The American Journal of Chinese Medicine, Vol. 33, No. 2, 307–314
© 2005 World Scientific Publishing Company
Institute for Advanced Research in Asian Science and Medicine

Atropine-Induced HRV Alteration is Not Amended by Electroacupuncture on Zusanli

Chi-Hsien Chang,[*] Jin-Long Huang,[†,¶] Chih-Tai Ting,[†,¶] Chi-Sen Chang[‡,§] and Gran-Hum Chen[‡,¶]

[*]*Institute of Food Science, National Chung Hsing University*

[†]*Division of Cardiology and* [‡]*Division of Gastroenterology, Taichung Veterans General Hospital*

[§]*Institute of Internal Medicine, Chung Shan Medical University*

Taichung, Taiwan

[¶]*Institute of Clinical Medicine, National Yang-Min University*

Taipei, Taiwan

Abstract: Acupuncture is known to influence autonomic nervous activity. Acupuncture on Zusanli points has been shown to enhance the regularity of gastric myoelectrical activity and accelerate gastric emptying, partly through the vagal pathway, in dogs. The aim of this study was to evaluate whether atropine-induced autonomic nervous alteration, measured by heart rate variability (HRV), could be amended by electroacupuncture on Zusanli points. HRV measurements were recorded in 15 healthy volunteers before, during and after electroacupuncture. Each subject was studied for three sessions in a randomized sequence, which included electroacupuncture on the Zusanli (St 36) points with or without premedication of atropine and placebo stimulation on a non-acupoint. The analysis of low frequency (LF), high frequency (HF) and LF/HF ratios were compared between different sessions. Serum levels of gastrin, motilin and pancreatic polypeptide (PP) levels were also measured. There was an increase in the LF/HF ratio (indicating increased sympathetic activity) during the post-acupuncture period with 2 Hz of electrical stimulation on the Zusanli acupoints. When IV atropine was used immediately before the electroacupuncture, there was a decrease in the LF power and HF power during the acupuncture and post-acupuncture periods. In addition, there was a significant increase in the LF/HF ratio during the acupuncture and post-acupuncture periods. There was a significant decrease in serum PP in the post-acupuncture period after premedication with IV atropine. In conclusion, atropine-induced HRV change might be mediated via the vagal pathway. However, atropine-induced HRV alteration is not amended by electroacupuncture on Zusanli points.

Keywords: Electroacupuncture; Atropine; Heart Rate Variability; Autonomic Nerve Function.

Correspondence to: Dr. Chi-Sen Chang, Division of Gastroenterology, Taichung Veterans, General Hospital, 160, Sec. 3, Chung-Kang Rd., Taichung, Taiwan 407. Fax: (+886) 4-2374-1318, E-mail: changcs@vghtc.gov.tw

Introduction

The influence of acupuncture is complex, for the effects depend upon the species studied, the acupoints employed, the methods of manipulation, and the underlying functional activity of the organs. Several studies have shown that acupuncture decreased sham feeding-stimulated acid output through naloxane-sensitive opioid mechanisms involving vagal efferent pathways (Li *et al*., 1989; Lux *et al.*, 1994). Furthermore, acupuncture applied to the Ximen point (PC 4) decreased heart rate via facilitation of cardiac vagal activity and suppression of cardiac sympathetic nerve activity (Nishijo *et al.*, 1997). Therefore, a potential role of the autonomic nerve system in mediating the effects of acupuncture on various organ systems is convincing.

Our previous study showed that after intravenous (IV) atropine was given in a dose that induced a parasympatholytic effect, the percentage of normal frequency of gastric myoelectrical activity decreased significantly (Chang *et al*., 2002). Blockage of vagal cholinergic activity by atropine as the cause of gastric dysrhythmia was hypothesized, and atropine-induced gastric dysrhythmia was not corrected by acupuncture. To elucidate further whether atropine-induced autonomic nervous alteration could be amended by electroacupuncture on Zusanli points, we measured the heart rate variability (HRV) (Eckberg 1985; Hayano *et al.*, 1991) with or without premedication with atropine in healthy volunteers. In this study, we also monitored the levels of several gastrointestinal peptides, including serum gastrin, motilin and pancreatic polypeptide (PP) (Taylor *et al*., 1978) in these volunteers.

Subjects and Methods

Subjects

A total of 15 healthy male volunteers (aged 23–30 years, mean 26.8 ± 3.8 years) were enrolled in this study. No subjects had a history of cardiovascular, pulmonary or gastrointestinal disorders, and none had taken any medications in the last 2 weeks before the study. Screening for *Helicobacter pylori* infection was performed using a serologic test for IgG antibodies to *H. pylori* (Pylori ELISA II, Bio Whittaker, Walkersville, MD, USA), and all subjects tested negative for *H. pylori*. The subjects fasted for at least 8 hours before the experiment to avoid compounding effects of meal ingestion on heart rate variability (Cox *et al.*, 1995, Lu *et al.*, 1999). Each subject was studied for three sessions in a randomized order with 3 days between sessions. In session 1, HRV was performed for 30 minutes before electroacupuncture as the baseline, 30 minutes during electroacupuncture on the Zusanli (St 36) points, and for an additional 30 minutes post-electroacupuncture. Session 2 was the same as session 1 except that atropine IV bolus was given immediately before electroacupuncture. Session 3 was the same as session 1 except that placebo stimulation on a non-acupoint was conducted. Informed consent was obtained from each subject, with the ethics review committee of the Taichung Veterans General Hospital approving the study protocol.

Electrical Stimulation of Acupuncture Points

Acupuncture needles were used for electroacupuncture. Two acupuncture needles (0.3 mm in diameter, 30 mm long, Chian-Huei Acupuncture Appliance Co., Taipei, Taiwan) were inserted into the Zusanli (St 36) points and manipulated until a "chi"-sensation was reported (a deep, cramp-like sensation). The Zusanli points are located about 10 cm below the patella and 2 cm laterally from the anterior rest of the tibia. The acupuncture needles were connected to a 9-V battery-powered electrical acupuncture instrument (Model-04S, Gwo-Jih Medical Instrument Factory, Taipei, Taiwan). Biphasic, square electrical stimulation of 2 Hz frequency pulses, with a wave width of 0.16 ms duration at the greatest tolerated strength without discomfort, were delivered for 30 minutes.

Placebo stimulation on a non-acupoint was conducted by inserting acupuncture needles subcutaneously 2 cm lateral to the Zusanli points. No manipulation was performed. The leads to the needles were connected to an inactive channel of the acupuncture stimulator. The stimulator was turned on, but electrical pulses were delivered to an unconnected channel. Hence, the subject experienced the same audio-visual stimuli.

Vagal Blockade

According to the atropine dose-sinus node response relation obtained in healthy subjects (Porter *et al.*, 1990), a dose of 9 μg/kg is considered to cause the parasympatholytic effect. After the baseline EGG recording, an atropine sulfate (9 μg/kg, Sintong, Taoyung, Taiwan) IV bolus was given to each subject before electroacupuncture during session 2.

Assessment of HRV

During the experiment, ventricular rhythm was continuously recorded with a Marquette Holter recorder (Series 8500, Marquette Inc., Milwaukee, USA). Three ECG leads were used: modified leads V1, V5 and aVF. After recording of baseline rhythm for 30 minutes, two additional 30-minute recordings were continued during and after acupuncture. To avoid any artifacts during acupuncture, HRV analysis was conducted for 10 minutes during needle insertion and the initiation of electrical stimulation (to exclude the first and last 10 minutes from each 30-minutes period). The RR intervals were viewed on the computer monitor by two experienced observers. Only those RR intervals related to normal sinus beats were included in the final analysis. HRV analysis was performed in accordance with the recommendations from the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The recordings were processed by a Marquette Laser Holter system (Series 8000XP, Marquette Inc., Milwaukee, USA). Discrete Fourier transformation was used for the analysis of the frequency domain parameters. Analysis of the power spectra was performed on two frequency ranges, revealing a low frequency (LF) component between 0.04 and 0.15 Hz and a high frequency (HF) component between 0.15 and 0.40 Hz. The LF/HF ratio, an

estimate of sympathovagal balances in which a high ratio indicates greater sympathetic activity and a low ratio indicates greater parasympathetic activity, was also calculated. The analysis of LF, HF and LF/HF ratios were compared between different stimulation frequencies to evaluate the effect of acupuncture on autonomic nerve activity.

Assay of Serum Gastrointestinal Hormones

Serum gastrin, motilin and human pancreatic polypeptide (PP) levels were measured with commercial ELISA kits (Euro-Diagnostica, Malmo, Sweden). Ten milliliters of venous blood was collected in tubes without additives at 30-minute intervals at the end of each stage (i.e. baseline, acupuncture and post-acupuncture stages). The blood samples were placed on ice and centrifuged at 4°C at 3000 rpm for 10 minutes. The serum was separated and stored below −20°C before analysis. Serum values were expressed as picograms per milliliter (pg/ml) for gastrin, and as picomolars per liter (pmol/L) for motilin and PP.

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD) for serum motilin, gastrin and PP levels; and mean ± standard error (SE) for LF power, HF power and LF/HF ratio. Statistical analyses were performed to investigate the effects of acupuncture on the HRV parameters and hormones during each session, using repeated measurement analysis of variance (ANOVA) and the Wilcoxon matched-pairs signed ranks test. A $p < 0.05$ was considered statistically significant.

Results

Effects of Acupuncture

The baseline LF and HF power in each session was taken as its own control to evaluate the effect of electroacupunture. There were no significant changes in the power of LF and HF or LF/HF ratio in the control group during the acupuncture and post-acupuncture periods. When 2 Hz of electrical stimulation was applied on the Zusanli points, there was an increase in the LF power during the acupuncture and post-acupuncture periods (baseline, acupuncture and post-acupuncture: 1130.2 ± 442.2, 1170.2 ± 282.2 and 1446.8 ± 360.2, respectively). The difference between the baseline and post-acupuncture periods was statistically significant ($p < 0.05$) (Table 1). The HF power decreased slightly during the acupuncture and post-acupuncture periods with 2 Hz of electrical stimulation on the Zusanli points (baseline, acupuncture and post-acupuncture: 1042.2 ± 158.2, 1018.1 ± 166.2 and 1019.5 ± 140.2, respectively). However, the change was not significant when compared to the baseline period. The LF/HF ratio, an estimate of sympathovagal balance, was also calculated and compared. There was an increase in the LF/HF ratio during post-acupuncture of 2 Hz of electrical stimulation on the Zusanli points (baseline versus post-acupuncture 1.03 ± 0.34 versus 1.51 ± 0.28, $p < 0.01$).

Table 1. LF Power (0.04–0.15 Hz, Ms^2), HF Power (0.15–0.40 Hz, Ms^2) and LF/HF Ratio in Each Group at Baseline, During Acupuncture and Post-Acupuncture

Group	Baseline (mean ± SE)	Acupuncture (mean ± SE)	Post-Acupuncture (mean ± SE)
Control			
LF power	1482.6 ± 490.2	1340.2 ± 302.4	1396.2 ± 380.1
HF power	1246.2 ± 166.2	1211.6 ± 240.2	1145.2 ± 163.3
LF/HF ratio	1.18 ± 0.22	1.16 ± 0.20	1.17 ± 0.32
Zusanli group			
LF power	1130.2 ± 442.2	1170.2 ± 282.2	1446.8 ± 360.2
HF power	1042.2 ± 158.2	1018.1 ± 166.2	1019.5 ± 140.2
LF/HF ratio*	1.03 ± 0.34	1.24 ± 0.32	1.51 ± 0.28
Atropine group			
LF power†	1636.9 ± 348.5	492.9 ± 296.3	672.4 ± 192.2
HF power†	1580.8 ± 232.4	53.8 ± 22.9	322.6 ± 92.0
LF/HF ratio†	1.04 ± 0.17	9.28 ± 2.70	4.17 ± 1.09

*Zusanli group, LF/HF ratio: baseline versus post-acupuncture, $p < 0.05$, by paired t-test; †Atropine group, LF power, HF power and LF/HF ratio: baseline versus acupuncture and post-acupuncture, $p < 0.01$; by one-way ANOVA (repeated).

Effect of Atropine

When IV atropine was used immediately before the electroacupuncture, there were marked decreases in the LF power during the acupuncture and post-acupuncture periods (baseline, acupuncture and post-acupuncture: 1636.9 ± 348.5, 492.9 ± 296.3 and 672.4 ± 192.2, respectively, $p < 0.01$). HF power were also marked decreases during the acupuncture and post-acupuncture periods (baseline, acupuncture and post-acupuncture: 1580.8 ± 232.4, 53.8 ± 22.9 and 322.6 ± 92.0, respectively, $p < 0.01$). Furthermore, the LF/HF ratio increased significantly during the acupuncture and post-acupuncture periods (baseline, acupuncture and post-acupuncture: 1.04 ± 0.17, 9.28 ± 2.70 and 4.17 ± 1.09, respectively, $p < 0.01$) (Table 1).

Change of Serum Hormone Levels

The serum levels of motilin did not change during any study period among all three sessions (Table 2). The serum levels of gastrin increased significantly during the post-acupuncture period with electro-stimulation on the Zusanli points (post-acupuncture versus baseline and acupuncture: 39.20 ± 17.15 pg/ml versus 34.42 ± 15.85 pg/ml and 32.98 ± 15.53 pg/ml, respectively, $p < 0.01$). Interestingly, there was a significant decrease in serum PP during pre-medication with atropine (baseline versus acupuncture and post-acupuncture: 22.64 ± 9.27 pmol/L versus 16.37 ± 8.25 pmol/L and 15.80 ± 8.39 pmol/L, respectively, $p < 0.01$).

Table 2. Serum Motilin (pmol/L), Gastrin (pg/ml) and Pancreatic Polypeptide Levels (pmol/L) in Each Group During Baseline, Acupuncture and Post-Acupuncture

Group	Baseline (mean ± SD)	Acupuncture (mean ± SD)	Post-Acupuncture (mean ± SD)
Control			
Motilin	63.80 ± 22.71	64.36 ± 26.46	59.69 ± 19.37
Gastrin	34.30 ± 14.92	36.20 ± 15.78	37.41 ± 16.21
PP	26.28 ± 12.07	26.30 ± 12.70	21.22 ± 11.66
Zusanli			
Motilin	60.81 ± 21.84	61.58 ± 22.32	69.18 ± 23.88
Gastrin*	34.42 ± 15.85	32.98 ± 15.53	39.20 ± 17.15
PP	24.24 ± 12.64	22.81 ± 13.47	25.55 ± 17.22
Atropine			
Motilin	56.85 ± 23.75	49.77 ± 20.90	56.04 ± 23.92
Gastrin	34.79 ± 18.53	36.12 ± 18.26	37.27 ± 19.31
PP†	22.64 ± 9.27	16.37 ± 8.25	15.80 ± 8.39

PP: Pancreatic polypeptide, *gastrin, Zusanli group: post-acupuncture versus baseline and acupuncture, $p < 0.01$; †pp, Atropine group: baseline versus acupuncture and post-acupuncture, $p < 0.01$.

Discussion

According to Porter *et al*. (1990), a dose of 9 μg/kg atropine is considered to cause a parasympatholytic effect in healthy subjects. Atropine, a competitive antagonist of acetylcholine and other muscarinic agonists, can efficiently inhibit the vagal impulses. In this study, there was a significant decrease in serum PP when patients were pre-medicated with an IV of atropine in a dose that induced a parasympatholytic effect. This finding may indicate that atropine can effectively inhibit the abdomino-vagal activity (Schwartz, 1983; Koop *et al*., 1984). The results of HRV studies showed that after an IV of atropine in a dose that induced a parasympatholytic effect, the power of LF (sympathetic nerve activity) as well as HF (parasympathetic nerve activity) decreased significantly during the electroacupuncture and post-acupuncture periods. In addition, there was a significant increase in the LF/HF ratio during the acupuncture and post-acupuncture periods, indicating increased sympathetic activity. The phenomenon probably is secondary to the higher extent of suppression in cardio-vagal activity.

No significant changes in LF power or HF power were observed with electroacupuncture on Zusanli acupoints. However, an increase in the LF/HF ratio (indicating greater sympathetic activity) during post-acupuncture of 2 Hz of electrical stimulation on the Zusanli points was observed. This observation was somewhat different from previous results done with Ximen acupoint (PC 4) (Nishijo *et al*., 1997). In that study, acupuncture stimulation applied to the Ximen point (P4) decreased heart rate via facilitation of cardiac vagal activity and suppression of the cardiac sympathetic nerve.

The serum levels of motilin did not change during any study period among all sessions. This indicated that neither electroacupuncture nor IV atropine had any effect on serum motilin levels. There was a significant increase in serum gastrin during the post-acupuncture period which was not observed in the control group and when a patient was premedicated with an IV of atropine. This finding may indicate that electroacupuncture increases the abdomino-vagal activity (Stoddard *et al.*, 1981).

Acupuncture has been used to treat GI symptoms in Asian countries for centuries (Vickers *et al*., 2002). The change in the sympathetic and parasympathetic nervous system activities after acupuncture is dependent on the site of stimulation and period of observation (Haker *et al*., 2000). Several studies have shown that acupuncture decreases sham feeding-stimulated acid output through naloxane-sensitive opioid mechanisms involving vagal efferent pathways (Li *et al*., 1989; Lux *et al.*, 1994). According to Ouyang *et al*. (2002), electroacupuncture on Neiguan (PC6) and Zusanli (St 36) points performed in dogs implanted with a duodenal cannula and serosal electrodes revealed improvement of gastric emptying. In their study, vagal activity assessed from the spectral analysis of HRV was markedly increased after electroacupuncture. In addition, Xu (1994) reported that the regulatory effect of electroacupuncture on gastric dysrhythmia in rabbits was abolished after vagotomy. Therefore, a pivotal role of vagal activity mediating the effect of acupuncture is acceptable. However, this phenomenon was not observed in our study on humans. This discrepancy may be due to the differences in species studied, the acupoints employed, and the methods of manipulation.

The autonomic nervous system is activated primarily by centers located in the spinal cord, brain stem and hypothalamus. The effects of parasympathetic nerve stimulation are in many ways opposite to the effects of sympathetic response. The parasympathetic system, however, is not normally activated as a whole. Stimulation of separate parasympathetic nerves can result in slowing of the heart, dilation of visceral blood vessels, and increased activity of the digestive tract. Therefore, it is possible that acupuncture on the acupoints within the heart meridian, such as Neiguan (PC 6) and Ximen (PC 4), could induce a more significant cardiovagal effect than within the stomach meridian, such as Zusanli (St 36) (Nishijo *et al*., 1997; Ouyang *et al*., 2002). A study by Muth *et al*. (1998) supported the hypothesis that there were different presentations in the autonomic system between cardiac and gastric vagal activity. In conclusion, the results of our study indicated that atropine-induced HRV change might be mediated via the vagal pathway. However, atropine-induced HRV alteration is not amended by electroacupuncture on Zusanli points.

Acknowledgments

This study was supported by a grant (No.TCVGH-923305C) from the Taichung Veterans General Hospital, Taichung, Taiwan. The authors would like to thank the Biostatistics Task Force of Taichung Veterans General Hospital for their help in statistical analysis.

References

Chang, C.S., J.W. Chou, C.Y. Wu, Y.H. Chang, C.W. Ko and G.H. Chen. Atropine-induced gastric dysrhythmia is not normalized by electroacupuncture. *Dig. Dis. Sci.* 47: 2466–2472, 2002.

Cox, H.S., D.M. Kaye, J.M. Thompson, A.G. Turner, G.L. Jennings, C. Itsiopoulos and M.D. Esler. Regional sympathetic nervous activation after a large meal in humans. *Clin. Sci.* (*Lond.*) 89: 145–154, 1995.

Eckberg, D.L. Human sinus arrhythmia as an index of vagal cardiac outflow. *J. Appl. Physiol.* 54: 961–966, 1985.

Haker, E., H. Egekvist and P. Bjerring. Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. *J. Auton. Nerv. Syst.* 79: 52–59, 2000.

Hayano, J., Y. Sakakibara, A. Yamada, M. Yamada, S. Mukai, T. Fujinami, K. Yokoyama, Y. Watanabe and K. Takata. Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects. *Am. J. Cardiol.* 67: 99–204, 1991.

Koop, H., R. Arnold and W. Creutzfeldt. Pancreatic polypeptide and gastrin release during sham feeding in man. *Digestion* 30: 47–52, 1984.

Li, Y.Y., G. Tougas, S.G. Chiverton and R.H. Hunt. Mechanisms of acid inhibition by electroacupuncture. *Gastroenterology* 96: A300, 1989.

Lu, C.L., X.P. Zou, W.C. Orr and J.D.Z. Chen. Postprandial changes of sympathovagal balance measured by heart rate variability. *Dig. Dis. Sci.* 44: 857–861, 1999.

Lux, G., J. Hagel, P. Backer, G. Backer, R. Vol, H. Ruppin, S. Domschke and W. Domschke. Acupuncture inhibits vagal acid secretion stimulated by sham feeding in healthy subjects. *Gut* 35: 1026–1029, 1994.

Muth, E.R., J.F. Thayer, R.M. Stern, B.H. Friedman and C. Drake. The effect of autonomic nervous system activity on gastric myoelectrical activity: does the spectral reverse hypothesis hold for the stomach? *Biol. Psychol.* 71: 265–278, 1998.

Nishijo, K., H. Mori, K. Yosikawa and K. Yazawa. Decreased heart rate by acupuncture stimulation in humans via facilitation of cardiac vagal activity and suppression of cardiac sympathetic nerve. *Neurosci. Lett.* 227: 165–168, 1997.

Ouyang, H., J. Yin, Z. Wang, P.J. Pasricha and J.D.Z. Chen. Electroacupuncture accelerates gastric emptying in association with changes in vagal activity. *Am. J. Physiol.* 282: G390–396, 2002.

Porter, T.R., D.L. Eckberg, J.M. Fritsch, R.F. Rea, L.A. Beightol, J.F. Schmedtje Jr and P.K. Mohanty. Autonomic pathophysiology in heart failure patients: sympathetic-cholinergic interrelations. *J. Clin. Invest.* 85: 1362–1371, 1990.

Schwartz, T.W. Pancreatic polypeptide: a hormone under vagal control. *Gastroenterology* 85: 1411–1425, 1983.

Stoddard, C.J., R.H. Smallwood and H.L. Duthie. Electrical arrhythmias in the human stomach. *Gut* 22: 704–712, 1981.

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

Taylor, I.L., M. Feldman, C.T. Richardson and J.H. Walsh. Gastric and cephalic stimulation of human pancreatic polypeptide release. *Gastroenterology* 75: 432–437, 1978.

Vickers, A., P. Wilson and J. Kleijnen. Acupuncture. *Qual. Health Care* 11: 91–97, 2002.

Xu, G. Regulating effect of electro-acupuncture on dysrhythmia of gastro-colonic electric activity induced by erythromycin in rabbits. *Chen Tzu Yen Chiu* 19: 71–74, 1994.

Copyright of American Journal of Chinese Medicine is the property of World Scientific Publishing Company and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.