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Experimental research

Effect of electroacupuncture on the expression of PGC-1α and UCP-1 in the brown adipose tissue of obese rats☆

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ABSTRACT

Objective: To explore the effect of peroxisome proliferators-activated receptor γ coactivator-1α (PGC-1α) and uncoupling protein-1 (UCP-1) in the brown adipose tissue (BAT) of obese rats in the process of acupuncture treatment for obesity.

Methods: Fifty clean-grade male Wistar rats with the age of 3 months were randomly divided into high-fat diet group ($n=40$) and normal diet group (control group, $n=10$). Nutritional obesity animal models were established through feeding with high-fat diet. Twenty-four animal models in the high-fat diet group were established successfully, and then they were randomly divided into model group, acupuncture group and non-acupoint group, with 8 rats in each group. In acupuncture group, Zúsānlǐ (足三里ST 36) and Tiānshū (天枢ST 25) were selected. In non-acupoint group, the non-acupoints located on 5 mm beside ST 36 and ST 25 were selected as acupuncture points, and electroacupuncture intervention was adopted for 5 times/week for 8 weeks. The body mass of obese rats was measured, the body fat ratio in BAT of rats was calculated, and the expression levels of PGC-1α and UCP-1 in BAT of rats were detected by immunohistochemical method.

Results: ① After treatment, the body mass of rats in acupuncture group reduced significantly, which increased in the other three groups. The changing value of body mass of rats in acupuncture group was higher than that in model group ($P < 0.05$), the changing value of body mass of rats in acupuncture group was higher than that in non-acupoint group ($P < 0.05$) and the difference in body mass changing value of rats between non-acupoint group and model group was not statistically significant ($P > 0.05$), the body fat ratio in BAT of rats in non-acupoint group was lower than that in acupuncture group, and the differences were statistically significant ($P < 0.05$). ② Compared with model group, the body fat ratio in BAT of rats in acupuncture group increased significantly ($P < 0.05$), and the difference in body fat ratio in BAT of rats between non-acupoint group and model group was not statistically significant ($P > 0.05$). ③ Compared with model group, the PGC-1α and UCP-1 levels in BAT of obese rats in acupuncture group increased ($P < 0.05$), and the difference in expression levels of PGC-1α and UCP-1 in BAT of rats between non-acupoint group and model group was not statistically significant ($P > 0.05$), the expression levels in non-acupoint group were lower than that in acupuncture group, and the differences were statistically significant ($P < 0.05$).

Conclusion: Electroacupuncture at “ST 36” and “ST 25” can effectively up-regulate the expression levels of PGC-1α and UCP-1 of diet induced obesity rats, it indicates that “ST 36” and “ST 25” have close relationship with obesity and it is may be one of the effect mechanisms of electroacupuncture in losing weight through facilitating the “browning reaction” of white adipose tissue.

As early as 1997, obesity was clearly defined as a disease by WHO, and has been listed as one of the world’s epidemic diseases which harms human health. The disease is one of the four largest medical social problems in the world along with alcoholism, smoking and AIDS [1]. As a safe method of losing weight,

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acupuncture-moxibustion can improve a series of symptoms of obesity through several ways. It has been proved according to current studies that acupuncture-moxibustion can effectively regulate the hypothalamic feeding center [2,3], promote gastrointestinal digestive function [4], improve the neuroendocrine state [5,6], and affect the energy consumption. However, the effect mechanism of acupuncture-moxibustion in losing weight has been still not completely clear.

With the gradually in-depth understanding of obesity, it is currently believed that the adipose tissues in mammals are composed of white adipose tissue (WAT) and brown adipose tissue (BAT), and the occurrence of obesity is closely related to WAT. In the process of becoming obesity, the excessive accumulation of white adipose cells causes the excessive storage of energy in the body, which results in the formation of obesity, and causes a series of complications, such as hypertension, hyperlipidemia, atherosclerosis and so on, thus causing great harm to health [7,8]. Previous literature [9,10] has confirmed that WAT has strong plasticity and can turn into brown under certain conditions. the browning WAT has a strong anti-obesity effect. Therefore, inhibiting the differentiation and maturation of white adipose tissue, promoting the browning reaction of white adipose tissue and improving lipid metabolism are the new ways to treat obesity. Peroxisome proliferators-activated receptor γ coactivator-1α (PGC-1α) and uncoupling protein-1 (UCP-1) are widely found in BAT. They are recognized as the marker proteins of brown adipose cells, and their levels determine whether the brown adipose cells are active or static [11]. In this study, the diet induced obesity (DIO) rat model was established. Electroacupuncture intervention at bilateral Zúsānlǐ (足三里ST 36) and Tiānshū (天枢ST 25) was adopted in order to observe the change of body mass, the body fat ratio of brown adipose tissue and the expression levels of PGC-1α and UCP-1 in brown adipose tissue, so as to clarify the mechanism of acupuncture in losing weight, and provide a more powerful scientific basis for future clinical acupuncture-moxibustion treatment of obesity.

Materials and methods

Animals

Fifty SPF Wistar rats with the age of 3 months, and body mass of 180–220 g were provided by the Laboratory Animal Center of Hubei Institute of Medical Science [License No. SCXK(Hubei)2015-0018]. After adaptive feeding for 7 days, DIO models were established. The rats were fed in the animal room of Acupuncture-moxibustion Institute, Hubei University of Chinese Medicine. Feeding conditions: ambient temperature 20–24 °C, humidity 30%−50%, illumination time 12 h/day. All the rats can drink and eat at liberty. The requirements of *Guidance on the Treatment of Experimental Animals* [12] issued by the Ministry of Science and Technology were strictly followed during the experiment.

Reagents and instruments

PGC-1α and UCP-1 antibodies(Santa Cruz Company, the U.S.), 0.25 mm × 13 mm *Hwato* filiform needles(Suzhou Medical Devices Co., Ltd.), HANS-200 model electroacupuncture device(Beijing Huayun Ante Technology Co., Ltd.), electronic balance(Sartorius, Germany), rat anti PGC-1α and UCP-1 antibodies standard substance(Shanghai Yuanye Bio-Technology Co., Ltd.), enzyme linked immunosorbent assay apparatus(iMARK, BIO-RAD), PGC-1α and UCP-1 immunohistochemical kits(Abcam Company, the U.S.), Axiovert 40CFL microscope (ZWISS, Germany), HMIAS-2000 high definition color medical image analysis system(Wuhan Champion Image Technology Co., Ltd.).

Grouping and modeling

DIO animal model was used. Forty rats were given high-fat diet: normal feed 68%, lard 10%, sugar 15%, protein powder 5%, cholesterol 1%, salt 0.8%, sodium cholate 0.2%. Another 10 rats were randomly given normal feed as control. All rats can drink and eat at liberty during the period of modeling, and the feed and water was replaced for once a day. All rats were fed for 12 weeks. The successful modeling of simple obesity rats referred to that the body mass in obesity group exceeded 20% of the body mass in normal diet group. Twenty-four rats were modeled successfully (7 rats died during the process of modeling, and 9 rats were excluded due to the unqualified weight), and they were randomly divided into model group, acupuncture group and non-acupoint group, with 8 rats in each group.

Intervention method

In order to facilitate the acupuncture operation, all rats were fixed with self-made rat garments. In acupuncture group, bilateral “ST 25” and “ST 36” were selected according to the *Study on Acupoints Atlas of Rats* [13] written by Hua Xing-bang, et al. The location of “ST 25”: 5 mm beside “Shénquè (神阙CV 8)”; “CV 8” is located in the median abdominal line, the point of intersection of upper 2/3 and lower 1/3 on the ligature between xiphoid process and superior margin of pubic symphysis; perpendicular insertion was performed at “ST 25” with a depth of 5 mm; “ST 36” is located at about 5 mm under the fibular head in the infer lateral part of knee joint in the posterior limb, and perpendicular insertion was performed at “ST 36” with a depth of 3–5 mm. Perpendicular insertion was performed by adopting 0.30 mm × 13 mm *Hwato* filiform needles, HANS-200 model electroacupuncture device was connected to “ST 25” and “ST 36” with dilatational wave at the frequency of 2 Hz/15 Hz and intensity of 1 mA to the extent that the obvious vibration was found in rats’ limbs. The manipulation was conducted for 30 min/time, 5 times/week, and lasted for 8 weeks. In non-acupoint group, the non-acupoints located on 5 mm beside “ST 36” and “ST 25” were selected as acupuncture points, and the intervention was similar to that adopted in acupuncture group. The rats in model group and control group were not treated. The rats in the four groups were fed with normal diet during the experiment.

Indices detection and methods

Body mass detection: during the experiment, the body mass of rats in each group was measured and recorded after fasting for 6 h after treatment on Friday, and the body mass changes of the obese rats during the 8 weeks were observed. Calculation method for body mass changing value: body mass changing value = body mass after acupuncture treatment–body mass before acupuncture treatment.

Mass detection of brown adipose tissue: the rats were fasted for 12 h after treatment on the last day, then the rats were intraperitoneally injected with 2% pentobarbital sodium (0.2 mL/100 g), and they were quickly killed through cervical dislocation. The brown adipose tissue in the scapula region was quickly separated within 20 min, and then the tissue was washed in cold normal saline for removing the blood. After the tissue fluid was extracted by filter paper, it was weighed on electronic balance, and the body fat ratio of brown adipose tissue was calculated. Body fat ratio = (Mass of BAT ÷ Body mass) × 1000‰.

Immunohistochemical test: after intraperitoneal anesthesia, the rats were killed through opening the chest with the heart exposed. Firstly, the tube was quickly washed with 250 ml of 0.9% sodium chloride solution at 4 °C, then 500 ml of 4% paraformaldehyde solution was perfused at 4 °C. 150 ml of paraformaldehyde solution

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