Anti-hypertensive Effects of Electroacupuncture at ST36 in Spontaneously Hypertensive Rat

Hyun Jung Park, Hee Young Kim[1], Sung Ok Kim[2], Dae Hyun Hahm[3], Hye Jung Lee[3], Insop Shim*

Department of Integrated Medicine, College of Medicine, The Catholic University,
1: Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston,
2: Department of Oriental Medical Science, Graduate School of East-West Medical Science, Kyung Hee University,
3: Acupuncture and Meridian Science Research Center, Kyung Hee University

The present study investigated whether electroacupuncture (EA) can alleviate hypertension and concomitant disorders such as decreased kidney weight and elevation of serum creatinine in spontaneous hypertensive rats. EA (2 Hz, 3 mA, 10 min) was applied to Joksamni (ST36) once daily for 7 days. Body weight, blood pressure and heart rate were measured on Day 0, 2, 6, 8, 10 and 12, and kidney weight and serum creatinine levels were examined after sacrifice (on Day 12 after last examination). In the ST36 group, the blood pressure were significantly decreased from 6thdays and its effects lasted up to Day12 (up to 5days after cessation of acupuncture), compared to control. And, significant decreases of the heart rates after EA at ST36 were also observed on Day 2, 6, and 8. However, there were no significant differences in daily body weight, kidney weight and serum creatinine between acupuncture and control group. These results showed that EA at ST36 caused anti-hypertension by decreasing blood pressure and heart rates in spontaneous hypertenstive rats, although it failed to alleviate concomitant disorders such as the decreased kidney weight and elevation of serum creatinine shown in hypertensive rats.

Key words : Joksamni (ST36), spontaneously hypertensive rat, electroacupuncture

Introduction

Systemic arterial hypertension is a common disorder worldwide[1,3,9,15]. Without proper management, it can cause numerous complications such as heart disease, stroke, hypertension, kidney failure, eye disease, and nervous system disorders[4,13,18]. Spontaneously hypertensive rats (SHR) have been used as an animal model of arterial hypertension; these animals reflect the characteristics of hypertension and its side effects similar to the manifestations observed in humans[1,9,15].

The hypertension in these rats is associated with increases in sympathetic activity. The sympathetic nervous system plays an important role in regulating blood pressure; it controls blood pressure by modulating peripheral vascular resistance and cardiac output[5]. Similar to animal models of hypertension, primary human hypertension is also associated with increases in sympathetic activity and blunted arterial baroreflexes[2,12]. An effective cure for hypertension has not yet been found.

Acupuncture is an ancient therapeutic technique that has been used for the treatment and prevention of a variety of diseases[6,7]. ST36 is one of the most commonly used points in clinic. Oriental medicine describes that ST36 strengthens the S spleen and stomach, regulates the intestines and stabilizes the mind and emotions, which can treat the deficiency of heart and spleen. Few studies have reported that EA at ST36 has antihypertensive effects in hypertension animal models.

The aim of the present study was to explore whether EA at ST36 can decrease hypertension and alleviate the concomitant kidney disorders including decreased kidney weight and elevation of serum creatinine.

* To whom correspondence should be addressed at : Insop Shim, Department of Integrative Medicine, College of Medicine, Catholic University of Korea, Seoul 137-701, Korea
· E-mail : ishim@catholic.ac.kr, · Tel : 02-2258-7385
· Received : 2009/02/03 · Revised : 2009/05/29 · Accepted : 2009/06/07

Materials and Methods

1. Experimental animals

Eight-week-old spontaneously hypertensive rats (SHR; Orient Inc. Korea) weighing approximately 250 g were used for the study. Each animal was housed at a controlled temperature (22±2℃) and was maintained under light-dark

cycles, each cycle consisting of 12 hrs of light and 12 hrs of darkness (lights on from 07:00 to 19:00 h), with food and water made available ad libitum. All rats were housed for at least 7 days under these conditions prior to any experimental manipulations. The experimental procedures were performed in accordance with the animal care guidelines of the NIH and the Catholic University of Korea Institutional Animal Care and Use Committee. SHR rats (n=20) were divided randomly into two groups of control (n=10) and acupuncture (n=10), For the EA stimulation, each rat was placed in a plastic holder with tail and hip protruding. One pair of acupuncture needle (10 mm long, 0.15mm diameter) was inserted about 3 mm deep into the left side ST36 and the other needle into the right side ST36. The needles were connected with the output terminals of the stimulator (LH800, Han's acupoint nerve stimulator, China) that delivered square waves at 2 Hz, a 0.3 ms pulse width, and 3 mA intensity for 10 min. After the EA stimulation, the rats returned to their cages. Acupuncture group received total 7 treatments, once day for 7 days. Control group received the same manipulation as acupuncture group without EA. ST36 is located about 10 mm below the knee joint and about 5mm lateral to the midline on the anterior surface of the hind leg in a rat.

2. Measurements

Body weights, blood pressure and heart rate were measured on Day 0, 2, 6, 8, 10 and 12. All rats were sacrificed after the last treatment (Day 12) to examine kidney weight and serum creatinine. On Day 0, 2, 6, 8, 10 and 12, blood pressure (BP) and heart rate (HR) were measured 3 times (2 min intervals) for one average data in each animal after EA at ST36 using physiography and a tail cuff sensor[17]. To measure blood pressure, the rats were prewarmed at 32℃ for 5-10 min in a warming box. Briefly, the rat placed in a plastic holder with tail and hip protruding and received EA for 10 min. The rats were stabilized for 5-10 min after EA and the tail was occluded with an appropriate sized tail cuff sensor connected to the physiography (Grass79, Grass, USA) and the pulse was detected as the cuff pressure was lowered. The only rats with blood pressure over 180 mmHg were used in this study. In each animal, blood pressure and heart rate were expressed as percentage of baseline (pretreatment value on Day 0). To measure serum creatinine level, 1 ml blood was withdrawn via cardiac puncture. The proteins were isolated from the blood sample using the Folin-Wu method[14] and then creatinine levels were ascertained by spectrophotometer measurements.

3. Statistical analysis

All data are presented as mean ± SEM (standard error of the mean). The data were analyzed by one- or two-way repeated-measurement analysis of variance (ANOVA), followed by post hoc testing using the Holm-Sidak method. P values £0.05 were considered statistically significant.

Results

The changes in body weights are shown in Fig. 1. The body weights tend to increase in acupuncture group, but, there were no significant difference between control and acupuncture groups. In acupuncture group, the significant changes in blood pressure(Fig. 2). Its effects lasted up to Day 12 (5 days after cessation of EA). This finding indicates that EA at ST36 had a long-term suppression effect on the high blood pressure in the SHRs. The heart rate after EA at ST36 was significantly decreased on Day 2, 6, and 8, compared to control, but its values returned to those of control on Day 10 and 12 after cessation of EA(Fig. 3).

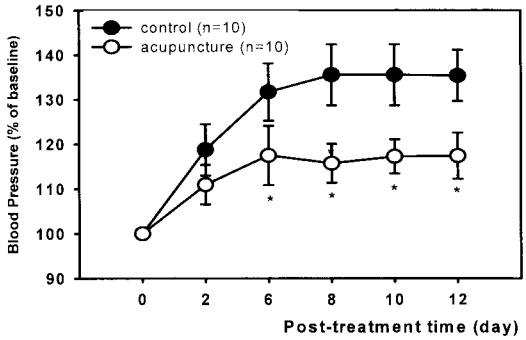

Fig. 1. Change in mean blood pressure during the experimental procedure. Each value reoresents the mean±S.E.M. The results of blood pressure were analyzed by performing two-way repeated-measurement analysis of variance (ANOVA), followeo by post hoc testing using the Holm-Sidak method. *p<0.05 vs. control group.

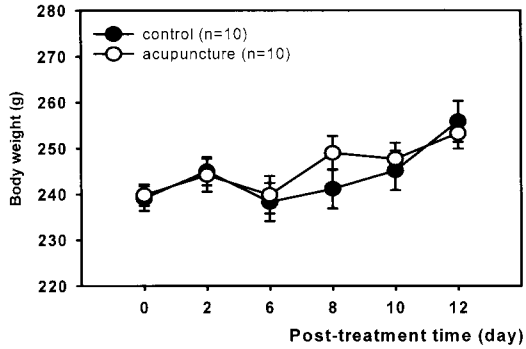

Fig. 2. Change of body weights in rats. Each value represents the mean±S.E.M.

This result suggests that EA at ST36 can attenuate the increase of heart rate during acupuncture treatment. On the other hands, there were no significant differences in the kidney

weight and serum creatinine levels between control and acupuncture groups. It may indicate that EA at ST36 does not have any effects on concomitant kidney disorders from hypertension.

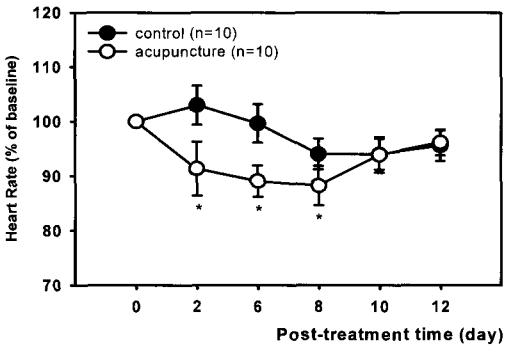

Fig. 3. Change in mean heart rate during the experimental procedure. Each value represents the mean±S.E.M. The results of heart rate were analyzed by performing separate The results of blood pressure were analyzed by performing two-way repeated-measurement analysis of variance (ANOVA), followed by post hoc testing using the Holm-Sidak method. *p<0.05 vs. control group.

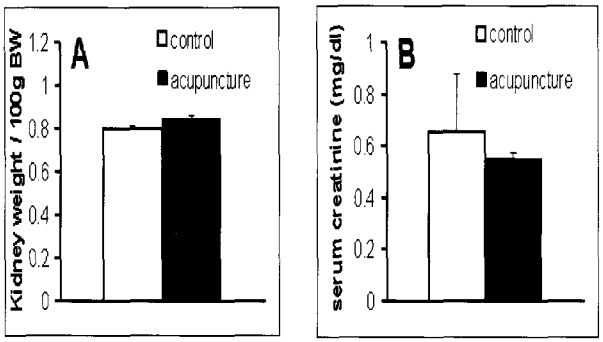

Fig. 4. A) The effect of electroacupuncture at ST36 on Kidney weights. Each value represents the mean±S.E.M. B) The effect of electroacupuncture at ST36 on creatinine concentration in blood. Each value represents the mean±S.E.M.

Discussion

The results of the current study showed that EA at ST36 effectively decreased the blood pressure and the heart rate in the SHRs, especially, it had long-term suppression effects on blood pressure in SHRs. Hypertensive animal models such as SHRs, renal hypertensive rats, DOCA salt hypertensive rats, and NaCl-fed-hypertensive rats have been used extensively as human hypertension-mimetic models. Among them, SHRs are widely accepted as an animal model for the study of essential hypertension in humans. In acupuncture researches using SHR model, it was reported that manual acupuncture at LI[11], LIV3, LI4 and ST36 decreases the blood pressure, and improves the bulbar conjunctiva microcirculation in SHRs[18]. Zhou Y et al (1995) reported that acupuncture at bilateral ST36 plus LI[11] remarkably droped the blood pressure in the SHRs [19]. Huang YL et al demonstrated that the curative mechanism of acupuncture on stress-induced hypertension was related to the changes of systolic blood pressure8. They suggested that the acupuncture at ST36 had a similar suppression effect in stress-induced hypertensive rats. Consistent with the previous reports, the present study confirmed that the repeated EA treatment at ST36 significantly decreased the blood pressure and heart rate in the SHRs.

Generally, hypertension is related to the kidney function. Many reports have shown that patients with hypertension have decreased kidney weight and increased creatinine[10,11]. Creatinine is a protein metabolite excreted by the kidney. Once the kidneys are functioning normally, the serum creatinine level should remain constant and normal. Serious renal disorders, such as glomerulonephritis, pyelonephritis and urinary obstruction, will cause abnormal elevations in the creatinine. In our preliminary study, compared to normal Sprague-Dawley rats, decreased kidney weight and increased serum creatinine were observed in SHRs (data not shown). In the present study, EA at ST36 failed to alleviate the decreased kidney weight and elevation of serum creatinine in SHRs, compared to control. It may suggest that EA exerts anti-hypertension effect via other mechanisms (i.g., sympathetic activity), not due to modulation of kidney functions or EA did not have any effects on the concomitant kidney disorders by hypertension. It is not clear how EA at ST36 caused long-term suppression on blood pressure in SHRs and it is important subject for future study.

In conclusion, this study showed that EA at ST36 had a potent antihypertensive effect and induced prolonged suppression effects on high blood pressure in SHRs.

Acknowledgments

This study was supported by the Grant of the SRC program of KOSEF(R11-2005-014), Republic of Korea. The first two quthors contributed equally to this study.

References

1. Barbagallo, M., Quaini, F., Baroni, M.C., Barbagallo, C.M., Boiardi, L., Passeri, G., Arlunno, B., Delsignore, R. and Passeri, M. Histological evidence of increased turnover in bone from spontaneously hypertensive rats: Cardioscience, 2(1):15-17, 1991.
2. Brook, R.D. and Julius, S., Autonomic imbalance, hypertension, and cardiovascular risk : Am J Hypertens, 13

(6 Pt 2):112S-122S, 2000.

3. Cappuccio, F.P., Meilahn, E., Zmuda, J.M. and Cauley, J.A. High blood pressure and bone-mineral loss in elderly white women: a prospective study. Study of Osteoporotic Fractures Research Group: Lancet, 354(9183):971-975, 1999.
4. Chrousos, G.P. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture: Ann N Y Acad Sci, 851: 311-335, 1998.
5. Esler, M. The sympathetic system and hypertension: Am J Hypertens, 13(6 Pt 2):99S-105S, 2000.
6. Goldstein, D.S. Plasma norepinephrine in essential hypertension. A study of the studies: Hypertension, 3(1):48-52, 1981.
7. Guimaraes, C.M., Pinge, M.C., Yamamura, Y. and Mello, L.E. Effects of acupuncture on behavioral, cardiovascular and hormonal responses in restraint-stressed Wistar rats: Braz J Med Biol Res, 30(12):1445-1450, 1997.
8. Huang, Y.L., Fan, M.X., Wang, J., Li, L., Lu, N., Cao, Y.X., Shen, L.L. and Zhu, D.N. Effects of acupuncture on nNOS and iNOS expression in the rostral ventrolateral medulla of stress-induced hypertensive rats: Acupunct Electrother Res, 30(3-4):263-273, 2005.
9. Izawa, Y., Sagara, K., Kadota, T. and Makita, T. Bone disorders in spontaneously hypertensive rat : Calcif Tissue Int, 37(6):605-607, 1985.
10. Kumai, T., Tanaka, M., Watanabe, M. and Kobayashi, S. Elevated tyrosine hydroxylase mRNA levels in the adrenal medulla of spontaneously hypertensive rats: Jpn J Pharmacol, 65:(4)367-369, 1994.
11. Maemura, S., Niwa, M. and Ozaki, M. Characteristic alteration in adrenal catecholamine contents in SHRs: Japanease Heart Journal, 23(4):593-602, 1982.
12. Mancia, G., Grassi, G., Giannattasio, C. and Seravalle, G. Sympathetic activation in the pathogenesis of hypertension and progression of organ damage: Hypertension, 34(4 Pt 2):724-728, 1999.
13. Masood, A., Banerjee, B., Vijayan, V.K. and Ray, A. Modulation of stress-induced neurobehavioral changes by nitric oxide in rats: Eur J Pharmacol, 458(1-2):135-139, 2003.
14. Miles, L., Tanner, T., Coates, A. A taste of honey-critical analysis of the folin wu method of blood glucose estimation: N Z Med J, 63(1):232-236, 1964.
15. Oshima, T. and Young, E.W. Systemic and cellular calcium metabolism and hypertension: Semin Nephrol, 15(6):496-503, 1995.
16. Romanova, T. P., Karpel, G.G., Brill, G.E. and Markov KhM. Mechanism of disorders of the cerebral blood supply during stress in spontaneously hypertensive rats: Patol Fiziol Eksp Ter, 3: 5-8, 1994.
17. Ruben, D.B., Jason, B. Tail-cuff blood pressure measurement without external preheating in awake rats: Hypertension, 4: 898-903, 1982.
18. Zhou, Y., Chen, Q., Hou, Z. and Chen, Y. Experimental research on treatment of hypertension with acupuncture: J Tradit Chin Med, 13(4):277-280, 1993.
19. Zhou, Y., Wang, Y., Fang, Z., Xia, C., Liu, B., Chen, Q. and Zhang, F. Influence, of acupuncture on blood pressure, contents of NE, DA and 5-HT of SHR and the interrelation between blood pressure and whole blood viscosity: Zhen Ci Yan Jiu, 20(3):55-61, 1995.