Effects of auricular point pressing + Danshen Dropping Pill on platelet function, inflammatory response and protease expression in patients with stable angina pectoris

Chen-Ming Hu†, Ji-Hong Liu, Yu Wu

1. Introduction

Fatigue-induced myocardial ischemia, the resulting sternal and surrounding discomfort are called as angina pectoris. Some patients are accompanied by cardiac dysfunction but without myocardial necrosis, and the rest or sublingual nitroglycerin can rapidly relieve the discomfort, which is classified as stable angina pectoris. The number of people with stable angina has been rising, and it has become one of the main cardiovascular and cerebrovascular diseases that would threaten the people’s health and life safety in our country, and how to control the population and prevent disease development into myocardial infarction and death, is the focus and hot spot of the clinical research[1]. Traditional Chinese medicine treatment of stable angina pectoris has a long history, compound danshen dropping pill is a Chinese patent medicine that promotes blood circulation to remove blood stasis as well as regulates qi to alleviate pain, its role in anti-myocardial ischemia-reperfusion injury, anti-inflammation, anti-oxidation and other aspects has got the recognition of modern pharmacology, and it is a common drug for TCM treatment of stable angina pectoris[2,3]. In addition to oral traditional Chinese medicine preparation, there are many non-invasive TCM methods that are beneficial to the cardiac function, and auricular point pressing is the one with wide attention at present. It has been confirmed in many literaturee that auricular point pressing can be used to treat cholelithiasis, insomnia, bronchial asthma, abdominal pain, hypertension, and so on, but at present, there are not many relevant studies about its benefit for patients with stable angina pectoris.

In this study, compound danshen dropping pill combined with

**ARTICLE INFO**

**ABSTRACT**

**Objective:** To investigate the effects of auricular point pressing plus Danshen Dropping Pill on platelet function, inflammatory response and protease expression in patients with stable angina pectoris. **Methods:** A total of 92 patients with stable angina pectoris who were treated in this hospital between November 2014 and November 2017 were divided into the auricular point pressing group (n=46) and routine group (n=46) by random number table method. Routine group were treated with routine western medicine plus Danshen Dropping Pill, auricular point pressing group were treated with auricular point pressing on the basis of the therapy of routine group, and both groups had been continuously treated for 1 month. The differences in platelet function, inflammatory response and protease expression were compared between the two groups before and after treatment. **Results:** Before treatment, there was no statistically significant difference in the platelet activation index levels, serum inflammatory factor contents or protease expression between the two groups. After 1 month of treatment, peripheral blood platelet activation index CD42b level of auricular point pressing group was higher than that of routine group whereas CD63 and PAC-1 levels were lower than those of routine group; serum inflammatory factors IL-6, IL-10, IL-18 and hs-CRP contents were lower than those of routine group; serum proteases MMP-1, MMP-2 and MMP-9 contents were lower than those of routine group. **Conclusion:** Compound Danshen Dropping Pill combined with auricular point pressing can further optimize the platelet function and improve the systemic inflammatory state and protease expression in patients with stable angina pectoris.
auricular point pressing was adopted, and the optimization effect of the therapy on the condition of patients with stable angina was discussed, which is elaborated as follows.

2. Information and methods

2.1 Case information

A total of 92 patients with stable angina pectoris who were treated in this hospital between November 2014 and November 2017 were included in this study, and inclusion criteria were: (1) in accordance with the diagnostic criteria for stable angina pectoris; (2) without previous history of auricular point pressing; (3) cooperating with the treatment and signing the informed consent. Exclusion criteria: (1) combined with allergy to compound danshen drop pills; (2) combined with infective endocarditis, severe atrial and ventricular septal defect and other cardiac disorders; (3) combined with influenza and other systemic infectious diseases; (4) combined with platelet malformations such as Henoch-Schönlein purpura.

According to the principle of random number table, the 92 patients were divided into auricular point pressing group (n=46) and routine group (n=46). Auricular point pressing group included 25 males and 21 females that were 49-77 years old; routine group included 24 males and 22 females who were 48-79 years old. There was no statistical difference in the above basic data distribution between the two groups, and the subsequent data results were comparable. The hospital ethics committee approved the study after reviewing the application and discussing it.

2.2 Therapy

Routine group received conventional western medicine plus compound danshen dropping pill therapy, including calcium antagonist, nitrates, Aspirin Enteric-coated Tablets as well as compound danshen dropping pill therapy, including calcium channel blockers, antagonists, nitrates, Aspirin Enteric-coated Tablets as well as compound danshen dropping pills 10 pills/time (27 mg/pill), 3 times/d, for 1 month in a row.

Based on the therapy for routine group, auricular point pressing group received auricular point pressing, which was as follows: acupoint selection (heart point, liver point, posterior coronary artery point, Shenmen, and so on), and the adhesive plaster of 0.5 cm 0.5 cm was covered with cowherb seed and applied after routine disinfection. Auricular point sticking was adopted to make the acupoints develop aching, numb, distending, painful and other sensations of "de qi", then patients did the pressing on their own, the above auricular point pressing was done 3-5 times a day, 2 min each time, the dressing was replaced every 3 d, and the treatment lasted for 1 month.

2.3 Platelet function indexes

Before treatment and after 1 month of treatment, fasting peripheral blood specimens were obtained from the two groups of patients, and flow cytometer was used to detect platelet activation function index levels, including platelet membrane glycoprotein CD42b, CD63 and PAC-1.

2.4 Inflammatory response indexes

Peripheral blood was obtained from the two groups of patients at the same point in time to separate serum, and Elisa kit was used to detect the levels of stable angina pectoris-related inflammatory factors, including interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-18 (IL-18) and hypersensitive C-reactive protein (hs-CRP).

2.5 Protease expression indexes

Serum-obtaining time point and method were the same as those in Table 1, and microplate reader was used to measure the expression of proteases, including matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9).

2.6 Statistical processing

The data involved in the study (platelet function indexes, inflammatory response indexes and protease expression indexes) were all measurement data and expressed as mean±standard deviation. The comparison between groups before treatment and after treatment was by grouping t test, the comparison between before and after treatment within group was by paired t test, the statistical software was SPSS 21.0 and P<0.05 was the standard of statistical significance in the study results.

Table 1.

Peripheral blood platelet function index levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>CD42b</th>
<th>CD63</th>
<th>PAC-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine group</td>
<td>46</td>
<td>Before treatment</td>
<td>65.18±0.74</td>
<td>4.09±0.53</td>
<td>30.72±3.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 1 month treatment</td>
<td>71.32±7.59</td>
<td>2.78±0.34</td>
<td>19.66±2.43</td>
</tr>
<tr>
<td>Auricular point pressing</td>
<td>46</td>
<td>Before treatment</td>
<td>65.06±0.96</td>
<td>4.11±0.48</td>
<td>30.69±3.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 1 month treatment</td>
<td>78.64±8.12</td>
<td>1.91±0.25</td>
<td>11.85±1.29</td>
</tr>
</tbody>
</table>

Note: compared with same group before treatment, *P<0.05; compared with routine group after 1 month of treatment, †P<0.05.

Table 2.

Serum inflammatory response index contents.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>IL-6</th>
<th>IL-10</th>
<th>IL-18</th>
<th>hs-CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine group</td>
<td>46</td>
<td>Before treatment</td>
<td>18.45±2.48</td>
<td>25.93±3.47</td>
<td>71.29±8.53</td>
<td>40.38±4.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 1 month treatment</td>
<td>12.18±1.74</td>
<td>17.04±1.88</td>
<td>50.74±5.39</td>
<td>27.59±3.18</td>
</tr>
<tr>
<td>Auricular point pressing</td>
<td>46</td>
<td>Before treatment</td>
<td>18.39±2.63</td>
<td>25.71±3.52</td>
<td>71.04±7.95</td>
<td>40.57±4.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 1 month treatment</td>
<td>78.64±8.12</td>
<td>9.11±0.25</td>
<td>11.85±1.29</td>
<td>18.73±2.09</td>
</tr>
</tbody>
</table>

Note: compared with same group before treatment, *P<0.05; compared with routine group after 1 month of treatment, †P<0.05.
Table 3.
Serum protease expression index contents (ng/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Time</th>
<th>MMP-1</th>
<th>MMP-2</th>
<th>MMP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine group</td>
<td>46</td>
<td>Before treatment</td>
<td>55.91±6.02</td>
<td>43.78±5.12</td>
<td>50.38±5.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 1 month</td>
<td>40.87±4.53*</td>
<td>30.16±4.39*</td>
<td>31.23±4.34*</td>
</tr>
<tr>
<td>Auricular point pressing group</td>
<td>46</td>
<td>Before treatment</td>
<td>55.76±5.91</td>
<td>43.26±5.09</td>
<td>50.71±5.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 1 month</td>
<td>23.49±3.12*</td>
<td>21.45±2.77*</td>
<td>18.45±2.12*</td>
</tr>
</tbody>
</table>

Note: compared with same group before treatment, \( P < 0.05 \); compared with routine group after 1 month of treatment, \( P > 0.05 \).

3. Results

3.1 Platelet function indexes

Comparison of peripheral blood platelet function indexes CD42b, CD63 and PAC-1 levels between the two groups of patients was as follows: before treatment, peripheral blood CD42b, CD63 and PAC-1 levels were not significantly different between the two groups \( (P>0.05) \); after 1 month of treatment, peripheral blood CD42b levels of both groups were higher than those before treatment whereas CD63 and PAC-1 levels were lower than those before treatment \( (P<0.05) \). After 1 month of treatment, peripheral blood CD42b level of auricular point pressing group was higher than that of routine group whereas CD63 and PAC-1 levels were lower than those of routine group \( (P<0.05) \), shown in Table 1.

3.2 Inflammatory response indexes

Comparison of serum inflammatory response indexes IL-6 (pg/mL), IL-10 (pg/mL), IL-18 (pg/mL) and hs-CRP (mg/L) contents between the two groups of patients was as follows: before treatment, serum IL-6, IL-10, IL-18 and hs-CRP contents were not significantly different between the two groups \( (P>0.05) \); after 1 month of treatment, serum IL-6, IL-10, IL-18 and hs-CRP contents of both groups were lower than those before treatment \( (P<0.05) \). After 1 month of treatment, serum IL-6, IL-10, IL-18 and hs-CRP contents of auricular point pressing group were lower than those of routine group \( (P<0.05) \), shown in Table 2.

3.3 Protease expression indexes

Comparison of serum protease expression indexes MMP-1, MMP-2 and MMP-9 contents between the two groups of patients was as follows: before treatment, serum MMP-1, MMP-2 and MMP-9 contents were not significantly different between the two groups \( (P>0.05) \); after 1 month of treatment, serum MMP-1, MMP-2 and MMP-9 contents of both groups were lower than those before treatment \( (P<0.05) \). After 1 month of treatment, serum MMP-1, MMP-2 and MMP-9 contents of auricular point pressing group were lower than those of routine group \( (P<0.05) \), shown in Table 3.

4. Discussion

Stable angina pectoris is clinical common cardiovascular disease, and the illness after acute attack can be quickly relieved by rest or sublingual nitroglycerin, but some patients failed to receive appropriate treatment, the myocardial cells are continuously injured, or sublingual nitroglycerin, but some patients failed to receive appropriate treatment, the myocardial cells are continuously injured, whereas CD63 and PAC-1 levels were lower after treatment, and the above mentioned function and change characteristics of each marker show adding auricular point pressing therapy can further reduce the platelet activity, and this is also the important symbol of stable condition in patients with stable angina, and confirms the feasibility of the traditional Chinese medicine treatment and its positive role in and "cardiac pain", its occurrence is related to cold evil invasion, eating disorders, unchecked emotion, overstrain and internal injuries as well as old and weak, the pathogenesis is both deficient and excessive, and oral administration of traditional Chinese medicine, acupuncture point massage, auricular point pressing and so on are all main approaches of Chinese medicine treatment of the disease[8,9]. Auricular point pressing is less reported in clinic at present, but it has been maturely used in many diseases such as cholelithiasis and hypertension, and some scholars have pointed out that it is also beneficial to heart disease. Auricular point pressing is to accurately attach the adhesive plaster to the auricular point and make it produce ache, numbness, distending, pain and other stimuli-responsive after moderate petriassage and pressing so as to achieve the therapeutic purpose. In the study, based on conventional western medicine, oral compound danshen dripping pill and other treatments, traditional Chinese medicine treatment auricular point pressing was added, and the changing effect of the treatment on patients’ internal environment was explored to clarify its feasibility in the treatment of chronic stable angina.

Many studies have shown that the myocardial ischemia extent in patients with coronary heart disease is directly correlated with fibrinogen level and blood viscosity. In the case of acute disease attack, the changes in coronary local hemodynamics cause the platelet activation function to be activated and release a large number of active factors, they promote the platelets to locally aggregate and form thrombus, and this is the main pathological basis of unstable angina and myocardial infarction[10,11]. The platelet activity is generally kept in normal level when patients with stable angina pectoris have good control of the illness, but when the illness is deteriorated, the platelet activity changes and coronary thrombotic events happen, so the detection of patients’ platelet activity is an effective means to assess their condition and current treatment effect. CD42b, CD63 and PAC-1 are the platelet activation markers with much clinical application at present, CD42b is one of the main glycoproteins in platelet, it is evenly distributed in the platelet membrane surface, and it moves quickly to the outside of cell membrane when platelets are activated, which leads to the reduction of its content in platelet surface[12]; CD63 is a lysosome granule membrane protein. When platelets are activated, the lysosomal membrane is dissolved, and it is expressed in platelet membrane after fusion with platelet serosa. It has been found that the CD63 expression increases in the acute phase of acute cerebrovascular events, and the application of its antibodies can reduce brain injury[13,14]; PAC-1 is platelet membrane glycoprotein IIb/IIIa complex, it exists in the form of monomer under physiological condition and is unable to bind to the ligand, its configuration changes after platelet activation, and it can be specifically combined with a variety of adhesion proteins and accelerate the platelet - fibrinogen - platelet aggregation[15]. The study showed that CD42b level on platelets of auricular point pressing group was higher whereas CD63 and PAC-1 levels were lower after treatment, and the above mentioned function and change characteristics of each marker show adding auricular point pressing therapy can further reduce the platelet activity, and this is also the important symbol of stable condition in patients with stable angina, and confirms the feasibility of the traditional Chinese medicine treatment and its positive role in
Coronary heart disease is a chronic inflammatory reaction process, and the patients with stable angina, unstable angina and even myocardial infarction are accompanied by varying degrees of systemic inflammatory response[5]. The existence of inflammatory response is an important foundation for coronary artery atheromatous plaque formation and its volume increasing, IL-6, IL-18 and hs-CRP are typical pro-inflammatory factors, and the increase of their contents can cause the thickening of atheromatous plaque and aggravate its condition; IL-10 has an anti-inflammatory effect. When the pro-inflammatory factors increase, the body reacts to release IL-10 in order to control the overall level of inflammation, so IL-10 is positively correlated with pro-inflammatory factor levels[16,17]. The results of this study showed that serum IL-6, IL-10, IL-18 and hs-CRP contents of auricular point pressing group were lower after treatment, it means that auricular point pressing can inhibit the overall state of inflammation in patients with stable angina, and this is an important link for it to inhibit the progression of coronary pathological changes and also illustrates again the effectiveness of the therapy.

Fibrous cap is an important part of atheromatous plaque, the fibrous cap thickness and collagen content directly determine the plaque stability, and the damaged synthesis or accelerated degradation of collagen can weaken the fibrous cap and make the plaque unstable, which increases the risk of plaque detachment. Matrix metalloproteinases are the main substances to degrade extracellular matrix, they include MMP-1, MMP-2, MMP-9 and many other subtypes, and they have already been confirmed to participate in the occurrence and development of coronary heart disease are mainly related to plaque formation and stability[18,19]. When the contents of matrix metalloproteinases increase, their ability to degrade extracellular matrix is enhanced, and to specifically reduce their contents has become one of the methods to treat coronary heart disease[20,21]. The results of the study showed that serum MMP-1, MMP-2 and MMP-9 contents of auricular point pressing group were lower after treatment, which indicates that auricular point pressing can effectively reduce the atheromatous plaque fibrous cap degradation and increase its stability and can also inhibit the progression from stable angina to unstable angina and even myocardial infarction.

To sum up, it can be concluded that auricular point pressing therapy helps to stabilize the condition of patients with stable angina, which is specifically manifested in its regulation on platelet activity, inflammation, plaque stability and other aspects; it is a feasible and reliable treatment of traditional Chinese medicine. In the treatment of stable angina in the future, auricular point pressing therapy can be added to the overall treatment to further stabilize the patients’ condition and optimize the treatment outcome.

References


