EXPERIMENTAL STUDY

Effect of electro-acupuncture on gene expression in heart of rats with stress-induced pre-hypertension based on gene chip technology

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Abstract

OBJECTIVE: To explore electro-acupuncture's (EA's) effect on gene expression in heart of rats with stress-induced pre-hypertension and try to reveal its biological mechanism based on gene chip technology.

METHODS: Twenty-seven Wistar male rats were randomly divided into 3 groups. The stress-induced hypertensive rat model was prepared by electric foot-shocks combined with generated noise. Molding cycle lasted for 14 days and EA intervene was applied on rats in model + EA group during model preparation. Rat Gene 2.0 Sense Target Array technology was used for the determination of gene expression profiles and the screened key genes were verified by real-time quantitative polymerase chain reaction (RT-PCR) method.

RESULTS: Compared with blank control group, 390 genes were changed in model group; compared with model control group, 330 genes were changed in model + EA group. Significance analysis of gene function showed that the differentially expressed genes are those involved in biological process, molecular function and cellular components. RT-PCR result of the screened key genes is consistent with that of gene chip test.

CONCLUTION: EA could significantly lower blood pressure of stress-induced pre-hypertension rats and affect its gene expression profile in heart. Genes that related to the contraction of vascular smooth muscle may be involved in EA's anti-hypertensive mechanism.

INTRODUCTION

Stress-induced hypertension refers to high blood pressure that caused by the long-term tension and stress.[1] A modern life style with fast pace can cause psychological and emotional stress, which may develop into a gradual increase in hypertension in young adults.
Present studies showed that stress-induced hyperten-

sion is of complicated pathogenesis which mainly involve the hypothalamus-pituitary-adrenal cortex (HPA) axis,[2] sympathetic nerve-adrenal medulla (SAM) system,[3] renin-angiotensin-aldosterone system (RAS).[4]

The 7th report released by the Joint National Committee report on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) pointed out that, pre-hypertension is a transition stage during which the blood pressure rises from normal to diagnostic hypertension. In this stage, the systolic blood pressure ranges between 120-139 mm Hg and (or) diastolic blood pressure between 80-89 mm Hg.[5] The study found that incidence of cardiovascular events in people with pre-hypertension in future time was as two times as people with normal blood pressure.[5] It is also proved that stress-induced hypertension is preceded by damage of target organs like blood vessels, heart, brain, and changes of the expression of relative proteins and genes.[6,7] Also, studies showed that treatment in the pre-hypertension stage can significantly reduce admission rate and mortality of hypertensive patients, and enhance the protection of target organs of the disease.[8]

Electro-acupuncture therapy was widely applied in the treatment of various kinds of hypertension-related cardiovascular diseases in clinical practices.[9] Lots of researches confirmed that acupuncture reduces blood pressure by immune system, nervous system, and vascular endothelial cell.[10,11]

Gene chip technology, characterized by high sensitivity and throughput, is a new method to analyze gene expression. It could detect multiple gene expression at the same time which provides a platform for the study of multiple genes influenced hypertension.[12] However, previous researches on treatment of hypertension were more concentrated on anti-hypertensive treatment and protection of target organs, which are applied in the middle and late stages. Fewer researches were focused on gene expression and intervention of pre-hypertension.

In this study we will explore the effect of acupuncture on stress-induced pre-hypertension, and try to systematically reveal its biological mechanisms from the aspect of gene by monitoring key genes and targets with gene chip technology, thereby, providing a new idea for the prevention and treatment of hypertension by acupuncture.

MATERIALS And METHODS

Animals preparation
Specified pathogen free (SPF) Wistar ratswere purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., License number: SCXK (Beijing) 200223. Experimental animals were raised in a controlled environment with a temperature of (20 ± 1) ℃, humidity of 50%, and 12-h light-dark cycle was maintained throughout the whole study. All procedures for animal experiments were conducted in accordance with World Health Organization's International Guiding Principles for Biomedical Research Involving Animalsand were approved by the Animal Research Ethics Board of Beijing University of Chinese Medicine.

Grouping
Twenty-seven 9-week-old Specified Pathogen Free (SPF) Wistar male rats of (220 ± 30) g were randomly divided into 3 groupsby random number table method: blank control group, model control group, and model + EA group (*n* = 9 per group).

Model preparation
The stress-induced hypertensive rat (SIHR) model was established by electric foot-shocks combined with generated noise. Rats in model control group and model + EA group were placed in a cage (22 cm × 22 cm × 26 cm) with a grid floor and received electric foot-shocks (30 V, 5 ms duration, 2-25 s intervals)and noises (80-100 db) produced by a buzzer (MG-2TYPE, Huai Bei Zheng Hua, Anhui, China) randomly delivered by a computer. The procedure repeated twice a day (8:00 to 10:00 in the morning and 2:00 to 4:00 in the afternoon). Rats in blank control group were put into the same cage in the same time period, with no foot-shocks or noise.

Intervention
All the rats in three groups were loosely immobilized in a specially made restrainer with four limbs exposed. Taichong (LR 3) is located in the dorsum of the foot, in the depression anterior to the junction of the first and second metatarsals. Quchi (LI 11) is located at the proximal end of radius, in the depression lateral and anterior to the elbow joint.

Model + EA group: the needles (0.32 mm × 25 mm, purchased from Suzhou Acupuncture Goods Co., Ltd., Suzhou, China) were directly inserted into Taichong (LR 3) and Quchi (LI 11) bilaterally for about 1.5-2 mm and 4 mm respectively. After the insertion, Hans (LH202H, Si Sheng Da, Beijing, China) was connected with Quchi (LI 11) and Taichong (LR 3) to form a circulation of 1mA with a frequency of 2 Hz. The needles were then withdrawn after a 20 minutes' retention. The acupuncture intervention took once a day (from 5 pm) since the first day of model preparation for 14 days. All the intervention was given by the same person. Blank control group and model control group: rats were immobilized in the same restrainer for 20 min without acupuncture intervention.

Samples obtaining
After the measurement of caudal artery blood pressure at the 15th day, rats were sacrificed after anesthetized by intraperitoneal injection of 100 g/L chloral hydrate