The Effect of Acupuncture on Morphological Structure and Function of the Rats Spleen after Exhaustive Exercise

Xia ZHENG¹,a, Qiang Li²,b, Bo-Gao BIAN³,c
¹Shantou Polytechnic, Shantou, Guangdong, China
²Huizhou Faculty, Huizhou, Guangdong, China
³Guangzhou University of Traditional Chinese Medicine, Guangzhou, Guangdong, China

Keywords: Acupuncture, Exhaustive Exercise, Rat, Spleen.

Abstract. Objective: to study the change of structure and function of spleen morphology in rats after exhaustive exercise, to explore the protective mechanisms of acupuncture on the spleen. Methods: 30 SD rats were divided into three groups: control group, model group and acupuncture group. Model group and acupuncture group received six-weeks exhaustive swimming training. The acupuncture group received acusector in their “Sanyinjiao”, “Shenmen”, “Neiguan”, “Baihui” and “Sishencong” before an hour training. The last training later the spleen damage was examined. Results: Compared with the control group, model group’s pathological of spleen morphology changed more significantly, number of spleen apoptosis and activation of caspase-3 activity, GSH and MDA contents increased more dramatically (p<0.01), spleen T-SOD activity, levels of IL-2 and IFN-γ decreased significantly(p<0.01). Compared with the model group, acupuncture group’s pathological of spleen morphology changed less obvious, spleen apoptosis and caspase-3 activity, GSH and MDA contents decreased more significantly (p<0.01). Spleen T-SOD activity, levels of IL-2 and IFN-γ increased more significantly(p<0.01). Conclusion: There was obvious pathological state in morphological structure and function of spleen in rats after exhaustive exercise. However, rats’ “Sanyinjiao”, “Shenmen”, “Neiguan”, “Baihui” and “Sishencong” received acupuncture can improve their spleen damages in some degree.

Introduction

Through six-weeks exhaustive swimming training to design model rats with exercise-induced injury. According to traditional Chinese Medicine principle that BuyiXinpi, JiannaoYangshen to make acupuncture prescription (“Sanyinjiao” as the main acupoint, assisted with “Shenmen”, “Neiguan”, “Sishencong”), and morphological structure of spleen (under the light microscopy), spleen apoptosis, caspase-3 activity of spleen, GSH and MDA contents, T-SOD activity, levels of IL-2 and IFN-γ were took out to indicated, to systematical explore acupuncture on the protective mechanism of spleen impairment in rats after exhaustive exercise.

Materials and Methods

Laboratory Animals and Feeding Conditions

There were 30 SPF male SD rats (10 weeks old, weigh 180-220g), which bought from Guangzhou University of Traditional Chinese medicine laboratory animal center. Certificate of quality number of experimental animals were SCXK(Yue)2008-0002. They were bred in different cages, each with 6 rats. Their food was complete nutritional pellet and distilled water. They could eat with water freely. The room temperature kept 21 - 24 Celsius degree, relative humidity was kept 40 - 55 percent, illumination time lasted 12 hours.

Grouping and Experimental Programs

Rats were randomly divided into control group, model group and acupuncture group, each group
with 10 rats. Referred to Zhang Pengyun's methods to establish exhaustive swimming model. Rats swam in a pool with the size of 100cm*60cm*50cm, 50cm water depth and 30±3 celsius degree temperature last six weeks, six days a week. They experienced exhaustive exercise six days a week, until their weigh of tail became 3 percent of total body weight. Standard for rats after exhaustive swimming was that they couldn’t keep balance obviously and float autonomously after sank underwater with 10 seconds [1]. The control group was bred normally without movement. Model group and acupuncture group received six-weeks exhaustive swimming training. The acupuncture group’s “Sanyinjiao”, “Shenmen”, “Neiguan”, “Sishencong” were received acupuncture in a way of Pingbuxiefa before 1 hour training. The last training later rats’ spleen was taken out to examine their splenic impairment.

**Primary Reagent**

Detection of Caspase-3 activity, MDA content, SOD activity, GSH content, levels of IL-2 and IFN-γ. All Kits are provided by Nanjing jiancheng Bioengineering Institute.

**Testing Index and Method**

**Observation of spleen morphologic.** After the experiment, we used ether to anaesthetize rats, took spleen tissue, conventional fixation, dehydration, dipping wax, embedding, sectioning and observed under light microscope after staining.

**Detection of number of apoptotic cells (positive cells) and the Caspase-3 activity.** At first, we took spleen tissue and made tissue homogenate with 10% density. Then clear liquid top of tissue homogenate was selected to detect number of positive cells by TUNEL method and Caspase-3 activity by Bradford method. Detection of contents of MDA and GSH, and SOD activity.

We extracted tissue homogenate to examine MDA content by Thiobarbituric Acid Colourimetry Method, detect GSH content by DTNB Colorimetric Method and SOD activity by Xanthine Oxidase Method.

**Detection of levels of IL-2 and IFN-γ.** We firstly extracted spleen tissue to make cell suspension, then detected level of IL-2 by H3-TdR Incorporation Method and examined level of IFN-γ by Crystal violet staining assay.

**Statistical Method.** All measurement data dealt with by SPSS17.0 statistics software with the average standard error of plus or minus (\(\bar{x} \pm s\)). Groups were compared by analysis of variance (One-Way ANOVA). Took \(p<0.01\) significant level.

**Results and Analysis**

**The Effect of Acupuncture on the Morphology Structure of Spleen in Rats after Exhaustive Exercise**

As shown in Figure 1, Lymphocytes in the red and white pulp of the rats’ spleen in control group are arranged regularly, intensively and with complete forms. While model Group’s red pulp expanded and white pulp shrunk, lymphocytes decreased significantly and ranged sparsely. They tended to group. The acupuncture group’s pathological changes were lighter and their morphology were more similarly to the control group.

![A. Normal group](image1.png) ![B. Model group](image2.png) ![C. Acupuncture group](image3.png)

Figure 1. Morphology Structure of Spleen (*200).
The Effect of Acupuncture on Splenocytes Apoptosis in Rats after Exhaustive Exercise

As shown in Table 1, the model group’s splenocytes apoptosis number and caspase-3 activity were significantly higher than control group (p<0.01). However, the acupuncture group’s splenocytes apoptosis number and caspase-3 activity were dramatically slower than model group(p<0.01).

Table 1. Statistic of Splenocytes Apoptosis (x ± s, n=10).

<table>
<thead>
<tr>
<th>Group</th>
<th>number of positive cells</th>
<th>caspase-3 activity (U/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>0.77±0.27</td>
<td>4.24±0.21</td>
</tr>
<tr>
<td>Model group</td>
<td>8.85±3.09**</td>
<td>11.17±1.74**</td>
</tr>
<tr>
<td>Acupuncture group</td>
<td>1.90±0.33#</td>
<td>6.86±1.37##</td>
</tr>
</tbody>
</table>

Note: compared with normal group, **means P<0.01; compared with model group, ## means P<0.01

The Effect of Acupuncture on Oxidative Damage of Spleen in Rats after Exhaustive Exercise

As shown in Table 2, the model group’s MDA content of spleen was dramatically higher than normal model, T-SOD activity and GSH content were significantly slower than normal group (p<0.01). While compared with model group, the acupuncture group’s spleen MDA content reduced dramatically, T-SOD activity and GSH content ascended significantly(p<0.01).

Table 2. Statistic of Oxidative Damage of Spleen in Rats (x ± s, n=10).

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/mg prot)</th>
<th>T-SOD (U/mg prot)</th>
<th>GSH (U/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>2.006±0.138</td>
<td>7.206±0.106</td>
<td>1.094±0.066</td>
</tr>
<tr>
<td>Model group</td>
<td>2.715±0.116**</td>
<td>6.523±0.129**</td>
<td>0.836±0.078**</td>
</tr>
<tr>
<td>Acupuncture group</td>
<td>2.127±0.107##</td>
<td>7.074±0.112##</td>
<td>1.037±0.098##</td>
</tr>
</tbody>
</table>

Note: compared with normal group, **means P<0.01; compared with model group, ## means P<0.01

The Effect of Acupuncture on Levels of Spleen Cells Secreted in Rats after Exhaustive Exercise.

As shown in Table 3, levels of IL-2 and IFN-γ of spleen cell secreted by model group were significantly slower than normal group (p<0.01). Levels of IL-2 and IFN-γ of spleen cell secreted by acupuncture group were dramatically higher than model group (p<0.01).

Table 3. Statistic of Levels of Spleen Cell Secreted in Rats (x ± s, n=10).

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-2 (cpm)</th>
<th>IFN-γ (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>25053.3±1247.7</td>
<td>0.472±0.049</td>
</tr>
<tr>
<td>Model group</td>
<td>17445.4±1785.2**</td>
<td>0.353±0.042**</td>
</tr>
<tr>
<td>Acupuncture group</td>
<td>23292.9±1670.7##</td>
<td>0.424±0.026##</td>
</tr>
</tbody>
</table>

Note: compared with normal group, **means P<0.01; compared with model group, ## means P<0.01

Discussion

The Effect of Exhaustive Exercise on the Morphology of Spleen and Splenocytes Apoptosis in Rats

Previous studies have shown that in some certain pathologic conditions (such as poisoning, radiation, exercise stress and so on), immune function would be effected obviously. There would appear pathological symptoms obviously in spleen morphology, whole structure of white pulp became unclear and with abnormal area. The Lymphocytes became abnormal, or increased
obviously and intensively, or decreased significantly and to be sparse. There would be obviously hyperaemia in red pulp, number of tissues increased and paramorphia. The lymphocytes in the marginal zone decreased significantly, number of macrophages increased, and cells ranged relatively sparse each other[2-3]. What’s more, with the pathological changes of spleen morphology, spleen cells apoptosis excessively and the expression activity of caspase-3, which as an important regulating apoptosis protein, increased significantly[4]. However, after designing sport model with six-weeks exhaustive swimming, model group’s red pulp enlarged, white pulp became narrow, lymphocyte decreased significantly, queued sparsely and tended to group. Meanwhile, number of spleen apoptosis and caspase-3 activity were significantly higher than normal group, which indicated that long-time heavy load exercise training would obviously lead to structural damage of spleen morphology.

The Effect of Exhaustive Exercise on Oxidative Damage of Spleen in Rats

The Relationship between exercise and oxidative impairment attached greatly attention of domestic academic. Many researchers explored the relationship between heavy load exercise and oxidative lesions of vital organs (or tissues), like liver, heart, kidney, skeletal muscle and brain, by detecting index of MDA, SOD, GSH and GSH-PX and so on, which can most sensitively reflect the body's metabolism. But, there are a few reports about the state of spleen function with sport oxidative damage in domestic. The study showed that after designing sport model with six-weeks exhaustive swimming, model group’s spleen MDA content increased significantly, while T-SOD activity and GSH content both decreased dramatically. Results indicated that exhaustive exercise would lead to spleen oxidative impairment.

The Effect of Exhaustive Exercise on Secretion Function of Spleen Cells

IL-2 is a cell factor generated by active CD4+ and CD8+, which is able to maintain the ability of T cells to differentiate and proliferate for a long time and promote B cells to proliferate. IFN-γ is capable of important immunoregulatory activity, which can promote T and B cells’ function in some certain conditions. Likewise, it also can be used as the effect factor of activation of IL-2 receptors on the cell surface to make IL-2 to be active [5-6]. Zhao Ke Wei concluded that the impacts of different movements (like heavy exercise, moderate exercise) and cycles on immunization factor were different [7]. While Lu Yong Mei and her collaborators reported that long-time heavy load exercises would lead to immunodepression, which resulted in levels of IL-2 and IFN-γ in Rats serum decreased significantly [8]. However, there are few research reported about spleen secret IL-2 and IFN-γ under the state of sports immunosuppressive. However, the research showed that after designing sport model with six-weeks exhaustive swimming, levels of IL-2 and IFN-γ secreted by spleen cells in model group were significantly slower than normal group. Results showed that exhaustive exercise decreased rats’ immune function.

The Effect of Acupuncture and Moxibustion on the Structure and Function of Spleen Morphology in Rats after Exhaustive Exercise

Recently, the academic community generally agrees that long-time heavy load exercises lead to decreasing immune function. Because heavy load exercises would lead to neuroendocrine-immune dysfunction, activate immune suppressor cells and generate immune suppressor factor, and disturb the balance of free radical and so on. Focusing on the immune suppression problem and solve the exercise-induced immunodepression in exercises, physical teaching and public recreation has become the focus in academic [9].

Acupuncture and moxibustion is valuable heritage of traditional Chinese medicine. It’s simple and safe. It can prevent and treat many diseases effectively. Chinese physicians think that human’s health depends on balance of internal and external environment, In other words, “YinpingYangmi, JingshenNaizhi”. Main and collateral channels play a part in contacting internal and external body, linking the whole body, promoting the movement of qi and blood, coordinating yin and yang, preventing diseases, reflecting symptom, conveying perception, adjusting Xushi and so on. It’s the main channel of operating Qi and blood. Acupuncture and moxibustion can accelerate the recovery
of the body's normal immune function by dredging meridians, BuqiZhuyang, ZiyinBuye, ShujinXingqi, Huoxue and relieving pain, activating functions of viscera.

The research claimed that exercise-induced immunosuppression mainly including organ dysfunction of the heart, spleen and brain. We selected “Sanyinjiao”, “Shenmen”, “Neiguan”, “Baihui” and “Sishencong” to experiment basis on the traditional Chinese Medicine principle that. The main acupoint “Sanyinjiao” referred to spleen channel of Foot-taiyin and channel of Foot-sanyinjiao. Modern researches showed that Sanyinjiao acupoint could adjust immune function. While assistant acupoint “Shenmen”, “Neiguan” can adjust heart function, “Baihui” and “Sishencong” were able to XingshenJiannao, AnshenDingzhi. The study treated rats with exhaustive exercise by acupuncture the referred five acupoints in a way of PingbuPingxie. The results showed that the acupuncture group’s pathological changes of spleen morphology were lighter than model group, number of apoptotic cells of spleen, caspase-3 activity, GSH and MDA content were significantly slower than model group, while T-SOD activity of spleen, levels of IL-2 and IFN-γ were dramatically higher than model group.

Conclusion

1) After designing sport model with six-weeks exhaustive swimming, the model group’s pathological of spleen morphology changed significantly, number of apoptotic cells of the spleen, caspase-3 activity, GSH and MDA contents increased significantly, while T-SOD activity of spleen, levels of IL-2 and IFN-γ decreased dramatically. They were in a state of immunocompromised obviously.

2) Acupuncture “Sanyinjiao”, “Shenmen”, “Neiguan”, “Baihui” and “Sishencong” could improve pathological of spleen morphology. They could receive the effects of adjusting immunologic function by significantly decreased number of apoptotic cells, caspase-3 activity, GSH and MDA contents and dramatically increased T-SOD activity of spleen, levels of IL-2 and IFN-γ.

References


