Effects of smilax bockii warb polysaccharide on inflammatory mediators, apoptosis and immune cell function in rats with chronic pelvic inflammatory disease

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ABSTRACT

Objective: To study the effects of smilax bockii warb polysaccharide on inflammatory mediators, apoptosis and immune cell function in rats with chronic pelvic inflammatory disease. Methods: Female SD rats were chosen as experimental animals and divided into control group, pelvic inflammation group and smilax bockii warb group, pelvic inflammation group and smilax bockii warb group were made into pelvic inflammation models, and smilax bockii warb group were given intragastric administration of smilax bockii warb. The contents of inflammatory mediators and the expression of apoptosis genes in uterine muscle as well as the contents of immune cells in peripheral blood were determined 21 d after molding and intervention. Results: TLR4, NF-κB, TNF-α, ICAM-1 and IL-8 levels as well as Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression in uterine muscle tissue and CD3+CD8+T cell in peripheral blood of pelvic inflammation group were higher than those of control group whereas peripheral blood CD3+CD4+T cell, red blood cell C3b receptor rosette rate and erythrocyte immune complex rosette were lower than those of control group; TLR4, NF-κB, TNF-α, ICAM-1 and IL-8 levels as well as Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression in uterine muscle tissue as well as CD3+CD8+T cell in peripheral blood of smilax bockii warb group were lower than those of pelvic inflammation group whereas peripheral blood CD3+CD4+T cell, red blood cell C3b receptor rosette rate and erythrocyte immune complex rosette were higher than those of pelvic inflammation group. Conclusion: Smilax bockii warb polysaccharide for chronic pelvic inflammatory disease intervention can inhibit the inflammatory mediator secretion, apoptosis and regulate the immune cell function.

1. Introduction

Pelvic inflammatory disease is a chronic inflammatory disease of the female reproductive system, and it mainly involves the reproductive organs as well as the surrounding connective tissue and pelvic peritoneum, it is with long course, difficult radical cure and complicated clinical symptoms, and it can cause infertility, exfetation and ectopic pregnancy and affect family life[1,2]. The etiology of pelvic inflammation is the ascending infection of pathogen, common pathogens include chlamydia trachomatis, neisseria gonorrhoeae and mycoplasma hominis, and persistent pathogen infection can cause the activation of local tissue inflammation by pattern recognition receptors, then increase the secretion of a variety of inflammatory cytokines and cause apoptosis process and immune response disorder. Smilax bockii warb is common Chinese medicine for clinical treatment of pelvic inflammatory disease, and its effective ingredients are saponins and flavonoids, and it has significant anti-inflammatory and immunomodulatory activity[3,4], but the mechanisms for the treatment of pelvic inflammatory disease are still not completely clear. In the following study, we chose the rat model with chronic pelvic inflammatory disease as the research object and specifically analyzed the effects of smilax bockii warb polysaccharide on inflammatory mediators, apoptosis and immune cell function in rats with chronic pelvic inflammatory disease.
2. Experimental materials and research methods

2.1 Experimental materials

The experimental animals were female SD rats purchased from the experimental animal center of the medical school of Xi’an Jiaotong University, with no copulation and body mass of 200-240 g. Smilax bocckii warb capsules were purchased from Hubei Furen Pharmaceutical Company, Elisa kits were purchased from Shanghai Westang Company, gene mRNA expression quantity detection kits were bought from Beijing Tiangen Company, and peripheral blood immune cell detection kits were purchased from Shanghai Tongwei Biotechnology Company.

2.2 Experimental methods

2.2.1 Model making and invention

SD rats were randomly divided into control group, pelvic inflammation group and smilax bocckii warb group, and pelvic inflammation group and smilax bocckii warb group were made into pelvic inflammation models according to the following method: they were put in the supine position after pentobarbital anesthesia, a median incision of 1 cm was made in lower abdomen to separate bilateral fallopian tube, 0.2 mL of 30% hydroxybenzene mucilage saline solution was injected in the central part of the ovary, and then the incision was sutured; control group were only given pentobarbital anaesthesia and abdominal incision without injection of hydroxybenzene mucilage saline solution in the ovary. Smilax bocckii warb group were given intragastric administration of 540 mg/kg/d Smilax bocckii warb capsules for 20 consecutive days. On day 21, three groups of rats were killed to separate peripheral blood and uterine muscle tissue, which were stored at -80℃.

2.2.2 Cytokine content detection

Moderate amount of uterine muscle tissues of the three groups of rats were taken, joined by PBS buffer and ground to get tissue grinding fluid, and the contents of TLR4, NF-κB, TNF-α, ICAM-1 and IL-8 were detected according to the instructions of Elisa kit.

2.2.3 Gene mRNA expression detection

Right amount of uterine muscle tissues of three groups of rats were taken, joined by Trizol lysate, then ground and joined by trichloromethane and isopropanol for extraction and centrifuged to get RNA, then fluorescence quantitative PCR kit manual was referred to configure the reaction system, the PCR reaction was done and then Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression were calculated.

2.2.4 Peripheral blood immune cell detection

The peripheral blood of three groups of rats was taken, the CD3, CD4 and CD8 fluorescent antibody were incubated to detect CD3+CD4+T cell and CD3+CD8+T cell contents, the C3b-sensitized frozen cerevisin suspension and unsensitized frozen cerevisin suspension suspension were added to determine red blood cell C3b receptor rosette rate and erythrocyte immune complex rosette.

2.3 Statistical methods

Software SPSS 20.0 was used to input data, the differences in measurement data among three groups were analyzed by variance analysis and P<0.05 indicated statistical significance in the differences.

3. Results

3.1 Inflammatory molecule levels in uterine muscle

Analysis of inflammatory molecules TLR4 (ng/mL), NF-κB (pg/mL), TNF-α (ng/mL), ICAM-1 (pg/mL) and IL-8 (ng/mL) levels in uterine muscle among the three groups of rats was as follows: TLR4, NF-κB, TNF-α , ICAM-1 and IL-8 levels in uterine muscle tissue of pelvic inflammation group were higher than those of control group (P<0.05), and TLR4, NF-κB, TNF-α , ICAM-1 and IL-8 levels in uterine muscle tissue of smilax bocckii warb group were lower than those of pelvic inflammation group (P<0.05).

Table 1.
Comparison of inflammatory molecules in uterine muscle among the Three Groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TLR4</th>
<th>NF-κB</th>
<th>TNF-α</th>
<th>ICAM-1</th>
<th>IL-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>6</td>
<td>0.83±0.11</td>
<td>174.4±20.6</td>
<td>6.83±0.73</td>
<td>203.6±33.7</td>
<td>2.14±0.33</td>
</tr>
<tr>
<td>Pelvic inflammation group</td>
<td>6</td>
<td>3.27±0.56</td>
<td>452.7±58.2</td>
<td>22.41±2.95</td>
<td>585.1±71.3</td>
<td>6.41±0.78</td>
</tr>
<tr>
<td>Smilax bocckii warb group</td>
<td>6</td>
<td>1.65±0.22</td>
<td>248.1±31.5</td>
<td>10.37±1.37</td>
<td>314.5±42.7</td>
<td>3.43±0.47</td>
</tr>
</tbody>
</table>

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

Table 2.
Comparison of apoptosis genes in uterine muscle among the three groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Fas</th>
<th>TNFR-1</th>
<th>GRP78</th>
<th>CHOP</th>
<th>Caspase-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>6</td>
<td>1.02±0.14</td>
<td>0.99±0.11</td>
<td>1.04±0.16</td>
<td>0.98±0.13</td>
<td>1.03±0.16</td>
</tr>
<tr>
<td>Pelvic inflammation group</td>
<td>6</td>
<td>2.52±0.37</td>
<td>2.03±0.31</td>
<td>2.17±0.35</td>
<td>1.89±0.24</td>
<td>2.70±0.41</td>
</tr>
<tr>
<td>Smilax bocckii warb group</td>
<td>6</td>
<td>1.67±0.22</td>
<td>1.46±0.20</td>
<td>1.41±0.18</td>
<td>1.30±0.16</td>
<td>1.64±0.22</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; #: compared with pelvic inflammation group, P<0.05.
start the expression of a variety of inflammatory cytokines κ pattern molecules and then start the downstream MyD88-dependent important pattern recognition receptor that can identify the pathogen in local tissue with pathogens could activate the inflammatory inflammation, and the combination of pattern recognition receptors infection of pathogens is the basic pathological change of pelvic

The chronic pelvic tissue inflammation caused by the retrograde infection of pathogens is the basic pathological change of pelvic inflammation, and the combination of pattern recognition receptors in local tissue with pathogens could activate the inflammatory response through downstream signaling pathways. TLR4 is an important pattern recognition receptor that can identify the pathogen pattern molecules and then start the downstream MyD88-dependent and non-dependent pathways to cause the transcription factor NF-κ B to be dissociated with inhibitors, transfer into the nucleus and start the expression of a variety of inflammatory cytokines(5–7). TNF-α , ICAM-1 and IL-8 are cytokines regulated by NF-κ B, TNF-α is a pro-inflammatory factor secreted by mononuclear macrophages, and it mediates the cascade activation process of inflammatory response[8]. ICAM-1 is a cytokine that mediates intercellular adhesion and can promote the inflammatory cell adhesion and infiltration to the pelvic inflammation area, IL-8 is a cytokine with chemotactic activity and can promote the chemotactic movement of inflammation cells towards the pelvic inflammation area, and the two can work together to promote the amplification of inflammatory response[9,10]. Analysis of the changes of above inflammatory molecules in the uterine muscle of rats with pelvic inflammation showed that TLR4, NF-κ B, TNF-α , ICAM-1 and IL-8 levels in uterine muscle tissue of pelvic inflammation group were higher than those of control group. This indicates that the excessive activation of inflammation mediated by TLR4 pathway is closely related to the occurrence of pelvic inflammatory disease. Further analysis of the effect of smilax bockii warb on the activation of TLR4 inflammation pathway in the uterine muscle showed that TLR4, NF-κ B, TNF-α , ICAM-1 and IL-8 levels in uterine muscle tissue of smilax bockii warb group were lower than those of pelvic inflammation group. This indicates that smilax bockii warb has inhibitory effect on the inflammatory response mediated by TLR4 in the course of chronic pelvic inflammatory disease.

The continuous activation of inflammatory response in the course of chronic pelvic inflammatory disease can cause damage to local tissues through multiple pathways, and the excessive apoptosis is a pathological link closely related to tissue injury. Death receptor apoptosis pathway and endoplasmic reticulum apoptosis pathway are the main mechanisms that regulate apoptosis in the process of chronic inflammations, and the two pathways can start the cascade amplification activation process of caspase family molecules, and finally activate caspase-3 to cause DNA breakage and karyopyknosis and lead to apoptosis. Fas and TNFR-1 are the two death receptors on the cell membrane, which can identify the ligands FasL and TNF-α of their own and then recruit FADD to activate the apoptosis cascade mediated by caspase[11]. GRP78 is the upstream signaling molecule of endoplasmic reticulum stress, which activates the downstream PERK, ATF6 and IRE1 pathways to increase CHOP expression, which finally activate caspase-3 to cause DNA breakage and karyopyknosis and lead to apoptosis. Analysis of the changes of above apoptosis genes in the uterine muscle of rats with pelvic inflammation showed that Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression in uterine muscle tissue of pelvic inflammation group were higher than those of control group, which indicates that the excessive activation of death receptor apoptosis pathway and endoplasmic reticulum apoptosis pathway is closely related to the occurrence of pelvic inflammation. Further analysis of the influence of smilax bockii warb on death receptor apoptosis pathway and endoplasmic reticulum apoptosis pathway in uterine muscle showed that Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression in

### Table 3.

Comparison of immune cells in peripheral blood among the three groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>CD3+CD4+T cell</th>
<th>CD3+CD8+T cell</th>
<th>Rbc C3b receptor rosette rate</th>
<th>Erythrocyte immune complex rosette</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>6</td>
<td>36.4±5.82</td>
<td>19.2±2.31</td>
<td>6.72±0.89</td>
<td>9.13±1.16</td>
</tr>
<tr>
<td>Pelvic inflammation group</td>
<td>6</td>
<td>20.1±3.24</td>
<td>31.6±3.85</td>
<td>3.3±0.46</td>
<td>4.79±0.38</td>
</tr>
<tr>
<td>Smilax bockii warb group</td>
<td>6</td>
<td>29.4±4.27</td>
<td>25.4±3.84</td>
<td>5.4±0.68</td>
<td>7.03±0.85</td>
</tr>
</tbody>
</table>

* compared with control group, P<0.05; # compared with pelvic inflammation group, P<0.05.

### 3.2 Apoptosis gene expression in uterine muscle

Analysis of apoptosis genes Fas, TNFR-1, GRP78, CHOP and Caspase-3 expression in uterine muscle among the three groups of rats was as follows: Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression in uterine muscle tissue of pelvic inflammation group were higher than those of control group (P<0.05), and Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression in uterine muscle tissue of smilax bockii warb group were lower than those of pelvic inflammation group (P<0.05).

### 3.3 Immune cell contents in peripheral blood

Analysis of immune cells CD3+CD4+T cell, CD3+CD8+T cell, red blood cell C3b receptor rosette rate and erythrocyte immune complex rosette in peripheral blood among the three groups of rats was as follows: peripheral blood CD3+CD4+T cell, red blood cell C3b receptor rosette rate and erythrocyte immune complex rosette of pelvic inflammation group were lower than those of control group whereas CD3+CD8+T cell was higher than that of control group (P<0.05), and peripheral blood CD3+CD4+T cell, red blood cell C3b receptor rosette rate and erythrocyte immune complex rosette of smilax bockii warb group were higher than those of pelvic inflammation group whereas CD3+CD8+T cell was lower than that of pelvic inflammation group (P<0.05).

### 4. Discussion

Smilax bockii warb is common Chinese medicine to treat chronic pelvic inflammation, but its functioning mechanism is still unclear. The chronic pelvic tissue inflammation caused by the retrograde infection of pathogens is the basic pathological change of pelvic inflammation, and the combination of pattern recognition receptors in local tissue with pathogens could activate the inflammatory response through downstream signaling pathways. TLR4 is an important pattern recognition receptor that can identify the ligands FasL and TNF-α B to be dissociated with inhibitors, transfer into the nucleus and start the expression of a variety of inflammatory cytokines(5–7). TNF-α , ICAM-1 and IL-8 are cytokines regulated by NF-κ B, TNF-α is a pro-inflammatory factor secreted by mononuclear macrophages, and it mediates the cascade activation process of inflammatory response[8]. ICAM-1 is a cytokine that mediates the interleukin-1 activity and can promote the chemotactic movement of inflammatory cells towards the pelvic inflammation area, and the two can work together to promote the amplification of inflammatory response[9,10]. Analysis of the changes of above inflammatory molecules in the uterine muscle of rats with pelvic inflammation showed that TLR4, NF-κ B, TNF-α , ICAM-1 and IL-8 levels in uterine muscle tissue of pelvic inflammation group were higher than those of control group. This shows that the excessive activation of inflammation mediated by TLR4 pathway is closely related to the occurrence of pelvic inflammatory disease. Further analysis of the effect of smilax bockii warb on the activation of TLR4 inflammation pathway in the uterine muscle showed that TLR4, NF-κ B, TNF-α , ICAM-1 and IL-8 levels in uterine muscle tissue of smilax bockii warb group were lower than those of pelvic inflammation group. This indicates that smilax bockii warb has inhibitory effect on the inflammatory response mediated by TLR4 in the course of chronic pelvic inflammatory disease.

The continuous activation of inflammatory response in the course of chronic pelvic inflammatory disease can cause damage to local tissues through multiple pathways, and the excessive apoptosis is a pathological link closely related to tissue injury. Death receptor apoptosis pathway and endoplasmic reticulum apoptosis pathway are the main mechanisms that regulate apoptosis in the process of chronic inflammations, and the two pathways can start the cascade amplification activation process of caspase family molecules, and finally activate caspase-3 to cause DNA breakage and karyopyknosis and lead to apoptosis. Fas and TNFR-1 are the two death receptors on the cell membrane, which can identify the ligands FasL and TNF-α of their own and then recruit FADD to activate the apoptosis cascade mediated by caspase[11]. GRP78 is the upstream signaling molecule of endoplasmic reticulum stress, which activates the downstream PERK, ATF6 and IRE1 pathways to increase CHOP expression, which finally activate caspase-3 to cause DNA breakage and karyopyknosis and lead to apoptosis. Analysis of the changes of above apoptosis genes in the uterine muscle of rats with pelvic inflammation showed that Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression in uterine muscle tissue of pelvic inflammation group were higher than those of control group, which indicates that the excessive activation of death receptor apoptosis pathway and endoplasmic reticulum apoptosis pathway is closely related to the occurrence of pelvic inflammation. Further analysis of the influence of smilax bockii warb on death receptor apoptosis pathway and endoplasmic reticulum apoptosis pathway in uterine muscle showed that Fas, TNFR-1, GRP78, CHOP and Caspase-3 mRNA expression in uterine muscle tissue of pelvic inflammation group were
utерине мышечной ткани симлякс боккьи варб группы были меньше, чем у тех, у кого был инфекционный процесс. Это указывает на то, что симлякс боккьи варб имеет ингибирующий эффект на избыточное выделение медиаторов воспаления и апоптоз.

In the occurrence of chronic pelvic inflammatory disease and the chronic infection process of pathogen, the immune response of the body is significantly abnormal, which is conducive to the elimination of pathogen through immune response. T cell is an important cell mass that mediates immune responses in the body, as it becomes mature CD3+CD4+T cell and CD3+CD8+T cell after positive and negative selection, the former mainly mediates cellular immune response and removes pathogen through the secretion of a variety of cytokines[13,14], and the latter mainly mediates humoral immune response and has inhibitory effect on cellular immune response, which is not conducive to the elimination of pathogen. Erythrocytes are newly discovered immunoactive cells in recent years, which form immune complexes through C3b receptors and remove pathogens[15,16]. Analysis of the change of above immune cells in the peripheral blood of rats with pelvic inflammatory disease showed that peripheral blood CD3+CD4+T cell, red blood cell CD3+ T cell receptor rosette rate and erythrocyte immune complex rosette of pelvic inflammation group were lower than those of control group whereas CD3+CD8+T cell was higher than that of control group. This indicates that T-cell and erythrocyte immune dysfunction are closely related to the occurrence of pelvic inflammatory disease. Further analysis of the influence of simlyax bockii warb on the immune cells in peripheral blood showed that peripheral blood CD3+CD4+T cell, red blood cell C3b receptor rosette rate and erythrocyte immune complex rosette of simlyax bockii warb group were higher than those of pelvic inflammation group whereas CD3+CD8+T cell was lower than that of pelvic inflammation group. This indicates that simlyax bockii warb can improve the immune function of T cells and erythrocytes in the course of chronic pelvic inflammatory disease.

The results of the above animal experiments indicated that there are excessive inflammatory mediator secretion and apoptosis activation as well as immune response disorder in chronic pelvic inflammatory disease model; the simlyax bockii warb polysaccharide intervention can inhibit the inflammatory mediator secretion and apoptosis and regulate the immune cell function.

References