180-220 g and 6-8 weeks of age were raised in the environment of 22-24 ℃ and humidity 50%-60%, and they were free to eat and drink. Animal experiments passed the ethical review of the hospital, and procedures were followed for animal experiments and the treatment of animals after death.

2.2 Experimental materials

Complete Freund's adjuvant, paraformaldehyde fixer were bought in the Sigma Company, acupoint stimulator was bought in Beijing Huawei Industrial Development Company, the RNA extraction kit, cDNA first-strand synthesis kit and fluorescence quantitative PCR kit were purchased from Dalian Takara Bio, and Elisa kit was bought in Shanghai Westang Company.

2.3 UC model building methods

The rats were randomly divided into control group, UC group and acupuncture group, 12 in each group. UC group and acupuncture group were made into UC models according to the following method: fresh colonic mucosa tissue was taken and made into tissue homogenate, it was frozen overnight and then centrifuged at 4 000 r/min for 30 min, the supernatant liquid was taken, used for protein quantification and then placed at -20 ℃ for spare use; cryopreserved spare homogenate was mixed with complete Freund's adjuvant into antigen emulsifier, 3.5 mg was taken on day 1 for plantar injection, and 7 mg was taken respectively on day 10, 17 and 24 for plantar, inguinal, dorsal and intraperitoneal injection; after that, 3% formalin fluid and 3 ml intestinal mucosa homogenate were used in turn for enema.

2.4 Acupuncture intervention methods

Acupuncture group of rats were intervened by acupoint stimulator, and the method was as follows: Tianshu and Zusanli were selected for acupuncture, the acupuncture frequency was 200 Hz, the intensity was 2 mA, and the acupuncture lasted for 20 min each time and was conducted once every day for continuous 14 d. No acupuncture intervention was performed for the control group and the UC group.

2.5 Index detection methods

14 d after intervention, the three groups of rats were put to death, blood specimen was collected and centrifuged to separate serum, HMGB-1, TNF-α, IL-1β, IFN-γ, IL-4, IL-5, IL-17 and TGF-β1 were detect by enzyme-linked immunosorbent assay kit, rats were put to death and anatomiced in the abdomen to separate diseased colonic mucosa tissue, RNA was extracted and synthesized into cDNA by reverse transcription, fluorescence quantitative PCR reaction was conducted to amplify NF-kB, HMGB-1, TNF-α, IL-1β, IFN-γ, IL-4, IL-5, IL-17 and TGF-β1 respectively and calculate the mRNA expression.

2.6 Statistical methods

SPSS 19.0 software was used to input and process data, data comparison among three groups was by variance analysis \( P<0.05 \) indicated statistical difference in differences.

3. Results

3.1 Inflammatory mediator expression in intestinal mucosa

14 d after intervention, analysis of inflammatory mediators NF-kB, HMGB-1, TNF-α and IL-1β expression in intestinal mucosa among three groups of rats was as follows: NF-kB, HMGB-1, TNF-α and IL-1β mRNA expression in intestinal mucosa of UC group were significantly higher than those of control group, and NF-kB, HMGB-1, TNF-α and IL-1β mRNA expression in intestinal mucosa of acupuncture group were significantly lower than those of UC group. Differences in pair-wise comparison of NF-kB, HMGB-1, TNF-α and IL-1β mRNA expression in intestinal mucosa were statistically significant among three groups of rats (\( P<0.05 \)).

Table 1. Inflammatory mediator mRNA expression in intestinal mucosa of three groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>NF-kB</th>
<th>HMGB-1</th>
<th>TNF-α</th>
<th>IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>12</td>
<td>1.03±0.18</td>
<td>0.97±0.11</td>
<td>1.05±0.13</td>
<td>0.99±0.14</td>
</tr>
<tr>
<td>UC group</td>
<td>12</td>
<td>2.77±0.36</td>
<td>3.18±0.42</td>
<td>3.49±0.57</td>
<td>2.51±0.32</td>
</tr>
<tr>
<td>Acupuncture group</td>
<td>12</td>
<td>1.69±0.23</td>
<td>1.54±0.18</td>
<td>1.75±0.25</td>
<td>1.80±0.24</td>
</tr>
</tbody>
</table>

\*: compared with control group, \( P<0.05 \); \&: compared with UC group, \( P<0.05 \).

3.2 Inflammatory mediator levels in serum

14 d after intervention, analysis of inflammatory mediators HMGB-1 (pg/mL), TNF-α (ng/mL) and IL-1β (ng/mL) levels
in serum among three groups of rats was as follows: HMGB-1, TNF-α and IL-1β levels in serum of UC group were significantly higher than those of control group, and HMGB-1, TNF-α and IL-1β levels in serum of acupuncture group were significantly lower than those of UC group. Differences in pair-wise comparison of HMGB-1, TNF-α and IL-1β levels in serum were statistically significant among three groups of rats (P<0.05).

### Table 2
Inflammatory mediator levels in serum of three groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HMGB-1</th>
<th>TNF-α</th>
<th>IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>12</td>
<td>69.68±9.24</td>
<td>24.21±3.58</td>
<td>12.56±1.88</td>
</tr>
<tr>
<td>UC group</td>
<td>12</td>
<td>261.25±34.27</td>
<td>68.69±8.79</td>
<td>38.38±5.24</td>
</tr>
<tr>
<td>Acupuncture group</td>
<td>12</td>
<td>125.62±15.48</td>
<td>37.41±5.24</td>
<td>19.24±2.46</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; #: compared with UC group, P<0.05.

### 3.3 Th1/Th2/Th17/Treg cytokine expression in intestinal mucosa

14 days after intervention, analysis of Th1/Th2/Th17/Treg cytokines IFN-γ, IL-4, IL-5, IL-17 and TGF-β1 expression in intestinal mucosa among three groups of rats was as follows: IFN-γ and IL-17 mRNA expression in intestinal mucosa of UC group were significantly higher than those of control group while IL-4, IL-5 and TGF-β1 levels in serum were significantly lower than those of control group, and IFN-γ and IL-17 mRNA expression in intestinal mucosa of acupuncture group were significantly lower than those of UC group. Differences in pair-wise comparison of IFN-γ, IL-4, IL-5, IL-17 and TGF-β1 levels in serum were statistically significant among three groups of rats (P<0.05).

### Table 3
Th1/Th2/Th17/Treg cytokine mRNA expression in intestinal mucosa of three groups of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>IFN-γ (ng/mL)</th>
<th>IL-4 (pg/mL)</th>
<th>IL-5 (pg/mL)</th>
<th>IL-17 (pg/mL)</th>
<th>TGF-β1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>12</td>
<td>1.07±0.14</td>
<td>1.03±0.16</td>
<td>0.98±0.11</td>
<td>0.94±0.13</td>
<td>1.04±0.18</td>
</tr>
<tr>
<td>UC group</td>
<td>12</td>
<td>2.25±0.38</td>
<td>0.38±0.06</td>
<td>0.30±0.05</td>
<td>2.79±0.34</td>
<td>0.28±0.05</td>
</tr>
<tr>
<td>Acupuncture group</td>
<td>12</td>
<td>1.52±0.18</td>
<td>0.77±0.11</td>
<td>0.69±0.08</td>
<td>1.52±0.18</td>
<td>0.70±0.07</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; #: compared with UC group, P<0.05.

### 3.4 Th1/Th2/Th17/Treg cytokine levels in serum

14 days after intervention, analysis of Th1/Th2/Th17/Treg cytokines IFN-γ (ng/mL), IL-4 (pg/mL), IL-5 (pg/mL), IL-17 (ng/mL) and TGF-β1 (pg/mL) levels in serum among three groups of rats was as follows: IFN-γ and IL-17 levels in serum of UC group were significantly higher than those of control group while IL-4, IL-5 and TGF-β1 levels were significantly lower than those of control group, and IFN-γ and IL-17 levels in serum of acupuncture group were significantly lower than those of UC group. Differences in pair-wise comparison of IFN-γ, IL-4, IL-5, IL-17 and TGF-β1 levels in serum were statistically significant among three groups of rats (P<0.05).

### 4. Discussion

The abnormal activation of inflammatory response in intestinal mucosa is the most prominent pathological feature of UC, and NF-kB is the most critical transcription factor that regulates inflammatory response in the body. In the progression of UC, NF-kB in intestinal mucosa tissue will be activated and transfer into the nucleus after regulated by upstream different pathological factors, and then it will be combined with the promoter regions of genes that express HMGB-1, TNF-α, IL-1β and other inflammatory mediators and start the gene expression[4-6]. In the study, analysis of expression of the above-mentioned inflammatory mediators in the intestinal mucosal tissue of UC group showed that NF-kB, HMGB-1, TNF-α and IL-1β mRNA expression in intestinal mucosa of UC group were significantly higher than those of control group. This shows that the UC model is successfully made, the inflammatory response in the intestinal mucosa is significantly activated and the expression of corresponding inflammatory mediators increases significantly. On the basis of establishing animal model of UC, Tianshu and Zusanli acupuncture were used for acupuncture intervention, and the analysis of acupuncture intervention effect on inflammatory mediator expression in the intestinal mucosa showed that NF-kB, HMGB-1, TNF-α and IL-1β mRNA expression in intestinal mucosa of
acupuncture group were significantly lower than those of UC group. This indicates that acupuncture intervention has a significant inhibitory effect on the inflammatory response of intestinal mucosa in UC.

After activated and transferring into the nucleus, the NF-kB can start the expression of HMGB-1, TNF-α, IL-1β and other genes, and the expression products have direct regulating effect on the cascade activation of inflammatory response in local tissue. HMGB-1 is a class of advanced inflammatory mediator that promotes the activation of mononuclear macrophages and secretes various pro-inflammatory factors[7]; TNF-α is an inflammatory mediator that changes in the early stage of inflammation, which is secreted by activated mononuclear macrophages, and can mediate the cascade amplification of inflammatory reactions and the recruitment of inflammatory cells[8]; IL-1β is a mediator with pro-inflammatory activity, which can directly mediate the process of inflammatory reaction[9]. In order to further define the inflammatory response change in UC course and the treatment value of acupuncture intervention, the levels of the inflammatory mediators that were released into the blood circulation were analyzed in the study, and the results showed that HMGB-1, TNF-α and IL-1β levels in serum of UC group were significantly higher than those of control group, and HMGB-1, TNF-α and IL-1β levels in serum of acupuncture group were significantly lower than those of UC group. This means that significant activation of inflammation in UC course can cause HMGB-1, TNF-α, IL-1β and a lot of inflammatory mediators to be released into the blood circulation, and acupuncture intervention has significant inhibitory effect on the expression and release of inflammatory mediators in UC course, and has a definite therapeutic value.

The abnormal activation of inflammatory response in UC disease is closely related to the disturbance of immune response. CD4+ T lymphocytes are important cell groups that regulate the immune response in intestinal mucosal tissue, and the balance of Th1/Th2 subgroups determines the equilibrium of the immune response[10]. The IFN-γ and IL-2 secreted by Th1 cells can mediate cellular immune response[11]; the IL-4 and IL-5 secreted by Th2 cells can mediate the humoral immune response[12,13]. In the study, analysis of the Th1/Th2 cytokine expression in intestinal mucosa as well as the Th1/Th2 cytokine levels in serum of UC rat was as follows: IFN-γ mRNA expression in intestinal mucosa and IFN-γ level in serum of UC group were significantly higher than those of control group while IL-4 and IL-5 mRNA expression in intestinal mucosa as well as IL-4 and IL-5 levels in serum were significantly lower than those of control group. This indicates that the Th1/Th2 balance shifting to Th1 is closely related to the progression of UC course. Further analysis of acupuncture intervention effect on Th1/Th2 balance in UC course showed that that IFN-γ mRNA expression in intestinal mucosa and IFN-γ level in serum of acupuncture group were significantly lower than those of UC group while IL-4 and IL-5 mRNA expression in intestinal mucosa as well as IL-4 and IL-5 levels in serum were significantly higher than those of UC group. This means that the acupuncture intervention can correct the disturbance of Th1/Th2 in UC course and promote the Th1/Th2 balance shifting to Th2.

Th17 and Treg are the new CD4+T cell subsets discovered in recent years, and the disorder of Th17/Treg is closely related to the abnormal immune response. Th17 is a kind of cell subset that specifically secretes cytokine IL-17, and the IL-17 secreted by Th-17 promotes the intestinal mucosal inflammation, and can also induce the infiltration of a variety of pro-inflammatory cytokines and chemokines in intestinal mucosa[14,15]. Treg cells have significant negative immunomodulatory effects, which can on the one hand, directly inhibit the activation of Th1, Th17 and other cells through intercellular contact, and on the other hand, can secrete TGF-β1, IL-10 and other inhibitory cytokines to suppress the activation of Th1, Th17 and other cells[16,17]. In the study, analysis of Th17/Treg cytokine expression in intestinal mucosa as well as Th17/Treg cytokine levels in serum of UC rats was as follows: IL-17 mRNA expression in intestinal mucosa and IL-17 level in serum of UC group were significantly higher than those of control group while TGF-β1 mRNA expression in intestinal mucosa and TGF-β1 level in serum were significantly lower than those of control group. This shows that the Th17/Treg balance shifting to Th17 is closely related to the progression of UC course. Further analysis of acupuncture intervention effect on Th17/Treg balance in the UC course showed that IL-17 mRNA expression in intestinal mucosa and IL-17 level in serum of acupuncture group were significantly lower than those of UC group while TGF-β1 mRNA expression in intestinal mucosa and TGF-β1 level in serum were significantly higher than those of control group. This indicates that acupuncture intervention can correct the disorder of Th17/Treg in UC course and promote the Th17/Treg balance shifting to Treg.

Acupuncture intervention can regulate the intestinal mucosal inflammatory response and immune response in animals with ulcerative colitis, inhibit the activation of inflammatory response and the secretion of inflammatory mediators, and regulate the Th1/Th2/Th17/Treg balance.

References
of IL-1 and IL-10 mRNA in colonic tissue of ulcerative colitis rats with abnormal sapra syndrome. World Chin J Digestol 2017; 9: 775-782.


