

*Full Length Research Paper*

# Effects of electroacupuncture at Quchi point on vascular smooth muscle cells apoptosis of two kidney one clip hypertensive rates (2K1C-RHR) in carotid artery

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To observe the effects of electroacupuncture at Quchi point on carotid artery vascular smooth muscle cells (VSMCs) apoptosis of 2K1C-RHR and expression of Bcl-2 and Bax. The therapy methods in electroacupuncture group and electroacupuncture control group were carried out through the Quchi point and the WaiGuan point, respectively, on base of 2K1C-RHR model. The expression of Bcl-2 and Bax protein in carotid artery was detected by immunohistochemical method; Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay was used to detect the apoptosis of carotid artery VSMCs. Compared with the normal control group, the apoptosis of carotid artery VSMCs of model control group increased, the expression of Bcl-2 increased and the expression of Bax decreased. The apoptosis rate of carotid artery VSMCs increased in the electroacupuncture group contrary to model control group ( $P < 0.05$ ), the expression of Bcl-2 decreased and the expression of Bax increased. There was not a significant difference in the apoptosis rate of carotid artery VSMCs between electroacupuncture control group and model control group ( $P > 0.05$ ). The vascular remodeling of hypertension rats in carotid artery can be influenced through the adjustment of VSMCs apoptosis by electroacupuncture.

**Key words:** Acupuncture, hypertension, Quchi point, Bcl-2, Bax.

## INTRODUCTION

Over the past years, the intensive research on pathogenesis of hypertension has shown that hypertension and the damage of related target organ, such as heart, brain, and kidney, were closely related to vascular remodeling (Hayashi et al., 2002). Study was carried to evaluate whether the aortic reflection of the ascending aortic pressure waveform is related to an increased risk of coronary heart disease (CHD) and found that both the inflection time and augmentation index were associated with an increased risk of CHD (Hayashi et al., 2002).

Acupuncture is the practice of inserting thin needles into specific body points to improve health and well-being (Li et al., 2010; Cheng, 2011). It originated in China more than 2,000 years ago. Research has shown that acupuncture reduces nausea and vomiting after surgery

and chemotherapy. It can also relieve pain. Researchers do not fully understand how acupuncture works. It might aid the activity of your body's pain-killing chemicals. It also might affect how body release chemicals that regulate blood pressure and flow. Acupuncture is an alternative medicine that treats patients by insertion and manipulation of needles in the body. Its proponents variously claim that it relieves pain, treats infertility, treats disease, prevents disease, promotes general health, or can be used for therapeutic purposes.

The acupuncture can dramatically reduce blood pressure (Jones et al., 2011; Yang, 2010). Research found that electroacupuncture on Quchi and Taichong has long-term antihypertensive effect and improves effectively day-night rhythm variation in young patients with hypertension. Quchi and Taichong are the effective

pair points for hypertension treated with acupuncture (Yang, 2010). It also can prevent and reverse the vascular remodeling of hypertension, which could be the mechanism for depressurization by acupuncture (Flachskampf et al., 2007). In this paper, we will investigate the effect of acupuncture on vascular smooth muscle cells apoptosis and further discuss the mechanism of acupuncture in the vascular remodeling of hypertension.

## MATERIALS AND METHODS

### Animals

Fifty healthy SD rats with half gender and weight  $200 \pm 20$  g were provided by Animal center of Tongji Medical College, Huazhong University of Science and Technology. The animals were housed at a controlled ambient temperature of  $20 \pm 2^\circ\text{C}$ . They were given food and water *ad libitum*. SD rats were randomly divided into five groups: normal group, sham operated group, model group, electroacupuncture group, and electroacupuncture control group with 10 in each group.

### Reagents and equipments

Immunohistochemistry and TUNEL assay kit (Beijing Zhongshan Goldenbridge Biotechnology Co., LTD); RBP-I type rat sphygmomanometer (Institute of China-Japan Friendship Hospital Clinical Medical Science); LH202H type HANS; Stainless-steel filiform needles sized  $0.5 \times 30$  (Suzhou Medical Appliance Factory).

### 2K1C -RHR model and screening

The renovascular hypertension rat (RHR) was induced using the two-kidney one-clip (2K1C) method. Briefly, the rat was anaesthetized with 5% sodium pentobarbital (45 mg/kg) by intra-peritoneal injection. A retroperitoneal flank incision was performed with sterile techniques. The left renal artery was exposed, and partly included by placing a U-form silver clip with an internal diameter of 0.20 or 0.25 mm on the vessel to induce hypertension, while the right renal artery was not received (Gul et al., 2009). Systolic blood pressure (SBP) was measured by rat tail artery blood pressure. SBP of the preoperative and postoperative after four weeks were measured in this experiment, respectively. We suggested this model is successful and valid if the postoperative SBP exceed 30 mmHg more than the preoperative SBP or the postoperative SBP is more than 20.0kPa (150 mmHg).

### Therapy

Bilateral Quchi point therapy was applied in electroacupuncture group, but WanGuan point in electroacupuncture control group. Acupoint selection and depth of needling based on the traditional methods.

The electroacupuncture parameter of LH202H type HANS consisted of density wave, frequency 2Hz/15Hz, current 1mA, and stimulating 20 min every time, once in a day. The therapeutic course was for 8 weeks. Normal control group: without any treatment and therapy, Sham operated group: similar treatment with model control group in addition that left renal artery was not clamped with U-form silver clip, Model control group: treatment but without acupuncture therapy.

### Sample preparation for paraffin section

The rats in each group were anesthetized with 5% sodium pentobarbital (45 mg/kg) by intra-peritoneal injection and then dissected quickly after therapy for 8 weeks. The collected samples were fixed in 4% paraformaldehyde immediately to prepare paraffin embedded section.

### Sample detection

#### Apoptosis detection of carotid artery VSMCs

Apoptosis detection was performed by TUNEL method according to the protocol provided with the TUNEL Kit. Two sections were chosen to observe in each animal and five visual fields at identical magnification factor ( $\times 400$ ) and luminous intensity. The apoptotic percentage of VSMCs was automatically counted by software, that is, positive apoptotic cell/total cell in visual field  $\times 100\%$ .

#### The expression of Bcl-2 and Bax proteins

The expression of Bcl-2 and Bax protein was detected with immunohistochemistry method (S-P immunohistochemical method) based on the kit instruction. The observation and counting methods of Bcl-2 and Bax positive cell were the same as above, namely, Bcl-2 or Bax positive cell / total cell in visual field  $\times 100\%$ .

### Statistical analysis

All the datum were processed by SPSS12.0 statistics software package and all results were demonstrated by mean + standard deviation ( $\bar{x} \pm s$ ).

## RESULTS

### The effects of electroacupuncture at Quchi point on VSMCs apoptosis of 2K1C-RHR in carotid artery

In the process of experiment, the number of dead rat in model control group, electroacupuncture group, and electroacupuncture control group was 1, 1, and 2, respectively (Table 1). The effects of electroacupuncture at Quchi point on the expression of Bcl-2 and Bax of 2K1C-RHR in carotid artery is shown in Table 2.

## DISCUSSION

### Effects of electroacupuncture at Quchi point on VSMCs apoptosis of 2K1C-RHR in carotid artery

Apoptosis dysfunction of cardiovascular tissue cell which is the importance cytological basis of hypertension, has related to the formation and progression of hypertension (Liu et al., 2000; Gonzalez et al., 2002); in addition to the proliferation of VSMCs, apoptosis dysfunction may also be suggested as one of hypertensive vascular remodeling mechanisms (Korshunov and Berk, 2008).

**Table 1.** The positive rate of carotid artery VSMCs apoptosis of rats in each group ( $\bar{x} \pm s$ , %).

Group	Number	The positive rate of VSMCs apoptosis
Normal control group	10	1.12±0.38
Sham operated group	10	0.95±0.11
Model control group	9	3.17±2.31 <sup>▲</sup>
Electroacupuncture group	9	8.37±2.97 <sup>▲★</sup>
Electroacupuncture control group	8	3.69±1.15 <sup>▲</sup>

Compared with normal control group, ▲ means P<0.05; compared with model control group, ★ means P<0.05.

**Table 2.** The expression of Bcl-2 and Bax of carotid artery of rats in each group ( $\bar{x} \pm s$ , %).

Group	Number	Bcl-2	Bax
Normal control group	10	2.23±0.46	2.83±0.37
Sham operated group	10	2.48±0.52 <sup>△</sup>	2.49±0.85
Model control group	9	4.21±1.31 <sup>▲</sup>	3.09±0.83
Electroacupuncture group	9	2.90±0.63 <sup>★</sup>	5.17±1.20 <sup>★</sup>
Electroacupuncture control group	8	4.02±1.00 <sup>▽</sup>	3.74±0.59

Compared with normal control group, △ means P>0.05, ▲ means P<0.05; compared with model control group, ▽ means P>0.05, ★ means P<0.05.

The imbalance between proliferation and apoptosis of VSMCs (the increase in cell proliferation and reduced apoptosis) participate in the development of hypertensive vascular remodeling process (Jung et al., 2000). Some studies showed that after atorvastatin treatment in spontaneously hypertensive rats (SHR), VSMCs apoptosis was increased but the proliferation was inhibited, thus the imbalance state was reversed between proliferation and apoptosis, vascular remodeling was improved, and blood pressure had been lowered finally (12).

In our study, abnormal apoptosis of VSMCs was identified in pathological changes of hypertensive vascular remodeling, namely, VSMCs apoptosis rate in hypertensive model rats was higher than the normal control group and sham operated group (P<0.05). After electroacupuncture therapy for 8 weeks, the apoptosis rate of electroacupuncture group was significantly higher than model control group (P<0.05). The apoptosis rate of electroacupuncture control group was also higher than the normal control group (P<0.05), but no significant difference compared with the model control group (P<0.05), and the apoptosis rate of electroacupuncture group was significantly higher than electroacupuncture control group (P<0.05). In general, the data indicated that electroacupuncture at Quchi point could inhibit proliferation of VSMCs through increasing its apoptosis level, which improved the imbalance between proliferation and apoptosis of VSMCs, alleviated the hypertensive vascular remodeling to achieve lower blood pressure.

### Effects of electro acupuncture at Quchi point on protein expression of Bcl-2 and Bax of 2K1C-HR in carotid artery

Bcl-2 and Bax are two important genes for apoptosis, and the ratio between these two proteins is a key factor to determine the strength of the apoptosis inhibition and decide the survival of cells (Youle and Strasser, 2008). Some reports have shown that the Bcl-2/Bax ratio (an inverse index of cell susceptibility to apoptosis) was lower (P<0.05) in untreated SHR than in Wistar-Kyoto rats, and the chronic administration of losartan was associated with the normalization of apoptosis, Bax expression, and the Bcl-2/Bax ratio in treated SHR (Fortuno et al., 1998; Rodriguez-Feo et al., 2000).

In our study, carotid artery tissue Bcl-2 positive staining rate in model control group showed significantly higher than its in normal control group (P<0.05), but Bax protein positive staining rate had no significant differences between model control group and normal control group (P<0.05), even if increasing to some extent. These results indicated that the change in apoptosis balance due to over-expression of anti-apoptotic genes and inactivation of apoptosis genes may be a major reason for the VSMCs accumulation in hypertension and bring about vascular remodeling. After electroacupuncture therapy for 8 weeks, Bcl-2 positive staining rate in electroacupuncture group decreased and showed lower than its in model control group (P<0.05); on the contrary, Bax positive staining rate in electroacupuncture group exhibited higher than its in model control group (P<0.05).

Furthermore, these results indicated that electroacupuncture could reverse the imbalance state between the proliferation and apoptosis of VSMCs, improve vascular remodeling and low blood pressure by activating apoptosis genes and (or) inhibiting the over expression of anti-apoptotic genes.

## Conclusions

Electroacupuncture at Quchi point could play an important role in improving vascular remodeling and reducing blood pressure in the form of regulating the abnormal expression between apoptosis-related gene Bcl-2 and Bax to inhibit abnormal apoptosis of VSMCs in hypertension pathological process.

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