Basic Investigations

Effects of Shenlian Extracts (参莲提取物) on Atherosclerosis by Inhibition of the Inflammatory Response

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Objective: Inflammation plays a critical role in atherosclerosis, and this inflammatory reaction is being intensively studied. Shenlian Extracts (参莲提取物), an active ingredient of Chinese medicinal herbs, is believed to have multiple therapeutic and preventive effects against human vascular diseases, including atherosclerosis. Our work investigated whether Shenlian Extracts serves as an anti-inflammatory agent during atherogenesis.

Methods: We established a model of atherosclerosis in rabbits using balloon angioplasty and a high cholesterol diet. The effects of Shenlian Extracts on vessel structure and inflammation were assessed by hematoxylin-eosin staining of the femoral artery, measurement of inflammation-related factors in serum or vascular tissue, and radioimmunoassay. Enzyme linked immunosorbent assays (ELISA), flow cytometry and western blots were also performed.

Results: We show that oral pre-treatment with Shenlian Extracts suppressed the pathological changes associated with atherosclerosis and that graded doses of Shenlian Extracts reduced total serum levels of cholesterol (90, 180 and 360 mg/kg), triglyceride (180 and 360 mg/kg), and LDL-c (90, 180 mg/kg). Various doses of Shenlian Extracts reduced serum content of TNF-α (180 and 360 mg/kg), CRP (90, 180 and 360 mg/kg) and IL-8 (360 mg/kg) (P<0.05), but led to no significant changes in IL-1β levels. Treatment with Shenlian Extracts also significantly reduced VCAM-1 levels (90 and 360 mg/kg) and IGF-1 levels (90 and 180 mg/kg) in vascular tissue but had no significant effect on ICAM-1 and MCP-1 levels. Finally, Shenlian Extracts significantly reduced the abnormal expression of CD18 in monocytes in a dose-dependent manner.

Conclusion: These results suggest that Shenlian Extracts may play a direct role in preventing and treating atherogenesis by inhibiting the inflammatory reaction, providing insights into the possible mechanism underlying the anti-atherosclerotic actions of Shenlian Extracts.

Keywords: Traditional Chinese Medicine; Shenlian Extracts; atherosclerosis; inflammatory; pathology; lipid; inflammatory factor; adhesion factor; growth factor
Extracts on early and long-term atherosclerosis were observed using a rabbit model of atherosclerosis. The extent and area of atherosclerosis plaque formation were used as an indication of the overall efficacy of the treatment. Adhesion factors, cytokines, growth factors, and chemotactic factors in Serum or vascular tissue were measured to obtain an indication of the severity of the inflammatory reaction.

**MATERIALS AND METHODS**

**Animals and Drug Administration**

Healthy male New Zealand rabbits weighting 2.2–2.8 kg were provided by the Beijing Tongli Laboratory Animal Breeding Center (Beijing, China). Each animal was housed in a separate cage in a room at temperature 22±0.5°C. All experiments conformed to the guidelines of the Animal Care Committee of the China Academy of Chinese Medical Sciences. Animals were grouped randomly into six groups of ten animals: a control group, a sham-operated group, and five atherosclerosis (model) groups; one group was treated with distilled water, three groups received different doses of Shenlian Extracts (90 mg/kg, 180 mg/kg, or 360 mg/kg), and one group received Simvastatin (10 mg/kg). Each group was administered its treatment intragastrically once per day. Shenlian Extracts, which contains tanshinone IIa (3%), salvianolic acid B (38%), tanshinol (1.6%) and andrographolide (20%), was provided by the Chemistry Laboratory of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences (Beijing, China).

**Induction of Atherosclerosis**

Our model of atherosclerosis in rabbits was established using a combination of balloon angioplasty and high cholesterol diet.\textsuperscript{11,12}

**Measurement of Blood Lipoprotein**

Ten weeks after initiation of atherosclerosis and drug administration, rabbits were fasted overnight and anaesthetized with diethyl ether before blood samples were collected directly from the heart chamber. Blood samples were collected into plain sample bottles and allowed to clot at room temperature for 4 h before they were centrifuged using a Uniscope Laboratory Centrifuge (Model SM 902B, Surgifriend Medicals, England, UK) at 10,000 × g at the same temperature for 20 min to separate the sera. Blood lipoproteins, including total cholesterol (SEKISUI, R1804RF, R2803RHF), triacylglycerols (Prodia Diagnostics, 04142), and high density lipoproteins (SEKISUI, R1805RF, R2807RJF), were assayed with a biochemical autoanalyzer (Olympus AU640) by the clinical laboratory of Dongzhimen Hospital. LDL-c = (TC - (HDL-c + (TG/5)).

**Measurement of CD18 in Peripheral Blood Monocytes**

Peripheral blood (0.5 mL) was sampled through the ear vein and anticoagulated with EDTA (10%) after 10 weeks of treatment. The expression of CD18 on monocytes (CD14 positive cells) was detected by flow cytometry (FCM) and western blot analysis.

**Histopathological Assessment**

After 10 weeks of treatment, all animals were sacrificed. A blood vessel (2 cm) between the abdominal aortic bifurcation and the femoral artery ligature was harvested immediately and fixed with 4% paraformaldehyde, then paraffin-embedded and stained with hematoxylin eosin.

**Measurement of Correlative Inflammatory Factors**

Blood was sampled to separate serum. Levels of TNF-α, IL-1β, IL-8 were tested by radioimmunoassay (RIA). C-reactive protein (CRP) levels were tested by immunoturbidimetry (ITM).

Vessels 1 cm above abdominal aortic bifurcation as well as 1 cm between abdominal aortic bifurcation and femoral artery ligature on the lesion side were preserved in liquid nitrogen. After homogenization using the liquid nitrogen grinding method, VCAM-I, ICAM-I, MCP-1 and IGF-I in injured vascular tissues were measured using an ELISA kit according to the manufacturer’s instructions.

**Statistical Analysis**

Data are expressed as mean ± SD. The statistical significance of inter-group differences was evaluated by one-way analysis of variance (one-way ANOVA) using software SPSS12.0 (SPSS Inc.). A value of $P<0.05$ was considered significant.

**RESULTS**

**Histopathological Assessment**

Compared with the sham-operated group, hematoxylin-eosin-stained histological sections from animals in the model group showed endothelial denudation and cell detachment from the femoral artery surface. Large amounts of lipid droplets and foam cells appeared under the common femoral artery and some lipid droplets even merged to form lipid pools. Based on this histopathological assessment, we concluded that the animal model could be considered to be in the advanced state of atherosclerosis. Treatment with Simvastatin and all doses of Shenlian Extracts improved these pathological changes to some extent (Figure 1).

**Blood Lipoprotein Measurement**

In the atherosclerosis model group, levels of serum cholesterol, triglyceride and low density lipoprotein were higher than those in the other groups, but high density lipoprotein was significantly lower than in sham-operated rabbits fed with a regular diet. Pre-treatment with Simvastatin and graded doses of Shenlian Extracts caused significant reductions in the
serum levels of total cholesterol (90, 180 and 360 mg/kg) 
(P<0.05, P<0.05, P<0.05), triglyceride (180 and 360 mg/kg) 
(P<0.05, P<0.01), and LDL-c (90 and 180 mg/kg) 
(P<0.05, P<0.05, Table 1).

Table 1. Effect of treatment with 10 mg/kg Simvastatin and 90-360 mg/kg Shenlian Extracts for 10 weeks on serum triglyceride, total cholesterol and LDL-c, HDL-c, in atherosclerotic rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum total cholesterol (mmol/L)</th>
<th>Serum triglyceride (mmol/L)</th>
<th>Serum HDL-c (mmol/L)</th>
<th>Serum LDL-c (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.39±0.68 b</td>
<td>1.14±0.21 b</td>
<td>0.71±0.25 b</td>
<td>1.51±1.32 b</td>
</tr>
<tr>
<td>II</td>
<td>15.15±3.63</td>
<td>3.10±1.08</td>
<td>0.44±0.07</td>
<td>12.39±4.36</td>
</tr>
<tr>
<td>III</td>
<td>8.51±0.61a</td>
<td>1.65±0.79</td>
<td>0.73±0.17 b</td>
<td>4.32±1.03 b</td>
</tr>
<tr>
<td>IV</td>
<td>11.12±3.79a</td>
<td>2.21±1.32</td>
<td>0.42±0.04</td>
<td>6.12±1.11 a</td>
</tr>
<tr>
<td>V</td>
<td>8.76±1.32a</td>
<td>1.12±0.55 a</td>
<td>0.47±0.03</td>
<td>8.31±4.34 a</td>
</tr>
<tr>
<td>VI</td>
<td>11.13±4.39a</td>
<td>0.89±0.13 b</td>
<td>0.50±0.09</td>
<td>9.18±3.10</td>
</tr>
</tbody>
</table>

Notes: a and b represent significant increases at P<0.05 and P<0.01, respectively, compared with Group II; I: treatment with distilled water 10 mL/kg/day + sham operation + ordinary diet; II: pre-treatment with distilled water 10 mL/kg/day + endothelial injury + high fat diet; III: pre-treatment with Simvastatin 10 mg/kg/day + endothelial injury + high fat; IV: pre-treatment with Shenlian Extracts 90 mg/kg/day + endothelial injury + high fat; V: pre-treatment with Shenlian Extracts 180 mg/kg/day + endothelial injury + high fat; VI: pre-treatment with Shenlian Extracts 360 mg/kg/day + endothelial injury + high fat.

**Correlative Inflammatory Factors Measurement**

After 10 weeks, serum levels of TNF-α, IL-8 and CRP were higher in the model group compared with the control group. Various doses of Shenlian Extracts reduced serum levels of TNF-α (180 and 360 mg/kg) (P<0.01, P<0.01), CRP (90, 180 and 360 mg/kg) (P<0.01, P<0.01, P<0.05) and IL-8 (360 mg/kg) (P<0.05). Serum IL-1β levels did not significantly change in any group (Table 2).

The levels of ICAM-1, VCAM-1, MCP-1 and IGF-1 in vascular tissues increased sharply in the model group in the 10th week. Pre-treatment with Simvastatin or Shenlian Extracts significantly reduced VCAM-1 levels (90 and 360 mg/kg) (P<0.05, P<0.01) and IGF-1 levels (90 and 180 mg/kg) (P<0.01, P<0.01), but did not cause a significant effect on ICAM-1 and MCP-1 levels (Table 3).

**Monocyte CD18 Measurement**

Expression of CD18 by peripheral blood monocytes increased sharply in animals of the model group in the 10th week (P<0.01). Various doses of Shenlian Extracts significantly reduced this abnormal expression of CD18, in a dose-dependent manner (P<0.05, P<0.05, P<0.01). Western blots showed the same increase of CD18 in the model group (P<0.01). Each of the three doses of Shenlian Extracts inhibited CD18 expression significantly (P<0.05, P<0.01, P<0.01), while Simvastatin showed no such effect (Figure 2).
Table 2. Effect of treatment with 10 mg/kg Simvastatin and 90–360 mg/kg Shenlian Extracts for 10 weeks on serum TNF-α, IL-1 β, CRP, IL-8 in a rabbit model of atherosclerosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum TNF-α (ng/mL)</th>
<th>Serum CRP (µg/mL)</th>
<th>Serum IL-1β (ng/mL)</th>
<th>Serum IL-8 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.82±1.10 b</td>
<td>0.82±0.32 b</td>
<td>1.79±0.39</td>
<td>1.14±0.20 b</td>
</tr>
<tr>
<td>II</td>
<td>4.10±1.69</td>
<td>3.24±0.36</td>
<td>2.00±0.89</td>
<td>1.87±0.46</td>
</tr>
<tr>
<td>III</td>
<td>1.25±0.74 b</td>
<td>1.04±0.54 b</td>
<td>2.37±0.65</td>
<td>1.20±0.31 b</td>
</tr>
<tr>
<td>IV</td>
<td>3.27±0.74</td>
<td>1.66±0.61 b</td>
<td>1.70±0.65</td>
<td>1.39±0.28</td>
</tr>
<tr>
<td>V</td>
<td>1.69±0.29 b</td>
<td>1.27±0.16 b</td>
<td>2.12±0.87</td>
<td>1.57±0.23</td>
</tr>
<tr>
<td>VI</td>
<td>1.62±0.28 b</td>
<td>2.11±1.35 a</td>
<td>2.82±0.89</td>
<td>1.01±0.31 a</td>
</tr>
</tbody>
</table>

Notes: a and b represent significant increases at \(P<0.05\) and \(P<0.01\), respectively, compared with Group II. For group definitions, see Table 1.

Table 3. Effect of treatment with 10 mg/kg Simvastatin and 90-360 mg/kg Shenlian Extracts for 10 weeks on vascular tissue VCAM-1, ICAM-1, IGF-1, MCP-1 in a rabbit model of atherosclerosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Vascular tissue ICAM-1 (ng/mg)</th>
<th>Vascular tissue VCAM-1 (ng/mg)</th>
<th>Vascular tissue MCP-1 (pg/mg)</th>
<th>Vascular tissue IGF-1 (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>28.70±7.33 b</td>
<td>16.68±5.14 b</td>
<td>90.35±11.46 a</td>
<td>25.01±7.37 b</td>
</tr>
<tr>
<td>II</td>
<td>42.89±12.82</td>
<td>38.67±10.32</td>
<td>122.80±21.01</td>
<td>45.51±12.07</td>
</tr>
<tr>
<td>III</td>
<td>38.45±12.97</td>
<td>23.41±11.45 b</td>
<td>92.41±26.45 a</td>
<td>26.41±8.16 b</td>
</tr>
<tr>
<td>IV</td>
<td>41.32±4.13</td>
<td>25.48±12.62 a</td>
<td>113.87±10.54</td>
<td>25.82±9.28 b</td>
</tr>
<tr>
<td>V</td>
<td>36.42±13.35</td>
<td>31.32±10.05</td>
<td>112.21±36.49</td>
<td>23.10±13.33 b</td>
</tr>
<tr>
<td>VI</td>
<td>39.15±12.32</td>
<td>28.41±4.90 a</td>
<td>99.98±43.12</td>
<td>34.34±6.75</td>
</tr>
</tbody>
</table>

Notes: a and b represent significant increases at \(P<0.05\) and \(P<0.01\), respectively, compared with Group II. For group definitions, Table 1.

Figure 2. Effect of treatment with 10 mg/kg Simvastatin and 90–360 mg/kg Shenlian Extracts for 10 weeks on CD18 expression in atherosclerotic rabbits. △ and △△ represent significant increases at \(P<0.05\) and \(P<0.01\), respectively, compared with Group II. For group definitions, see Table 1.

**DISCUSSION**

The injury-response theory and inflammatory reaction theory illustrate that atherosclerosis plaques form gradually on vessel walls during a process of chronic inflammatory damage and body repair responses. The inflammatory response is one of the key aspects of atherosclerosis pathology, and anti-inflammatory interventions can block this inflammatory response, thereby postponing the process or lessening the damage in the early and middle stages of the disease. Anti-inflammatory treatment also greatly reduces the incidence of acute cardio-cerebral-vascular incidents in advanced-stage atherosclerosis. Thus, anti-inflammatory intervention is theoretically and practically valuable for the prevention and treatment of atherosclerosis.

Traditional Chinese Medicine (TCM) has been practiced for thousands of years. In recent years, many Chinese medical specialists recognized from their clinical experiences that atherosclerosis was closely related to the
conditions endogenous heat toxin and the damage of brain collateral by toxins. At present, the methods of heat-clearing and detoxication, removing toxins and decreasing turbid pathogens, and detoxication and purgation have been widely used in Chinese medicine treatment of arteriosclerotic cardiovascular and cerebrovascular diseases.

Medical research has found that the pathogenesis of atherosclerosis as endogenous heat toxin in Chinese medicine is very similar to the current mainstream theories of injury-response theory and inflammatory reaction theory. This similarity provides an interesting conjunction of the theories on atherosclerosis pathogenesis from both Chinese and Western medicine systems. In Chinese medicine, intervention in endogenous heat toxin or inflammatory response by activating blood circulation to dissipate blood stasis and heat-clearing and detoxication have been discussed as a method for the prevention and treatment of atherosclerosis.

Based on this thinking, Shenlian Extracts is believed to have multiple therapeutic and preventive effects against human vascular diseases, including atherosclerosis. We previously found that Shenlian Extracts could inhibit atherosclerosis formation in rats by regulating lipid metabolism, inducing endothelial cells contraction and excretion, and improving hemorheology. In this study, we show that Shenlian Extracts significantly reduces the formation of atherosclerosis plaques. We suggest that the mechanism of the effects of Shenlian Extracts might be that it improves lipid metabolism, directly reduces lipid deposition and also indirectly inhibits lipid deposition via an anti-inflammatory. Shenlian Extracts might also inhibit the formation and abnormal release of inflammatory agents, reducing the concentration of proinflammatory cytokines, adhesion molecules, chemotactic factors and growth factors. Finally, it might also interrupt the abnormally high expression of CD18 in monocytes during atherosclerosis.

In conclusion, our results suggest that Shenlian Extracts can inhibit the inflammatory reaction in initiation and development of atherosclerosis, and may therefore be used to treat atherosclerosis. This study highlights the promising possibility of atherosclerosis prevention by TCM based on activating blood circulation to dissipate blood stasis and heat-clearing and detoxication therapies.

REFERENCES

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