Effect of lit-moxa stimulation of Guanyuan (CV 4) acupoint on levels of lactic acid and super-oxide dismutase in skeletal muscle of rats

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Abstract
OBJECTIVE: To observe the effect of lit-moxa stimulating acupoint Guanyuan (CV 4) on lactic acid and super-oxide dismutase (SOD) in skeletal muscle after exercise exhaustion.

METHODS: Twenty-eight adult male Sprague-Dawley rats were randomly divided into normal control group, exhausted control group, exercise group and moxibustion group using exercise training and mild heating with lit-moxa stick as treatment methods. The exhausted control group, moxibustion group and exercise group received an exhaustive swimming after 20 days of intervention. Swimming exhausted times were recorded. Lactic acid and SOD concentration in soleus muscle were detected and compared between every two groups.

RESULTS: The swimming exhausted times of the moxibustion group and the exercise group were significantly increased compare to the exhausted control group ($P < 0.05$). The lactic acid of the exhausted control group was significantly increased comparing with the normal control group ($P < 0.05$), and the lactic acid of the moxibustion group and the exercise group were significantly lower than that of the exhausted control group ($P < 0.05$). The SOD level of the exhausted control group was significantly decreased comparing with the normal control group ($P < 0.05$), and the SOD level of the moxibustion group and the exercise group were significantly lower than that of the exhausted control group ($P < 0.05$). The SOD level of the exhausted control group was significantly decreased comparing with the normal control group ($P < 0.05$), and the SOD level of the moxibustion group and the exercise group were significantly higher than that of the exhausted control group (both, $P < 0.05$). There was no statistical difference between the moxibustion group and the exercise group.

CONCLUSION: With lit moxa stick, heat stimulating acupoint of Guanyuan (CV 4) decreased the levels of lactic acid and SOD in rat’s skeletal muscle.
INTRODUCTION

Animal’s exercise tolerance relies on the continuous contraction of skeletal muscle, in which there is a rapid increase of lactic acid and free radicals that ultimately leads to exercise-induced fatigue. Super oxide dismutase (SOD), a specific anti-oxidant enzyme, is effective in scavenging oxygen free radicals, preventing lipid peroxidation and maintaining cell function. Recent experiment indicates that exercise training can prevent the decrease of SOD level in skeletal muscle causing by exhaustion. In China, applying moxibustion therapy at Guanyuan (CV 4) point is a traditional way to strengthen the body and prevent diseases. Our previous studies have shown that moxa heat stimulation had a positive effect on the SOD concentration in rat. In order to know whether the therapy at acupoint of Guanyuan can improve exercise tolerance, and if the effect is related with the changing of SOD level, we designed this experiment.

MATERIALS AND METHODS

Animals and grouping

Twenty-eight Sprague-Dawley male rats of clean grade, 3.5 months of age, 180-200 gram of weight, provided by Beijing Vital River Laboratory Animal Technology Co., Ltd., (Certificate of quality No. SCXK [Beijing] 2006-0009), fed with rat ordinary feed in clean cabinets with temperature of (20 ± 1) °C and humidity of 50% in the mechanism laboratory of acupuncture and moxibustion of Beijing university of Chinese medicine. After one week of acclimatization, an exhaustion swimming was carried out with random sequence and the exhaustion time of each rat was recorded. Then the rats were divided into four groups by random number table method: normal control group, exhaustion control group, moxibustion group, and exercise group. There was no difference in swimming ability between each groups.

Treatment methods

The normal control group received a sham treatment of 5 min of grasping along with 5 min of soaking in water every other day in twenty days. The exhaustion control group received the same sham treatment and an exhaustion swimming in the last day. The moxibustion group received ten minutes of moxibustion every other day in twenty days and an exhaustion swimming in the last day. The exercise group received ten minutes of swimming exercise training every other day in twenty days and an exhaustion swimming in the last day. All rats were executed and sampled in the last day immediately after the exhaustion swimming. All experimental protocols were approved by the Review Committee for the Use of Animal Subjects of Beijing University of Chinese Medicine.

Moxibustion

Smokeless moxa stick, one centimeter of diameter, nine centimeters of length, provided by Wolong Chinese Medicine Moxa Factory, was affixed to an iron holder, the height can be adjusted. Guanyuan (CV 4) point was located in the mid-point of the line connects the roots of the two hind legs of the rat. Rats of the moxibustion group, with their eyes covered, were turned over by the operators and laid down under the moxa holder. Guanyuan point of the rat was located directly under the burning point of the moxa stick. The distance between Guanyuan and the burning point was keeping in two centimeters by continuous adjusting of the moxa holder.

Swimming exercise training

The swimming place was an eighty centimeters high plastic bucket with diameter of fifty centimeters. The water temperature was (30 ± 1) °C and water depth was fifty centimeters. While swimming, a lead weight of 5% body weight was attached on each rat’s tail. Rats were being driven by the operator with a plastic rod. The swimming exercise time was half of the average swimming exhausted time before the grouping.

Modeling (exhausted swimming)

The swimming place and lead weight on tail used the same standard in the swimming exercise training. The exhaustion criterion was: the rat’s swimming movement is obviously uncoordinated, then sinks into the water for more than ten seconds before it is able to emerge from the water or unable to emerge.

Sampling

Rats were decapitated; the right side musculus soleus were taken out into individual cryovials and immediately put into liquid nitrogen for 5 min before removing into - 80 °C refrigerator.

Instruments and reagents

electronic scale; digital timer; JY99-Ⅲ B supersonic (Ningbo New Iris Branch Research institute, Ningbo, China); 5415R supercentrifuge (German Eppendorf Corporation, Hamburg, Germany); Lambda P40 UV vis spectrophotometry meter (US PE Corporation, Waltham, MA, USA).

Lactic acid biochemical kits produced in Nanjing Jiancheng Bioengineering Institute, batch number: 20100417 (Nanjing, China). SOD biochemical kits produced in Nanjing Jiancheng Bioengineering Institute, batch number: 20100429 (Nanjing, China).
**RESULTS**

**Average value comparison of swimming exhausted time after grouping**

The average value comparison of swimming exhausted time after grouping of normal control group, exhaustion control group, moxibustion group and exercise group is 16 ± 4, 18 ± 5, 16 ± 4, and 16 ± 4. There was no statistical difference among every group in swimming exhausted time.

**Average value comparison of final swimming exhausted time**

The average value comparison of final swimming exhausted time after grouping of normal control group, exhaustion control group, moxibustion group and exercise group is 10 ± 8, 28 ± 11, and 36 ± 18. The average exhausted times of the moxibustion group and the exercise group were significantly longer than that of the exhaustion control group (both P < 0.05).

**Average value comparison of lactic acid level in musculus soleus**

The average value comparison of lactic acid level in musculus soleus of normal control group, exhaustion control group, moxibustion group and exercise group is 6.9 ± 0.9, 10.5 ± 1.3, 8.8 ± 0.9, 8.0 ± 1.0. The average lactic acid level of the exhaustion control group was significantly higher than that of the normal control group (P < 0.05); and the average lactic acid levels of the moxibustion group and the exercise group were significantly lower than that of the exhaustion control group (P < 0.05).

**Average value comparison of SOD level in musculus soleus**

The average value comparison of SOD level in musculus soleus of normal control group, exhaustion control group, moxibustion group and exercise group is 61 ± 13, 36 ± 6, 49 ± 8, 57 ± 8. The average SOD level of the exhaustion control group was significantly lower than that of the normal control group (P < 0.01); and the average SOD levels of the moxibustion group and the exercise group were significantly higher than that of the exhaustion control group (P < 0.05).

**DISCUSSION**

Moxibustion therapy and acupuncture are traditional Chinese medical methods thought to be able to improve the immunity of the body. Its theory comes from The Inner Canon of Huangdi, which values the importance of disease prevention. Guanyuan (CV 4), point of Ren channel, front-Mu point of small intestine, crossing point where the three Yin channels of foot meets Ren channel, is closely related to the generation of Yuan Qi (original vital energy) and being considered by ancient Chinese doctors as the most important acupoint for health keeping. Our previous studies showed that applying moxibustion therapy at acupoint of Guanyuan (CV 4) had beneficial effects on certain disorder conditions in illnesses. The regulation was considered to be a comprehensive promoting effect involving multiple systems of the body.

Acute exhaustive exercise tolerance, as a manifestation of comprehensive biophysical ability of animal, can manifest the whole comprehensive regulative function of moxibustion therapy more appropriately than specific biochemical indicators. Muscle lactic acid, as one of the most important reasons to produce exercise-induced fatigue, has been commonly used to measure the motor ability of the body in experiments. In this experiment, the lit-moxa stimulation improved the swimming tolerance and decreased the lactic acid concentration after exhaustion.

SOD is one of the important matters to protect muscle cells from exercise-induced rising of oxygen radicals, its concentration level shows how well the muscle function can be protected. In this experiment, the results have shown the same changing pattern of SOD concentration in moxibustion group and exercise group, which suggests the lit-moxa stimulation at Guanyuan point might be beneficial to the muscle of the rats.

In conclusion, the stimulating the acupoint of Guanyuan in rats with lit-moxa stick may improve their swimming exercise tolerance, and decrease the levels of lactic acid and SOD in their skeletal muscle.

**REFERENCES**

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