

The Effects of Moxibustion Shenshu on Exercised Rats

艾灸腎俞對運動大鼠的作用

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Abstract

Objective: The effects of moxibustion on exercised rats were investigated. **Methods:** Rats were exercised using a swimming model and moxibusting shenshu (U. B.23) was applied every other day to treatment groups for six weeks. **Results:** It was found that moxibustion shenshu (MS) could attenuate exercise-induced changes in hypothalamus-pituitary-adrenal axis (HPA), decrease the level of enzymes in serum and relieve the decreased levels of SOD in both liver and muscle tissues. **Conclusion:** The application of MS could be applied to relieve exercise-induced fatigue in rats and its implications to human should be further studied.

Keywords: Acupuncture, Endocrine system, Fatigue, Recovery.

摘要

研究物件：採用運動大鼠研究艾灸腎俞的作用。**方法：**採用游泳大鼠運動模型訓練6周，對腎俞進行隔天艾灸。**結果：**艾灸腎俞可以緩解運動誘發的下丘腦-垂體-腎上腺軸功能的變化，降低血清酶的升高和緩解肌組織和肝組織超氧化物歧化酶的降低。**結論：**艾灸可用於緩解大鼠運動誘發的疲勞，其對人體的作用有待進一步的研究。

Introduction

Exercise-induced fatigue has been widely studied and many effective ways to prevent overtraining and exercise-induced fatigue were investigated. There are, however, only a few studies using acupuncture to relieve exercise-induced

fatigue have been reported. Acupuncture is a non-peculiar stressor that regulates dysfunction and rebalances the metabolism of the body through stimulating one's inherent mechanism (Andersson, et al.,1995; WHO 2002). The purpose of study was to investigate the effects of moxibustion Shenshu (MS -a form of acupuncture) on exercise-induced fatigue and to better understand the possible regulatory mechanisms in the function and metabolism of fatigue.

Methods

Subjects were 78 male SD rats, weighing between 190–210 gm. They were housed under controlled environmental conditions ($22^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, relative humidity 40%–60%, 12 hour alternate light/dark cycles, food and water ad libitum) for two days for adaptation in new cages. They were then divided randomly into four groups: control group (CON), exercise group (E), exercise + moxibustion shenshu (U. B.23) group (EM), exercise + moxibustion on non-point group (EN).

Exercised rats (E, EM, EN) were required to swim (water temperature around $29^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for the first 3 days for 30–60min, and then for 120min at the end of first week. The swimming duration was increased to 180 min at the end of second week, and by the third week, an attachment (at the tail) of 2% to 3% of the body weight was added to the exercised rats with the swimming time maintained between 30–90min - the training durations varied according to the stamina of exercised rats. It was maintained until the end of six weeks. Rats swam 6 days a week for 5 weeks and during the last week when they swam every other day. The swimming exercise was stopped when rats showed signs of fatigue (drowning) and the swimming time for the last week lasted 50 min (Zheng 2000; Tian. 2002).

During the period of swimming exercise, the CON group received neither exercise stress nor stimulation with MS. The EM group was given MS treatment bilaterally for 20 min every other day, and the EN group received the same procedure except the stimulating point was one cm away from the shenshu -where no acupuncture point was located. Anatomical location of stimulated acupoints were determined according to the rat acupoint atlas (Hua 1991).

All subjects were sacrificed by decapitation immediately at the end of six weeks. Trunk blood was collected in tubes (with EDTA for plasma) and then centrifuged (3000rpm) for 15 min at 4°C . The serum and plasma were collected and stored at -80°C until assayed. Muscle and liver tissues were weighted and homogenized in cold 0.9% NaCl, and stored in -40°C for assessment of superoxide dismutase (SOD). Creatine kinase(CK), lactic dehydrogenase (LDH), succinic dehydrogenase (SDH), and the supernatant for superoxide dismutase(SOD) were measured with procedures recommended by the manufacturer. Hypothalamus (H) was boiled in phosphate buffer for 10 min, then weighed and homogenized in cold 1mmol/l Acetic acid 0.5ml, and was centrifuged at 3000 rpm for 30min at 4°C . Then 1mmol/l NaOH 0.5ml, the supernatants were used for CRH analysis. Testosterone (T), cortisol (C), adrenocorticotrophic hormone (ACTH), and corticotrophin release factor (CRF) were measured with RIA kits using the procedures recommended by the manufacturer.

Data obtained are presented as means (\pm standard deviations). To evaluate the differences between groups, an analysis of variance (ANOVA) was conducted with a confidence level of $p < 0.01$ used.

Results

Body weight

Body weight was measured every week and it was found that from the third week, E was significantly lighter than CON ($P < 0.01$). At the end of six weeks, the body weight of EM was significantly higher than E ($P < 0.01$).

Table 1. Body Weight of Rats.

Group	Pre-exercise	1st week	2 nd week	3rd week	4 th week	5th week	6th week
CON	210.36±5.94	276.27±9.09	319.82±14.44	362.82±17.02	405.73±25.88	426.91±25.62	441.45±28.79
E	209.5±5.09	264.00±18.65	290.50±17.68*	302.25±26.24*	315.38±21.22*	324.88±19.64**	337.25±18.34**
EM	207.70±5.60	267.80±9.80	297.07±12.40	318.50±13.40*	337.10±21.42*	354.20±21.79*#	368.90±23.48*#
EN	209.89±3.10	265.44±9.38	289.44±11.92	308.44±20.16*	322.22±26.16*	330.67±26.41**	347.33±28.06**

P* < 0.01 ; ** < 0.001 compared with control group; P # < 0.01 ; compared with exercise group.

Serum levels of T, C and T/C

After exercise, E had significantly lower levels of T, T/C (P < 0.01), and EM was also higher than E (P < 0.01).

The levels of C of E was significantly higher than the CON group (P < 0.01), but there were no significant differences between EM and E.

Table 2. Serum Levels of T, C and T/C.

Group	N	T(ng/ml)	C(ng/ml)	T/C (×10 ⁻²)
CON	11	1.99±0.29	25.58±3.49	7.92±1.50
Exercise	10	0.93±0.21*	32.35±4.61*	2.31±0.54*
EM	10	1.73±0.20#	27.31±4.19	7.37±1.21#
EN	9	1.08±0.18	31.66±4.73	3.49±0.81

P* < 0.01: compared with control group; P # < 0.01: Compared with exercise group.

Serum Levels of ACTH and CRH in Hypothalamus

It was found that the serum level of ACTH increased

in E but was not significantly different from the CON group. Similar results were also observed in the level of CRH.

Table 3. Serum Levels of ACTH and CRH in Hypothalamus.

Group	N	ACTH(pg/ml)	CRH(pg/mg)
CON	11	34.12±5.92	176.56±44.78
E	10	40.86±6.57	235.33±67.84
EM	10	38.12±5.18	206.23±43.80
EN	9	40.52±4.61	233.63±57.00

Serum Enzymes

There were significant increases in serum enzymes such as CK, LDH, SDH in E and EN compared with CON

($P<0.01$), while the level in EM increased significantly compared with the CON, but the increased levels were much lower than E and EN ($p<0.05$).

Table 4. Serum Enzymes Levels in Different Groups.

Group	N	CK (U/L)	LDH (U/L)	SDH (U/L)
CON	11	817.91±113.20	659.37±123.53	35.91±8.01
E	8	1708.05±225.23**	1073.60±80.36**	78.13±7.99**
EM	10	1137.22±147.80##	804.64±72.42#	51.00±16.30##
EN	9	1492.60±4240.56	972.30±81.30#	67.22±10.03#

P* <0.05 ** <0.01 ; compared with control group, # <0.05 ## <0.01 ; compared with exercise group.

Levels of SOD in Gastrocnemius Muscles and Liver

Compared with CON, the level of SOD in gastrocnemius muscles and liver in E was significantly decreased ($P<0.05$,

$P<0.01$), while the levels in EM and EN were not significantly different to CON. The level of SOD in EM was significantly higher than E ($P<0.05$).

Table 5. SOD Activities of the Gastrocnemius Muscles and Liver.

Group	N	Gastrocnemius muscles (U/ml)	Liver (U/ml)
CON	11	161.88±36.99	59.47±8.87
E	8	123.91±22.78*	43.58±12.11**
EM	10	167.46±35.83#	72.26±34.02
EN	9	147.56±37.73	63.54±20.16

* : $P<0.05$ ** : $P<0.01$ compared with control group

: $P<0.05$ compared with exercise group

Discussion

Acupuncture is a traditional Chinese medical treatment that includes body needling, moxibustion, electric acupuncture, laser acupuncture, microsystem acupuncture and acupressure (George et al., 2002). Recent scientific studies revealed that the therapeutic effects of acupuncture were brought about through its regulatory actions on various systems. It can be regarded as a nonspecific therapy with a broad spectrum of indications, particularly helpful in treating functional disorders (Edward et al. 1999). In this study, the effects of MS on exercised rats were evaluated.

Results showed that E rats ate less in comparing with the CON group after several weeks of training but EM rats showed much better appetite and gained weight. It suggested that MS might contribute to relieving exercise stress, inducing lower food intake and controlling body weight. Body weight is a good indicator that reflects the reaction to intensive exercise. It was reported that exercise induced weight loss and a loss of three percent the body weight would be an indication of overtraining. Loss in body weight due to intensive exercise is attributed to increased energy expenditure, lowered food intake, and neuro-endocrine changes such as releases of anabolic/catabolic hormones induced by exercise.

In the present study, the level of serum corticosterone in E rats increased significantly compared with the CON group after intensive exercise. Corticosterone is a steroid hormone and its synthesis and secretion are stimulated by ACTH, which is secreted from the anterior pituitary gland. Release of ACTH was in terms stimulated by CRF from paraventricular nucleus (PVN) in hypothalamus.

Prolonged aerobic exercise appears to be a potent stimulator of the adrenocortical system. An increase secretion of cortisol was proportional to the intensity of exercise (Farrell 1983). Only when exercise is greater than 70% VO_2 max, a consistent increase in cortisol is observed (Few, 1974). Thus, our results suggested that the exercise intensity in the last week of exercise was higher than mild to moderate intensity.

The level of serum testosterone was also measured in the study and a significant decrease in the E rats was noted. It has been shown that when exercise reached 20-30 min in duration, significant elevations in testosterone secretion occurred (Wikerson, Horvath & Gutin, 1980). As the duration of exercise continued, testosterone levels would continue to

increase and then begin to decline toward baseline levels before exercise was completed. With three hours of exercise, significant declines (below resting levels) in testosterone have been reported (Dessypris, Kuoppasalmi & Sdlercreutz, 1976). Such low levels might continue for 48 hours post exercise (Urhaussen & Kindermann, 1987). In the present study, the level of testosterone in E, EM and EN rats were lowered than the level of CON group, supporting the mechanisms mentioned above. Another reason might be exercise decreased the hormone reaction to the acute exercise.

Increased serum enzyme activities were reported in intensive and prolonged exercise. The efflux of muscle enzymes was related with the cellular destruction and disturbances in cell volume or in energy state development (Rose et al., 1970). The results in the present study showed that activities of CK, LDH, and SDH in serum of E rats increased significantly compared with the CON group, while activities of enzymes in EM did not change, which might suggest that MS protected muscle cell from exercise-induced damage.

The effusing of the enzymes from muscle cells were triggered by Reactive radical oxygen species (ROS) that were generated during aerobic endurance stress. The major source of ROS was believed to be the mitochondria of active muscles (Ji, 1999). To prevent exercise-induced oxidative stress, the organism should be well equipped with antioxidant defense systems including enzymes such as superoxide dismutase (SOD), catalase (CA), and glutathione peroxidase (GSH-Px). In the present study, the levels of SOD in gastrocnemius muscles and liver in E rats decreased significantly after exercise, and the level of SOD was also significantly lower than EM rats. The results suggested that MS attenuated the exercise-induced oxidative stress.

It has reported that acupuncture treatment activated the somatic afferents and these effects were eliminated after hypothalamic lesions. Experimental and clinical evidence suggested that acupuncture could also affect the sympathetic system via mechanisms at hypothalamic and brainstem levels (Wang et al., 1990). Results in the present study supported such mechanism at the hypothalamic level.

Studies showed that the effects of afferent nerve stimulation are in two phases: First is excitation of the sympathetic system, the sympathetic inhibition is not evident during the ongoing activation due to excitatory input via certain afferent somatic fibers and metabolic effects on chemoreceptors (Reid et al., 1987). Second is the post-stimulatory sympathetic inhibition.

The sympathetic inhibition attains its maximum after a few hours and can have a total duration of more than 12 hours (Rowell, 1986). Moriyama (1987) found an initial increase in activity during acupuncture, but during prolonged acupuncture and in the post stimulatory period, the sympathetic activity decreased and the recovered gradually. Sympathetic inhibition could be elicited at several acupuncture points, suggesting that a general post-stimulatory inhibition might occur (Cao et al., 1983).

Considerable evidences showed that the hallmarks of the stress reaction are activation of the sympathetic-adrenal medullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis and SAM system act in concert and exert mutual control over each other's activity. Monoamines such as noradrenaline (NA), adrenaline (A) and serotonin (5-HT) play an important role in both systems. During stress the adrenal medulla is directly exposed to high levels of cortical glucocorticoids that activates the medullary enzyme phenylethanolamine -N-methyltransferase (PNMT). Thus stress-induced activation of the HPA axis influenced the SAM system by elevating PNMT activity with a concomitant increased conversion of NA to A. Furthermore there is evidence that adrenal-cortex-derived glucocorticoids dampens sympathetic neuronal activity under basal conditions and during stressful stimulation (Kvetnansky et al., 1993). Conversely, free plasma monoamines can stimulate ACTH release through a direct pituitary mechanism and glucocorticoid release directly from the adrenal cortex (Dinan, 1996).

Numerous studies indicated that the effects of stress are mediated through the central nervous system, probably residing in the sympathetic nervous system, and also including the hypothalamus and anterior pituitary gland. Research findings suggested that there is considerable neural interaction with CRF release, and because there is a direct proportional relationship between stress and sympathetic activity, it is possible that the neural response to the stress is the cause of the release (Gann, 1985).

In this study, there were no significant changes in the levels of ACTH and CRF in E rats, and this might be due to exercise and lowered HPA response (Halson & Jeukendrup, 2004).

The present study indicated that the effects of MS on E rats might be related with its modulating the function of SAM and HPA. The mechanism might be through post stimulatory sympathetic inhibition. Attenuated changes in HPA might be

related via its inhibition and decrease the SAM activity induced by exercise after its cessation. Since SAM and HPA play an important role in increasing energy metabolism and elevating body function, MS might have positive effects in improving recovery from induced fatigue. Results showed body weight, body functions, antioxidation function and effuse of enzymes were better in the EM group. Further studies on the mechanism of acupuncture on the sympathetic system of subjects after exercise would be recommended.

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