Zhou LG, Zhou XH, Xu XK, *et al*. Experimental study on the effect of moxibustion at Shenque (CV 8) for long-term exercise-induced fatigue. J Acupunct Tuina Sci, 2017, 15(6): 387-391
DOI: 10.1007/s11726-017-1033-8

Basic Study

Experimental study on the effect of moxibustion at Shenque (CV 8) for long-term exercise-induced fatigue

艾灸神阙穴抗长期运动疲劳作用的实验研究

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Abstract

Objective: To investigate the effect of moxibustion at Shenque (CV 8) on fatigue in rats with chronic exercise-induced exhaustion.

Methods: Thirty male Sprague-Dawley (SD) rats were randomly divided into a blank group, a model group and a moxibustion group, 10 rats in each group. Except rats in the blank group, the remaining rats were subjected to create long-term exhaustion models by repeated swimming. After successful modeling, rats in the moxibustion group received mild moxibustion at Shenque (CV 8) for 15 min, once every other day with a total of 10 times. Rats in the model group and the blank group did not receive moxibustion. At the end of the treatment, the exhausted times, and the body weight of rats before and after the experiment were compared among groups. The levels of blood malondialdehyde (MDA) and urea nitrogen (BUN), as well as the activities of aspartate transarninase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were also measured by the automatic biochemical analyzer, 24 h after the exhausting excise.

Results: The 10th swimming time was significantly longer in the moxibustion group than that in the model group (P<0.01). The increase rate of the body weight was lower in the rats of the moxibustion group than that in the model group before the 7th and the 10th exhausting excise (P<0.05, P<0.01). The levels of serum MDA and BUN, as well as the activities of AST, ALT and LDH in the model group were higher than those in the blank group (all P<0.01). The levels of serum MDA and BUN, as well as the activities of AST, ALT and LDH in the moxibustion group were lower than those in the model group (P<0.01).

Conclusion: Moxibustion at Shenque (CV 8) can decrease the serum levels of MDA and BUN, as well as activities of AST, ALT and LDH in the long-term fatigue rats, thus to improve the symptoms of fatigue.

Keywords: Moxibustion Therapy; Mild Moxibustion; Point, Shenque (CV 8); Exhaustive exercise; Fatigue; Rats

【摘要】目的：探讨艾灸神阙穴对长期力竭大鼠抗疲劳性运动作用的影响。**方法：**将30只Sprague-Dawley(SD)雄性大鼠随机分为空白组、模型组和艾灸组，每组10只。除空白组外，其余大鼠采用反复游泳实验制造长期力竭模型。造模成功后，艾灸组大鼠温和灸神阙穴15 min，隔日1次，共治疗10次。模型组和空白组大鼠不接受治疗。治疗结束后比较各组大鼠的力竭时间及实验前后的体质量，并采用全自动分析仪检测力竭运动24 h后血清中丙二醛(MDA)、血尿素氮(BUN)浓度及天门冬氨酸转氨酶(AST)、丙氨酸转氨酶(ALT)和乳酸脱氢酶(LDH)活性。**结果：**第10次游泳时间比较，艾灸组明显较模型组长(P＜0.01)。艾灸组大鼠第7次、第10次力竭运动前体质量增长速度慢于同期模型组(P＜0.05，P＜0.01)。模型组大鼠血清MDA和BUN含量及AST、ALT和LDH活性均较空白组升高(均P＜0.01)。艾灸组大鼠血清MDA和BUN含量及AST、ALT和LDH活性均较模型组降低(P＜0.01)。**结论：**艾灸神阙穴可以降低长期疲劳大鼠血清中的MDA和BUN含量及AST、ALT和LDH活性，改善疲劳症状。

【关键词】灸法；温和灸；穴，神阙；力竭运动；疲劳；大鼠

【中图分类号】R2-03　　**【文献标志码】**A

At the 5th International Conference on Exercise Biochemistry, the exercise fatigue is defined as that 'the body's physiological process cannot keep the body at a specific level or cannot maintain the intended exercise intensity', which includes physical fatigue and psychological fatigue. Physical fatigue is mainly manifested as decreased athletic ability, which can cause energy deficiency, decreased motor function, metabolic waste accumulation and other adverse

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effects. Physical fatigue falls under the category of deficiency overexertion in Chinese medicine mainly characterized by limb weakness, lassitude and fatigue caused by yang qi deficiency or exhaustion[1-2]. In recent years, Chinese medicine has made gratifying achievements in improving physical fatigue[3-6]. However, few researches have been done on the study of acupuncture and moxibustion on physical fatigue or improving the long-term fatigue. In this study, long-term fatigue model was established by repeated exhausting swimming. The effects of moxibustion at Shenque (CV 8) on serum levels of malondialdehyde (MDA) and urea nitrogen (BUN), as well as the activities of aspartate acid transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) were detected to explore the possible mechanism.

1 Materials and Methods

1.1 Laboratory animals and groups

Thirty clean grade male Sprague-Dawley (SD) rats, body weight (200±10) g, were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., China [license No.: SCXK (Beijing) 2012-0001]. Rats were randomly divided into three groups according to the random number table, a blank group, a model group and a moxibustion group, 10 rats in each group.

Rats were adaptively fed for 7 d, and swam once on day 3 and day 6 respectively, 3 min/time. All animal experiments followed the Experimental Animal Regulations of Hebei University of Chinese Medicine.

1.2 Main reagents and instruments

Kits of BUN, AST and ALT (Changchun Jiangli Biotechnology Co., Ltd., China); kits of MDA and LDH (Nanjing Jiancheng Institute of Bioengineering, China); self-made thermostatic water tank (200 cm×80 cm); fine moxa stick (7 mm in diameter, 117 mm in length, Henan Nanyang Chinese medicine moxa Co., Ltd., China); homemade moxibustion box for rats[7]; TDL-A-type centrifuge (Shanghai Anting Science Instrument Factory, China); AU400 automatic biochemical analyzer [Keler Gewani (Shanghai) Analytical Instrument Co., Ltd., China].

1.3 Model preparation

Rats in the model group and the moxibustion group were subjected to create exhaustive exercise model with reference to the literature[8-9]. The rats were placed in a thermostatic water tank [50 cm in depth, temperature at (30±2) ℃] with a piece of lead (weighing 5% of the body weight) wrapping around the tail (at 1-5 cm from the base of the tail] for the exhausting exercise (exhaustion criteria: rat swimming was abnormal and cannot continue; the nose was under water for 5 s and unable to return to the surface of water), once every other day for 10 continuous times.

1.4 Statistical methods

The SPSS 13.0 version software was used for statistical analysis. Measurement data in normal distribution were represented as mean ± standard deviation ($\bar{x}\pm s$). One-way ANOVA was used to compare between groups. All analyses were performed with two tailed test. $P<0.05$ indicated a statistical significance.

2 Intervention Methods

Moxibustion group: Rats were placed in the special rat moxibustion box immediately after the exhausting swim each time to perform mild moxibustion at Shenque (CV 8) for 15 min, once every other day with a total of 10 times.

Model group: Rats were placed in the special rat moxibustion box immediately after the exhausting swim each time without moxibustion for 15 min, once every other day with a total of 10 times.

Blank group: Rats did not receive exhausting swimming experiment or moxibustion intervention, only were placed in the special rat moxibustion box for 15 min, once every other day with a total of 10 times.

3 Observation Results

3.1 Observed indicators and methods

3.1.1 Exhausted time

The 10th exhausting swim time was recorded by designated person with the unit of second.

3.1.2 Body weight

The body weights of rats before the 1st, 4th, 7th, and 10th exhausting swim were measured by designated person with the unit of gram.

3.1.3 Serum biochemical markers

The changes of rat serum levels of MDA and BUN, activities of AST, ALT and LDH in each group were observed.

At 24 h after the 10th exhausting swim, rats in each group were anesthetized with ether and 5 mL blood was collected by decapitation. Serum was separated by centrifugation at 4 ℃ and 3 000 r/min for 10 min (8 cm in centrifugal radius) and kept at −80 ℃ cryopreservation to be measured. Concentrations of serum MDA and BUN, as well as activities of AST, ALT and LDH were measured by automatic biochemical analyzer. All tests were conducted by Research Center of Hebei University of Chinese Medicine.

3.2 Results

3.2.1 Changes of the exhaustion time and body weight before and after the experiment

The exhaustion time of the moxibustion group was significantly longer than that of the model group ($P<0.01$); the body weight increase of the model group was significantly lower than that of the blank group

($P<0.01$), which indicated that the long-term fatigue model was successful.

The rat body weight in each group was increased with the prolongation of the feeding time. The increase rate of the rat body weight in the model group was lower than that in the same time point blank group before the 7th, 10th exhausting swim ($P<0.05$). The increase rate of the rat body weight in the moxibustion group was slower than that at the same time point in the model group before the 7th and 10th exhausting swim ($P<0.05$ or $P<0.01$), (Table 1).

Table 1. Comparison of exhaustion time and changes of body weight before and after the experiment among groups ($\bar{x}\pm s$)

Group	n	Exhaustion time (s)	Body weight (g)			
			1st time	4th time	7th time	10th time
Blank	10	/	240.17±6.44	269.02±4.99	300.75±4.80	321.98±5.77
Model	10	370.57±34.68	240.32±6.23	264.88±9.93	285.05±14.95 1)	299.79±12.50 1)
Moxibustion	10	582.14±52.97 2)	239.61±6.19	260.79±9.05	274.64±9.25 2)	289.14±12.12 3)

Note: Compared with the blank group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$, 3) $P<0.05$

3.2.2 Changes of serum MDA and BUN levels

The levels of serum MDA and BUN in the model group were significantly higher than those in the blank group (all $P<0.01$). The levels of serum MDA and BUN in the moxibustion group were significantly lower than those in model group (all $P<0.01$), (Table 2).

3.2.3 Changes of serum AST, ALT and LDH activities

The activities of serum AST, ALT and LDH in the model group were significantly higher than those in the blank group (all $P<0.01$). The activities of serum AST, ALT and LDH in the moxibustion group were significantly lower than those in the model group ($P<0.01$), (Table 3).

Table 2. Comparison of serum MDA and BUN levels in each group ($\bar{x}\pm s$)

Group	n	MDA (umol/L)	BUN (mmol/L)
Blank	10	5.88±0.43	5.89±0.56
Model	10	7.58±0.33 1)	7.95±0.85 1)
Moxibustion	10	5.94±0.44 2)	6.01±0.40 2)

Note: Compared with the blank group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$

Table 3. Comparison of changes in activities of serum AST, ALT and LDH in each group ($\bar{x}\pm s$, U/L)

Group	n	AST	ALT	LDH
Blank	10	98.58±6.63	54.49±4.30	956.76±69.32
Model	10	119.47±6.32 1)	67.50±3.07 1)	1 277.16±57.51 1)
Moxibustion	10	98.65±4.10 2)	54.39±4.40 2)	975.13±90.84 2)

Note: Compared with the blank group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$

4 Discussion

Fatigue is related to long-term or high-intensity exercise causing energy consumption, metabolites accumulation and other factors in the body[10]. Studies have shown that early movement consumes glycogen in the body. With the continuation of movement and reduction of glycogen material, fat will be broke down to produce fatty acids. The oxygen free radicals and fatty acids will cause lipid peroxidation reaction to produce MDA. The increase of MDA during exercise is an important factor leading to exercise-induced fatigue[11]. The body will break down the protein to make up for insufficent energy in the body during further exercise, and the metabolism will produce BUN. BUN level increases with the increase of exercise load, which can be used to determine the degree of body fatigue[12].

Repeated exhaustive exercise in rats is the extreme movement, which will affect the blood distribution and make multiple organ ischemia, thus resulting in a hypoxia environment[13]. Meanwhile, a large number of lactic acid and lipid peroxidation during this process will cause tissue damage. A large number of intracellular AST, ALT, LDH and other substances are released into blood[14]. Loss of these enzymes leads to cellular energy metabolism disorders[15]. Studies have shown that MDA, BUN and other substances are significantly increased immediately after prolonged high intensity exercise. With the gradual extension of the recovery time, muscle glycogen can be restored; protein catabolism is significantly reduced; MDA, BUN and other substances are gradually reduced and restored to the normal range 24 h after the exercise[16]. Therefore, the main purpose of this study was to observe the anti-fatigue effect of moxibustion based on the autoregulation.

Material consumption and dysfunction belongs to the scope of deficiency syndrome in traditional Chinese medicine. The treatment principle is to tonify or reinforce the body. Therefore, many doctors use tonifying method to treat the deficiency. Experiments have shown that reinforcing kidney and strengthening spleen can effectively relieve exercise fatigue[17-18]. Shenque (CV 8) belongs to the Conception Vessel. Moxibustion at Shenque (CV 8) can benefit both the root of innate endowment and the foundation of acquired constitution[19-21].

Studies have found that overexertion easily damages the spleen. Energy consumed during the exercise is from the transportation and transformation of the spleen. Long-term exercise fatigue can cause the dysfunction or decline of transportation and transformation in the spleen, resulting in fatigue of limbs, lassitude and weakness, and other symptoms due to spleen qi deficiency[22-24]. Therefore, the body weight of the model group was higher than that of the moxibustion group in the current study.

The results of this study show that long-term fatigue exercise will increase the levels of plasma MDA and BUN, as well as the activities of AST, ALT and LDH, thus the body is in a state of fatigue. Moxibustion at Shenque (CV 8) can effectively improve the internal environment of long-term fatigue rats, reduce the levels of serum MDA and BUN, as well as the activities of AST, ALT and LDH, which provides a certain experimental basis for the clinical application of moxibustion at Shenque (CV 8) to treat the long-term exercise fatigue. In the future, the dynamic observation should be performed to further understand the mechanism of moxibustion at Shenque (CV 8) for anti-exercise fatigue more systematically.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

Acknowledgments

This work was supported by Science and Technology Support Project of Hebei Provincial Administration of Traditional Chinese Medicine (河北省中医药管理局科技支撑项目, No. 2016011, No. 2017011); Youth Scientists Fund of Natural Science Foundation of Education Department of Hebei Province (河北省教育厅青年自然科学基金项目, No. QN2016021).

Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria in this experiment.

Received: 2 June 2017/Accepted: 30 June 2017

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