

Effect of Acupressure Therapy on Anti-inflammatory Mechanism in Obesity-induced IR Rats

Xiaochao GANG, Mingjun LIU*, Tianjiao GAO, Yuxing TAI, Yi TAN, Dongmei ZHANG, Chongwen ZHONG, Shaotao CHEN, Rongsheng JIANG, Zhengri CONG, Likun ZHENG, Peizhe LI

College of Acupuncture and Tuina, Changchun University of Chinese Medicine, Changchun 130117, China

Abstract [Objectives] To study the effect of "acupressure therapy" on anti-inflammatory mechanism in obesity-induced IR rats. [Methods] 10 rats were randomly selected and fed with normal diet as blank group, and the remaining 30 rats were fed with high-fat diet for 8 weeks. The obesity-induced IR model was induced and divided into two groups: model group and acupressure group. The acupressure group was treated by "acupressure therapy" three times a week for 6 weeks; the model group was taken 3 times at the same time every week, and the supine binding experiment lasted for 10 min for 6 weeks. Morphological observation was carried out before and after the experiment, mainly observing the general conditions and body weight. After the last intervention, the animals were killed and samples were collected. Western Blot method was used to detect the expression of TNF- α and IL-6 protein in adipose tissue of the three groups. [Results] Compared with the blank group, the expression of TNF- α and IL-6 protein in adipose tissue of the model group increased significantly ($P < 0.01$); compared with the model group, the expression of TNF- α and IL-6 protein in adipose tissue of the acupressure group decreased significantly ($P < 0.01$). [Conclusions] After intervening in the obesity-induced IR rats with acupressure method, the status of insulin resistance in obese rats was improved by inhibiting the expression of inflammatory factors.

Key words Massage, Acupressure therapy, Obesity and insulin resistance, Anti-inflammatory mechanism

1 Introduction

Obesity is a chronic multi-factor and multi-systemic disease caused by the interaction of heredity and lifestyle. With the increasing incidence of obesity in the world year by year, it brings more and more social and economic burdens^[1–2]. Adipose tissue can release anti-tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), and obesity can cause insulin resistance (IR)^[3–4], and it can endanger the lives and health of critical patients, so it is urgent to seek effective and safe therapies. It is found that one of the core pathological changes in IR caused by obesity is to cause low-grade chronic inflammation of patients. Now, in accordance with the characteristic massage method "acupressure therapy" of Changbai Mountain characteristic school, the related experiment of this massage method was innovatively carried out from the inflammatory reaction level, and the mechanism of "acupressure therapy method" in treating obesity-induced IR rats was preliminarily discussed.

2 Materials and methods

2.1 Material instruments Forty 8-week-old SPF SD male rats, weighing (200 ± 20) g, were supplied by Liaoning Changsheng Experimental Animal Technology Co., Ltd. Transfer electrophoresis instrument, semi-dry film transfer instrument, 170-4481

(BIO-RAD Company); Gel Imaging Analysis System, Tanon 1600 (Shanghai Tianneng Company); rapid blood glucose meter, Performa (Roche Group); frozen centrifuge, M1324R (RWD Company); microplate reader, Varioskan LUX, ThermoFisher Scientific, etc.

2.2 Modeling and grouping All animals were kept adaptively in SPF animal room for 1 week. One week later, 10 animals were randomly given basic diet as blank group, and the remaining animals were given high-fat diet (5.4 kcal/g 38.5% carbohydrate, 15% protein and 46.5% fat) to make model. After feeding for 8 weeks, IR model was induced. The general conditions, body weight, body length (distance between anus and nose) were observed, and Lee's index was calculated to evaluate the model. The Lee's index and the standard that the body weight is more than 20% higher than the average of the blank group, were used to evaluate whether the obese animal model was successfully constructed. The successfully constructed obese animal models were randomly divided into two groups. Fasting blood glucose was measured by tail arteriovenous hyperinsulinemia-positive glucose clamp, and glucose infusion rate was calculated. The blank control group was taken as the baseline, and the IR model was regarded as successful when it was lower than the baseline by more than 20%. The animals were randomly divided into two groups, acupressure group and model group, excluding the rats removed from samples due to death and other causes, and 10 rats were randomly kept in each group.

2.3 Intervention methods In the blank group, only observation and detection were carried out, and no other intervention was given.

Model group: Every week from Monday to Friday, the rats were grabbed three times with grabbing interval of 1 d for 6 weeks

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* Corresponding author. E-mail: 1245883827@qq.com

to make 10-min binding experiment each time.

Acupressure group: The rat intervention was based on the treatment principle of "dredging, regulating and supplementing".

Acupoint selection: Qihai, Zhongwan, Guanyuan, Daheng and Tianshu were treated with "acupressure method" once a day at a fixed time, with an operation time of 10 min and three times a week. Refer to *Veterinary Acupuncture and Moxibustion* (Edited by Yang Ying. *Veterinary Acupuncture and Moxibustion (First Edition)*), *etc.* for acupoint selection.

Operation: (i) Dredging method—from the position of Conception Vessel and Stomach Meridian of Foot-Yangming in the abdomen 5 inches above the navel to the position 5 inches below the navel, rub the three meridian lines horizontally with the middle finger, and only drive the epidermal tissue when exerting force, operating for 3 min. (ii) Regulating method—press the Tianshu acupoint (double) and Daheng acupoint (double) of Stomach Meridian of Foot-Yangming with the thumb tip, and exert heavy force when exhaling and light force when inhaling; the pressing force measured by massage manipulation tester was about 0.5 kg; 1 min for each acupoint, 4 min in total. (iii) Supplementing method—rub Zhongwan, Qihai and Guanyuan acupoints clockwise with thumb, and drive subcutaneous muscle tissue for 3 min.

2.4 Index detection

2.4.1 Body weight. During the experiment, the general conditions of rats was observed and weighed regularly, and the body weight of rats was measured and recorded.

2.4.2 Expression of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) protein in adipose tissue. Westernblot was used. After extracting the total protein, 80 μ L of sample was mixed with 20 μ L of 1 : 5 sample treatment solution. RhoA rabbit anti-rat (Ab86297, Abcam) and ROCK2 rabbit anti-rat (Ab71598, Abcam) primary antibodies were prepared. GAPDH was used as internal reference (1 : 1 000, rabbit anti-rat, Ab9484, Abcam). After rewarming at 37 °C for 30 min, they were washed with Tris-Buffer saline (TBST) containing Tween 20 for 3 times, and then incubated. After washing with TBST, enhanced chemiluminescence (ECL) was added. Samples were developed and exposed in Sage Creation gel imaging system, and each group of Western blotting band was repeated 3 times.

2.5 Statistical methods SPSS 26.0 software was used for statistical analysis. Independent sample *t*-test was used for comparison between the two groups, one-way ANOVA was used for comparison among the three groups, and Dunnett method was used for multiple comparison. Drawing was done on GraphPad Prism 8.0. $P < 0.05$ indicated that the difference was statistically significant, and $P < 0.01$ indicated that there was statistically significant difference.

3 Results and analysis

3.1 Morphological observation The fur of animals in the blank group was smooth and shiny, and the mental state and activ-

ities showed good performance. The feces were ellipsoidal and moderately dry. After successful modeling, the hair gloss of some rats in model group and acupressure group decreased slightly. The skin color of part of bilateral groin area was darker than that before modeling, the skin was slightly rougher than that of the blank group, and the mental state was average or poor. Some obesity-induced IR rats showed slightly slower activity or inactivity than before, and some stool volume was less and slightly drier than that of blank group. After 6 weeks of continuous intervention, there was no obvious change in the general conditions of the model group, but the mental state, activity and defecation of the rats in the acupressure group improved obviously, and the fur state improved to a certain extent. The skin color in the inguinal area of some rats became lighter, but there were still some rats whose skin color did not change significantly by naked eye observation.

3.2 Body weight observation After 6 weeks of intervention, compared with the blank group, the body weight of the model group and the acupressure group increased significantly ($P < 0.01$); compared with the model group, the weight of rats in the acupressure group decreased significantly after massage intervention ($P < 0.01$). Before the intervention (0 week) and 1 to 6 weeks after the intervention, the body weight of the three groups of rats is shown in Fig. 1.

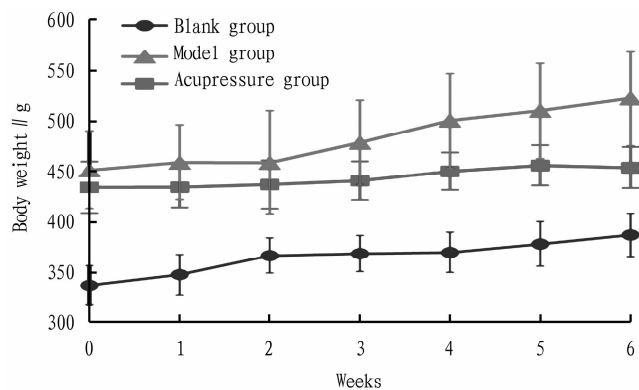
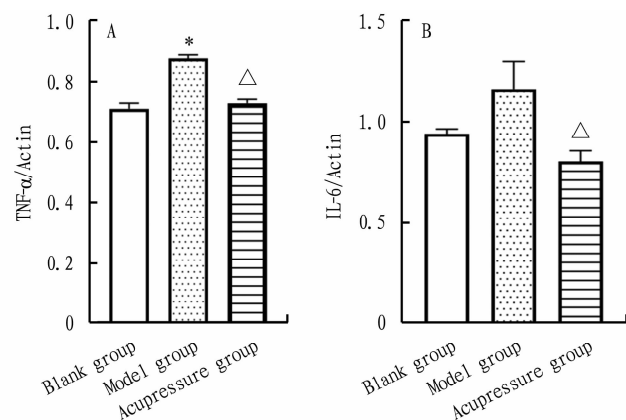


Fig. 1 Body weight of rats during intervention ($\bar{x} \pm s$, $n = 10$)

3.3 Observation of TNF- α and IL-6 protein expression After 6 weeks of intervention, the expression of IL-6 and TNF- α protein in adipose tissue of the model group was significantly higher than that of the blank group ($P < 0.01$). Compared with the model group, the expression of IL-6 and TNF- α protein in adipose tissue of rats in acupressure group decreased significantly ($P < 0.01$) (Fig. 2).

4 Discussion

IR induced by obesity is a common and frequently-occurring disease in clinic. IR related to glucose and lipid metabolism has posed a major challenge to the global public health system. The disease location of obesity is in the middle burner, where the viscera are damaged, the rising and falling are abnormal, maladjusted, and the fat and sweet accumulate, so that the meridians are blocked by blood stasis. After a long time, the middle burner will



Note: Comparing the model group with the blank group, * $P < 0.05$; comparing the acupressure group with the model group, $\triangle P < 0.05$.

Fig. 2 TNF- α protein expression (A) and IL-6 protein expression (B)

be full of dampness and heat, leading to "spleen-warm syndrome", which is equivalent to IR induced by obesity in western medicine. Therefore, based on the thought of "Changbai Mountain Manipulation School of Dredging Meridians and Regulating Viscera", which is widely spread and applied in Jilin Province, and based on the characteristics of "too much turbid ointment and blood stasis in viscera and meridians" in abdomen, the characteristic representative massage technique "acupressure therapy method" has been created in long-term clinical practice. This method has the advantages of smaller action area, more skillful treatment and simpler clinical operation, and can be used to clear the source, improve and treat IR induced by obesity. It has been widely verified by the clinical research of the previous project team, and it is one of the effective intervention measures for clinical treatment of obesity-induced IR^[5-7], but the mechanism of action has not been completely clear. Therefore, as an important continuation of the previous research of the team, this project focuses on the inflammatory reaction and carries out relevant experimental research.

Obesity-induced IR is a chronic low-level systemic inflammatory reaction. As inflammatory factors, TNF- α and IL-6 are related to the pathogenesis of obesity-induced IR^[8-9]. Modern studies have found that adipocytes increase in size under obesity, and mature adipocytes secrete adiponectin (APN), which plays an important role in the regulation of glucose and lipid metabolism. It acts on pancreatic β cells, blood vessels, liver, kidney and other organs, and improves IR by acting on lipid APN receptor R1 and APN receptor R2. Its concentration is positively correlated with insulin sensitivity^[10-11], and negatively correlated with IR degree. Under obesity, APN decreases, which promotes the expression of a small amount of TNF- α and monocyte chemotactic protein-1 (MCP-1), and then recruits immune cells such as macrophages into adipose tissue, and increases the adhesion of monocytes. Monocytes are the largest white blood cells and play an important role in the body's defense system. With the infiltration and accumulation of monocytes, the immune system is activated, and a

large number of inflammatory cytokines such as TNF- α , IL-6 and interleukin-1 (IL-1) are secreted, so that more monocytes infiltrate into adipose tissue and cause obesity-related chronic low-grade inflammation. Obesity-induced IR can increase ROS content in adipose tissue, activate inflammatory signaling pathways such as NF- κ B related pathway, promote the secretion of inflammatory factors TNF- α and IL-6, damage insulin sensitive tissues, affect insulin secretion, and aggravate obesity-induced IR state.

The results of this experiment showed that "acupressure" can effectively regulate the insulin sensitivity of obesity-induced IR rats, and the possible mechanism was that the levels of TNF- α and IL-6 were regulated. In addition, the changes of rat feces during the experiment suggested that high-fat diet may cause changes of intestinal flora in rats, while the "acupressure method" acting on rat abdomen may indirectly affect intestinal flora, thus causing changes in feces, and the clear mechanism needs to be confirmed by subsequent experiments.

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