The 2 Hz and 15 Hz electroacupuncture induced reverse effect on autonomic function in healthy adult using a heart rate variability analysis

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Running title: 2 and 15 Hz EA induced reverse effect on autonomic function

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Abstract

The purpose of the present study was to investigate effect of electro-acupuncture (EA) at different frequencies on autonomic function. Twenty healthy adult volunteers were studied, and underwent 4 sessions of EA (sham, 2 Hz, 15 Hz, and 50 Hz). Sham, 2 Hz, 15 Hz, and 50 Hz EA was applied to the bilateral Zusanli and Shanjuxu acupoints. The intensity of electrical stimulation was adjusted to obtain visible twitching of the anterior tibial muscle about 2.0-2.5 mA except sham without electrical stimulation. The components of heart rate variability (HRV) and blood pressure were measured before EA (BLP), EA (EAP), and post-EA periods (PEP). The results indicated that the natural logarithmic high frequency power (lnHF) of HRV was greater during PEP than during the BLP in the 2 Hz EA sessions. The natural logarithmic low frequency power (lnLF) of HRV was greater during the PEP than during the BLP in 15 Hz EA sessions, suggesting that 2 Hz EA apply to Zusanli and Shanjuxu acupoints increased vagal activity, whereas 15 Hz EA increased sympathetic activity.

Key words: Heart rate variability; Electro-acupuncture; Frequency; Autonomic function; Blood pressure

Introduction

Electro-acupuncture (EA) is now widely used for the treatment of disease worldwide. The needle stimulation at the ear point, Lu 1 (located at the left inferior hemi-conchae), can induce an increase in parasympathetic activity, while acupuncture at Hegu (LI 4; located at the middle of the right dorsal thenar muscle) can increase the activities of the sympathetic and parasympathetic nervous systems (Haker et al., 2000). Therefore, acupuncture applied to different specific sites can produce different activities of sympathetic or parasympathetic nerves. Our previous studies showed that 2Hz EA at both Zusanli acupoints (ST36) may maintain a prolonged effect in reduction of the pulse rate than a 100Hz EA (Hsieh et al., 1999). Several studies have reported that 2 and 15Hz EA induce the release of endomorphin, encephalin, and β-endomorphin in the central nervous system, and is mediated via μ and/or δ opoid receptor, whereas 15 and 100Hz induce the release of dynorphin, and is mediated via κ opoid receptor to produce an analgesic effect (Han, 2003; Ulett et al., 1998). Therefore, EA at a specific frequency delivered to the body site may cause specific neuropeptide release. Heart rate variability (HRV) analysis is considered a valuable method to assess autonomic function, and includes sympathetic and parasympathetic activities in normal healthy, diabetic, and cardiovascular patients (Malik, 1996a;1996b; van Ravenswaaij-Arts et al.,

1993). Time and frequency domain methods may be used to measure HRV, and these methods are divided into short- (5 min) and long-term (24 hr) recordings (Malik, 1996a;1996b). The high-frequency (HF) power (0.15-0.4 Hz) of HRV may represent vagal activity, while low frequency (LF) power (0.04-0.15) may reflect both sympathetic and vagal activities, or a sympathetic modulations (Guzzetti et al., 1998; Malliani et al., 1991; Yamasaki et al., 1991). The HF% of HRV represents an indicator of parasympathetic activity, and the LF/HF ratio may reflect the modulation of sympathovagal activity, or sympathetic nerve activity (Malik, 1996a;1996b). Acupuncture at Ximen (PC4) may cause a reciprocal modulation by reducing cardiac sympathetic and increasing cardiac parasympathetic activities, resulting in a decrease in heart rate (Nishijo et al., 1997). Acupuncture at Neiguan (PC6) may increase the normalized HF power of HRV (Huang et al., 2005). Williams et al. (1991) reported that acupuncture may reduce diastolic blood pressure in patients with diastolic hypertension (Williams et al., 1991), and Li et al. (2004) found that 2 Hz EA applied to Neiguan and Jianshi (PC5) may reduce the increase in mean artery pressure induced by exercise (Li et al., 2004), but Lee et al. (2009) concluded that no evidence supported a reduction in blood pressure by acupuncture (Lee et al., 2009). Therefore, the purpose of the present study was to investigate the effect of EA at different frequencies in specific sites on HRV and

blood pressure. We used 2 Hz, 15 Hz and 50 Hz EA, respectively, applied to bilateral Zusanli and Shangjuxu (ST37) acupoints, and HRV and blood pressure simultaneously recorded.

Materials and methods

Subjects

Twenty-two healthy adults were recruited between August 2008 and January 2009. The experiments were approved by the Institutional Review Board of China Medical University Hospital (IRB, DMR97-IRB-046). Signed informed consent was obtained from every subject after the experimental purpose and procedure was explained in detail prior to the experiments. The experiment was performed from 8:30-12:00 am, and from 13:30-17:00 pm in a quiet room with the temperature maintained at $24\pm1^{o}C$ by an air conditioner. The subjects were placed in the experimental bed in a supine position. Medications, coffee, and tea were prohibited 24 hr prior to the experiments. Acupuncture was performed by an experienced Chinese physician. The inclusion criteria were as follows: 1) 20-40 years of age; 2) female or male; and 3) physical examination within normal limits. The exclusion criteria were as follows: 1) hypertension (systolic pressure $\geqq$ 140 mmHg and diastolic pressure $\geqq$ 90 mmHg); 2) severe medical disease, including congestive heart failure, myocardial infarction, arrhythmia, chronic obstructive pulmonary disease, renal failure, cancer, and

pregnancy; and 3) psychiatric disorders.

Study design

The experiment was designed as a randomized complete block design according to the number of lots from a hard paper box. The sample size was calculated according to Dupont and Plummer (Dupont and Plummer, 1990); 20 subjects were required to obtain statistical significance.

Experimental procedure

Each subject completed 4 sessions (sham, 2Hz, 15Hz, and 50Hz); the interval between sessions was at least 1 week to prevent residual effect. Each session was divided into the pre-EA period (baseline period (BLP)), EA period (EAP), and post-EA period (PEP); each period was 20 min in length. Both HRV and blood pressure were recorded simultaneously in each period (Figure 1).

During the sham sessions, both the baseline HRV and blood pressure were recorded after the subjects had rested in a relaxed state as BLP recordings. The BLP recordings were followed by placement of the electrodes of the EA apparatus (HANS LY257; Healthronics, Singapore) were placed on the surface of the Zusanli (3 cun below the knee and one finger lateral to the anterior crest of the tibia) and

Shanjuxu (3 cun below Zusanli), bilaterally, and these electrodes were connected to the EA apparatus, but no electrical stimulation was delivered. The electrodes were taken off immediately after the EAP recordings were completed; both HRV and blood pressure were then recorded as PEP recordings.

During the 2Hz, 15Hz, and 50Hz sessions, the procedure was similar to the sham session, except the stainless acupuncture needles (7.5 cm in length, gauge #30; Yu Kuang, Taiwan) were inserted into Zusanli and Shanjuxu acupoints bilaterally and were twisted manually to elicit qi (the acupuncturist experiences a feeling like a fish biting on bait; the subject experiences a sensation of soreness, numbness, swelling, and heaviness around the inserting area). The cathode of the EA apparatus was connected to the Zusanli needles, whereas the anion was connected to the Shanjuxu needles. Electrical stimulation of 2Hz, 15Hz, and 50Hz EA was then delivered after BLP recordings. The intensity of electrical stimulation was adjusted to obtain visible twitching of the anterior tibial muscle about 2.0-2.5 mA.

HRV Analysis Recordings

A HRV analyzer (WG-MD-ANSA01 HRV analyzer; WeGene Technologies Inc., Jonghe City, Taiwan) was used to record the HRV. The electrodes were placed on the subject's right and left forearms and right lower leg. The HRV analyzer

converted time domain signals into frequency domain signals by fast fourier transformation (FFT), and the present study used frequency domain analysis with 5 min of short-term recording. The data of HRV recordings was storage and analysis in a personal computer. The individual component of HRV was definite according to the guidelines for HRV (Malik, 1996a;1996b). Frequencies between 0.15 and 0.40 Hz were high frequency (HF), and frequencies between 0.04 and 0.15 Hz were low frequency (LF).

Blood pressure recordings

The blood pressure was measured using a blood pressure apparatus (Spectrumpulse ^{TM} Heart Monitor, OSTAR Model: A2; Ostar Meditech Corp., Taiwan). The sensor of the blood pressure apparatus was placed at the elbow just below the brachial artery of the left arm, and the blood pressure was measured automatically. The blood pressure recording data were stored and analyzed in a personal computer.

Statistical analysis

The data are presented as the mean ± standard deviation (SD) and analyzed by SPSS 18.0 software. One-way analysis of variance (ANOVA) with LSD post hoc test was used to assess the differences among the periods. The significance level

was set at $\alpha = 0.05$.

Results

Basic data analysis

Twenty-two healthy adults were recruited in the present study, and 2 subjects were excluded due to arrhythmias, therefore there were 20 subjects (males, 15; females, 5) who finished the study. The ages ranged between 21 and 36 years (mean ± SD, 28.8± 4.3 years).

Effect of EA at different frequencies on HRV

The natural logarithmic HF power (lnHF) of HRV was greater during the PEP than during the BLP in the 2Hz EA sessions ($p < 0.05$; Table 1), whereas the lnHF was not significantly difference between the EAP and BLP, and between PEP and EAP (both $P > 0.05$; Table 1). The lnHF in the sham, 15Hz and 50Hz EA sessions were not significantly different between the EAP and BLP, between PEP and BLP, and between PEP and EAP (all $p > 0.05$; Table 1).

The natural logarithm LF power (lnLF) of HRV was greater during the PEP than during the BLP in 15Hz EA sessions ($p < 0.05$; Table 1), whereas the lnLF was not significantly different between the EAP and BLP, and between the PEP and EAP (both $P > 0.05$; Table 1). The lnLF in the sham, 2Hz and 50Hz sessions were not significantly different between EAP and BLP, between PEP and BLP, and between

PEP and EAP (all $p > 0.05$; Table 1).

The HF%, LF% and lnLF/HF ratio of the HRV in the sham, 2Hz, 15Hz and 50Hz sessions were not significantly different between PAP and BLP, between PEP and EAP, and between PEP and BLP (all $p > 0.05$; Table 1).

With regard to the natural logarithm very low frequency power (lnVLF), total power (lnTP), variance power (lnVAR), and R-R interval components of HRV, their results of the present study were summarized in Table 1.

Effect of EA at different frequencies on blood pressure

The systolic, diastolic and mean blood pressure in the sham, 2Hz, 15Hz, and 50Hz sessions was not significantly different between PAP and BLP, between PEP and EAP, and between PEP and BLP (all $p > 0.05$; Table 2).

Discussion

The 2Hz increase vagal activity, whereas 15Hz EA enhanced sympathetic activity

The results of the present study indicate that the lnHF was greater during the PEP than during the BLP in the 2Hz EA sessions, whereas no similar results occurred in the sham, 15Hz and 50Hz sessions, suggesting that 2Hz EA applied to the Zusanli and Shanjuxu acupoints increase vagal activity due to lnHF can represent vagal activity (Imai et al., 2008; Malik, 1996a;1996b); our results also indicated that lnLF was greater during PEP than during BLP in 15Hz EA sessions, but no similar results were noted in the sham, 2Hz and 50Hz sessions, suggesting that 15Hz EA applied to Zusanli and Shanjuxu acupoints can enhances sympathetic activity. Because lnLF is a parameter of both sympathetic and vagal activities, or a sympathetic modulations (Malik, 1996a;1996b), and lnLF is also an index of sympathovagal balance (Goldberger, 1999). Our results were consistent with other studies that EA applied to bilateral Zusanli acupoints can enhance gastric motility and increased vagal activity (Imai et al., 2008; Ouyang et al., 2002), and also similar to the results of our previous studies that 2Hz EA at Zusanli and Hegu may reduce gastric half-emptying time in diabetic patients with gastroparesis (Wang et al., 2008). In addition, the effect of EA is partially mediated via the opoid pathway to enhance vagal activity (Chen et al., 2008).

EA cannot change blood pressure

Our results indicate that blood pressure includes systolic, diastolic and mean arterial pressures were similar between EAP and BLP, between PEP and BLP, and between PEP and EAP in the 2Hz, 15Hz and 50Hz EA sessions, these results indicated that 2Hz, 15Hz and 50Hz EA applied to bilateral Zusanli and Shanjuxu acupoints cannot affect blood pressure. Iwa et al., (2005) report that EA delivered to Zusanli may reduce the increase in mean arterial pressure induced by rectal distension in conscious dogs, and suggest this effect of EA results from the opioid release of EA, and opioid reduces visceral pain (Iwa et al., 2005). Chen and Ma (2003) found that EA of 30Hz applied to bilateral Zusanli can reduce mean arterial pressure and heart rate in rats, and these effects of EA can be enhanced by L-arginine derived nitric oxide (NO) administration of the gracile nucleus, suggesting that EA at the Zusanli acupoint induces a hypotensive effect has a close relationship to NO (Chen and Ma, 2003). The hypotensive effect of EA at Zusanli is partly mediated via the arterial baroreflex reflex due to endogenous opioids may modulate the arterial baroreflex (Michikami et al., 2006). The 2Hz or 20Hz EA at the Zusanli acupoints do not affect blood pressure, while 2Hz EA at Hegu may induce a tonic effect to increase blood pressure, and 20Hz EA at Hegu may elicit a phasic effect to increase blood pressure

in normal and acute hemorrhage rat (Liao et al., 2006; Ting et al., 2002). The 2 Hz EA at Hegu may induce vasodilation and may reduce blood pressure in healthy young adults (Lin et al., 2003), therefore, the effect of EA on blood pressure in human needs further study.

Limitation

Although our results indicated that both 15Hz and 50Hz EA can increase lnVLF and lnTP components of HRV, and 50Hz EA also can increase lnVar component. The lnVLF is defined from 0.003-0.04 Hz, and its physiologic significance is less clear (Kuo et al., 1999; Malik, 1996a; 1996b). The lnTP influence LF and HF power and relate to the fractional distribution of the energy (Malik, 1996a;1996b). The lnVar of HRV is the variance of R-R intervals (Kuo et al., 1999).

Conclusion

Based on the results, 2Hz and 15Hz applied to Zusanli and Shanjuxu acupoints, may induce reverse effect on autonomic function. 2Hz EA increase vagal activity, where 15Hz EA enhances sympathetic activity. These results may provide scientific evidence for the selection of clinical treatment of disease.

Acknowledgments

This study was supported by grant CMU99-NSC-02 from the China Medical University, Taiwan, R. O. C. and also was supported in part by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH100-TD-B-111-004).

References

Chen, J., Song, G.Q., Yin, J., Koothan, T., Chen, J.D. 2008. Electroacupuncture improves impaired gastric motility and slow waves induced by rectal distension in dogs. American Journal of Physiology. Gastrointestinal and Liver Physiology. 295, G614-620.

Chen, S., Ma, S.X. 2003. Nitric oxide in the gracile nucleus mediates depressor response to acupuncture (ST36), Journal of Neurophysiology. 90, 780-785.

Dupont, W.D., Plummer, Jr W.D. 1990. Power and sample size calculations. A review and computer program. Controlled clinical trials. 11, 116-128.

Goldberger, J.J. 1999. Sympathovagal balance: how should we measure it? The American Journal of Physiology. 276, H1273-1280.

Guzzetti, S., Piccaluga, E., Casati, R., Cerutti, S., Lombardi, F., Pagani, M., Malliani, A. 1988. Sympathetic predominance in essential hypertension: a study employing spectral analysis of heart rate variability. Journal of hypertension. 6, 711-717.

Haker, E., Egekvist, H., Bjerring, P. 2000. Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. Journal of The Autonomic Nervous System. 79, 52-59.

Han, J.S. 2003. Acupuncture: neuropeptide release produced by electrical

stimulation of different frequencies. Trends in Neurosciences. 26, 17-22. Review.

Hsieh, C.L., Lin, J.G., Li, T.C., Chang, Q.Y. 1999. Changes of pulse rate and skin temperature evoked by electroacupuncture stimulation with different frequency on both Zusanli acupoints in humans. The American Journal of Chinese Medicine. 27, 11-18.

Huang, S.T., Chen, G.Y., Lo, H.M., Lin, J.G., Lee, Y.S., Kuo, C.D. 2005. Increase in the vagal modulation by acupuncture at neiguan point in the healthy subjects. The American Journal of Chinese Medicine. 33, 157-164.

Imai, K., Ariga, H., Chen, C., Mantyh, C., Pappas, T.N., Takahashi, T. 2008. Effects of electroacupuncture on gastric motility and heart rate variability in conscious rats. Autonomic neuroscience : basic & clinical. 138, 91-98.

Iwa, M., Strickland, C., Nakade, Y., Pappas, T.N., Takahashi, T. 2005. Electroacupuncture reduces rectal distension-induced blood pressure changes in conscious dogs. Digestive Diseases and Sciences. 50, 1264-1270.

Kuo, T.B., Lin, T., Yang, C.C., Li, C.L., Chen, C.F., Chou, P. 1999. Effect of aging on gender differences in neural control of heart rate. The American Journal of Physiology. 277, H2233-2239.

Lee, H., Kim, S.Y., Park, J., Kim, Y.J., Lee, H., Park, H.J. 2009. Acupuncture for

lowering blood pressure: systematic review and meta-analysis. American Journal of Hypertension. 2, 122-128.

Li, P., Ayannusi, O., Reid, C., Longhurst, J.C. 2004. Inhibitory effect of electroacupuncture (EA) on the pressor response induced by exercise stress. Clinical Autonomic Research: Official Journal of The Clinical Autonomic Research Society. 14, 182-188.

Liao, J.M., Ting, H., Lee, S.D., Yang, C.H., Liou, Y.M., Peng, M.L., Tsai, S.J., Lin, C.F., Lin, T.B. 2006. Electroacupuncture-induced pressor and chronotropic effects in anesthetized rats. Autonomic Neuroscience : Basic & Clinical. 124, 18-25.

Lin, C.F., Liao, J.M., Tsai, S.J., Chiang, P.Y., Ting, H., Tang, C.Y., Lou, K.L., Hsieh, L.C., Wang, D.W., Lin, T.B. 2003. Depressor effect on blood pressure and flow elicited by electroacupuncture in normal subjects. Autonomic Neuroscience : Basic & Clinical. 107, 60-64.

Malliani, A., Pagani, M., Lombardi, F., Cerutti, S. 1991. Cardiovascular neural regulation explored in the frequency domain. Circulation. 84, 482-492. Review.

Michikami, D., Kamiya, A., Kawada, T., Inagaki, M., Shishido, T., Yamamoto, K., Ariumi, H., Iwase, S., Sugenoya, J., Sunagawa, K., Sugimachi, M. 2006.

Short-term electroacupuncture at Zusanli resets the arterial baroreflex neural arc toward lower sympathetic nerve activity, American Journal of Physiology. Heart and Circulatory Physiology. 291, H318-326.

Nishijo, K., Mori, H., Yosikawa, K., Yazawa, K. 1997. Decreased heart rate by acupuncture stimulation in humans via facilitation of cardiac vagal activity and suppression of cardiac sympathetic nerve. Neuroscience Letters. 227, 165-168.

Ouyang, H., Yin, J., Wang, Z., Pasricha, P.J., Chen, J.D. 2002. Electroacupuncture accelerates gastric emptying in association with changes in vagal activity. American Journal of Physiology. Gastrointestinal and Liver Physiology. 282, G390-396.

Malik, M. 1996a. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. European Heart Journal. 17, 354-381.

Malik, M. 1996b. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 93, 1043-1065.

Ting, H., Liao, J.M., Lin, C.F., Chiang, P.Y., Chang, C.C., Kuo, D.Y., Lin, T.B. 2002.

Pressor effect on blood pressure and renal nerve activity elicited by electroacupuncture in intact and acute hemorrhage rats. Neuroscience Letters. 327, 5-8.

Ulett, G.A., Han, S., Han, J.S. 1998. Electroacupuncture: mechanisms and clinical application. Biological Psychiatry. 44, 129-138. Review.

van Ravenswaaij-Arts, C.M., Kollée, L.A., Hopman, J.C., Stoelinga, G.B., van Geijn, H.P. 1993. Heart rate variability. Annals of Internal Medicine. 118, 436-447.

Wang, C.P., Kao, C.H., Chen, W.K., Lo, W.Y., Hsieh, C.L. 2008. A single-blinded, randomized pilot study evaluating effects of electroacupuncture in diabetic patients with symptoms suggestive of gastroparesis, Journal of alternative and complementary medicine (New York, N.Y.). 14, 833-839.

Wang, J.D., Kuo, T.B., Yang, C.C. 2002. An alternative method to enhance vagal activities and suppress sympathetic activities in humans. Autonomic Neuroscience : Basic & Clinical. 100, 90-95.

Williams, T., Mueller, K., Cornwall, M.W. 1991. Effect of acupuncture-point stimulation on diastolic blood pressure in hypertensive subjects: a preliminary study. Physical Therapy. 71, 523-529.

Yamasaki, Y., Ueda, N., Kishimoto, M., Tani, A., Ishida, Y., Kawamori, R., Kamada,

T. 1991. Assessment of early stage autonomic nerve dysfunction in diabetic subjects- application of power spectral analysis of heart rate variability. Diabetes Research (Edinburgh, Scotland). 17, 73-80.

Legend

Figure 1. Experimental procedure. Baseline= before electroacupuncture period (baseline period); EA= electroacupuncture period; Post-EA= post electroacupuncture period; HRV= heart rate variability recordings; BP= blood pressure recordings

Table1. Effect of electroacupuncture at different frequencies on heart rate viability

Component	BLP	EAP	PEP
InHF (ms^2)			
sham	5.89±0.92	5.84±0.98	5.90±0.82
2	5.31±1.00	5.90±0.89	5.98±0.91*
15	5.25±1.00	5.70±0.90	5.83±1.00
50	5.48±1.20	5.91±1.02	6.00±0.97
InLF (ms^2)			
sham	6.42±0.95	6.40±1.05	6.54±1.03
2	6.02±1.13	6.28±0.87	6.51±0.78
15	5.79±1.04	6.14±0.18	6.51±0.92*
50	6.15±1.06	6.50±1.08	6.73±0.80
HF%			
sham	32.35±10.23	31.75±12.07	30.52±13.92
2	29.51±13.44	35.20±12.34	32.67±11.28
15	31.76±10.26	33.15±11.89	30.08±8.80
50	29.61±11.98	31.52±13.39	29.27±11.70
LF%			
sham	54.46±14.75	54.80±16.04	56.38±18.32
2	57.23±16.84	51.27±14.81	55.25±13.58
15	53.57±13.51	51.89±17.38	57.75±10.80
50	56.03±17.41	55.68±18.47	58.44±16.60
InLF/HF			
sham	1.10±0.12	1.10±0.13	1.11±0.15
2	1.15±0.17	1.07±0.12	1.10±0.11
15	1.11±0.12	1.08±0.14	1.12±0.94
50	1.16±0.23	1.11±0.17	1.14±0.16
InVLF(ms^2)			
sham	6.88±0.95	7.110.96	7.18±0.97
2	6.78±1.00	6.61±1.08	7.24±1.24
15	6.46±0.84	6.78±0.72	7.14±1.21*
50	6.58±1.13	7.24±0.97	7.33±0.92*
InTP (ms^2)			
sham	7.71±0.85	7.85±0.85	7.93±0.83
2	7.47±0.90	7.57±0.85	8.00±0.93
15	7.21±0.86	7.56±0.76	7.90±0.96*
50	7.46±0.94	7.93±0.94	8.07±0.78*

InVAR(ms^2)			
sham	7.85±0.85	8.00±0.83	7.91±0.77
2	7.51±0.89	7.65±0.86	8.05±0.95
15	7.47±0.81	7.58±0.67	7.91±0.94
50	7.54±0.86	7.97±0.79	8.12±0.79*
R-R (ms)			
sham	908.10±141.00	944.55±138.57	962.60±153.03
2	867.65±133.80	933.85±131.51	932.15±154.15
15	871.70±152.82	922.70±165.94	928.10±124.78
50	880.25±218.43	938.80±136.23	937.05±146.96

Mean ± standard deviation; BLP= recording of before electroacupuncture period; EAP= recordings
of Electroacupuncture period; PEP= recordings of post-electroacupuncture period; In= natural logarithms; HF= high frequency; LF=low frequency; LF/HF= LF'HF ratio; VLF= very low frequency; TP= total power; Var= variance of R-R intervals; R-R= R-R interval; sham= sham session;
2= 2Hz electroacupuncture session; 15= 15Hz Electroacupuncture session;
50= 50Hz electroacupuncture sessions. *$p < 0.05$ compared to BLP.

Table 2. Effect of electroacupuncture at different frequencies on blood pressure

	BLP	EAP	PEP
Systolic			
sham	108.75±10.76	108.80±9.89	109.65±10.01
2	113.25±9.08	112.05±9.52	114.50±11.25
15	111.45±11.85	109.25±11.73	109.95±11.73
50	110.70±10.94	109.75±10.63	111.30±10.21
Diastolic			
sham	67.90±8.71	68.75±9.65	70.45±9.58
2	73.10±8.92	72.25±9.34	74.05±7.54
15	71.25±8.54	70.15±8.36	70.60±8.95
50	70.05±7.14	67.50±7.80	69.65±8.42
Mean			
sham	81.52±8.50	82.10±8.98	83.52±8.88
2	86.48±8.42	85.52±8.62	87.53±7.81
15	84.65±9.20	83.18±9.14	83.72±9.29
50	83.60±7.80	81.58±7.96	83.53±8.18

Mean ± standard deviation; BLP= recording of before electroacupuncture period; EAP= recordings of Electroacupuncture period; PEP= recordings of post-electroacupuncture period; Systolic=systolic blood pressure; Diastolic=diastolic blood pressure; Mean= mean Arterial pressure; sham= sham session; 2= 2Hz electroacupuncture session; 15= 15Hz Electroacupuncture session; 50= 50Hz electroacupuncture session.

Figure 1