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2 Hz Electro-Acupuncture at Yinlingquan (SP9) and Ququan (LR8) Acupoints Induces Changes in Blood Flow in the Liver and Spleen

Wen-Cheng Chou,* Hsu-Jan Liu,* Yi-Wen Lin,* Chin-Yi Cheng,*
Tsai-Chung Li,† Nou-Ying Tang‡ and Ching-Liang Hsieh*,§,¶

*Graduate Institute of Acupuncture Science

†Graduate Institute of Biostatistics

‡School of Chinese Medicine

§Acupuncture Research Center
China Medical University, Taichung, Taiwan

¶Department of Chinese Medicine
China Medical University Hospital, Taichung, Taiwan

Abstract: According to the principles of traditional Chinese medicine, channels and collaterals within the body provide pathways through which qi and blood travel, and each channel or collateral is linked with a specific organ. The Yinlingquan (spleen 9, SP9) and Ququan (liver 8, LR8) acupoints represent the sea points of the spleen and liver meridians, respectively, from which qi and blood flow into their specific visceral organs. The purpose of this study was to investigate the changes in blood flow/perfusion in the liver and spleen resulting from the application of 2 Hz electro-acupuncture (EA) to the Yinlingquan (SP9) or Ququan (LR8) acupoints. A total of 18 Spragrue-Dawley rats were randomly divided into three groups of six rats each as follows: sham group receiving sham EA; Yinlingquan (SP9) group receiving 2 Hz EA, applied at bilateral Yinlingquan (SP9) acupoints; and Ququan (LR8) groups receiving 2 Hz EA, applied at bilateral Ququan (LR8) acupoints. The mean blood flow/perfusion of the spleen and liver was recorded using a laser Doppler blood flow monitor prior to EA (representing the baseline), during EA, and post-EA. Each measurement period lasted ten minutes. Nitric oxide levels were also measured from the right femoral arterial blood, following the conclusion of each series of blood flow/perfusion recordings. The results indicate that the sham EA did not increase the mean blood flow/perfusion in the

Correspondence to: Dr. Ching-Liang Hsieh, Graduate Institute of Acupuncture Science, China Medical University, 91 Hsueh-Shih Road, Taichung, 40402, Taiwan. Tel: (+886) 4-2205-3366 (ext. 3600), Fax: (+886) 4-2203-5191, E-mail: clhsieh@mail.cmuh.org.tw

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liver or spleen; 2 Hz EA at bilateral Yinlingquan (SP9) acupoints increased the mean blood flow/perfusion in the spleen, but not in the liver. In contrast, 2 Hz EA at bilateral Ququan (LR8) acupoints increased the mean blood flow/perfusion in the liver, but not in the spleen. Nitric oxide levels showed no significant difference between any of the groups at any stage of the measurements. According to the results, we conclude that EA at the Yinlingquan (SP9) and Ququan (LR8) acupoints can increase the blood flow in the spleen and liver, respectively.

Keywords: Mean Blood Flow Perfusion; Liver; Spleen; Electro-Acupuncture.

Introduction

In traditional Chinese medicine, channels and collaterals provide pathways through which qi and blood are transferred throughout the body. Channels and collaterals are connected with internal organs and external muscles, skin, and hair. The development of disease is thought to be the result of disturbances in the flow of qi or blood. Acupuncture employs a special metal needle that is used to pierce the body at specific points, whereupon the needle is twisted or otherwise manipulated to modulate the qi and blood along the meridians as a means of treating disease (Lin, 2009; Sun *et al.*, 2010). Ulett *et al.* (1998) described the analgesic effects and autonomic nervous regulation exhibited by acupuncture, claiming that electro-acupuncture (EA) is more effective than its manual counterpart. In addition, the frequency of EA selectively induces the release of various neuropeptides (Han, 2004). Many studies have shown that acupuncture influences gastric motility (Sato *et al.*, 1993), urinary bladder (Sato *et al.*, 1992), increased heart rate, and elevated blood pressure (Kimura *et al.*, 1995). Sato *et al.* (1997) suggested that acupuncture regulates the function of internal organs through the autonomic nervous system (Sato *et al.*, 1997). Acupuncture can increase the parietal blood flow and mean arterial pressure in the brain of rat (Uchida *et al.*, 2000), increase skin and uterine blood flow (Hotta *et al.*, 1999; Jan *et al.*, 2010), and reduce renal blood flow and the flow blood in skeletal muscles (Noguchi *et al.*, 1999). Stener-Victorin *et al.* (2004) suggested that low-frequency (2 Hz) EA significant increases in ovarian blood flow (OBF) in anaesthetized rats, and our previous study showed that both 2 and 15 Hz EA induced the increase of cerebral blood flow (Hsieh *et al.*, 2006). Therefore, 2 Hz EA was applied in this study. Nitric oxide (NO) is generated from nitric oxide synthase (NOS), and endothelial NOS (eNOS) play a role in neuro-protection by increasing blood flow (Choi, 1993; Samdani *et al.*, 1997).

According to the theory of channels and collaterals, each meridian is connected with a specific organ(s). In this study, we examined 2 Hz EA at Yinlingquan (SP9) acupoint located on the tibial aspect of the leg and Ququan (LR8) acupoint located on the medial aspect of the knee. The Yinlingquan (SP9) and Ququan (LR8) points represent the sea points of the spleen (SP), and liver meridians (LR), respectively. Laser Doppler ultrasound was used to simultaneously detect the perfusion of local mean blood flow in Sprague-Dawley (SD) rats' spleens and livers to explore the relationships between the meridian and the organs.

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Materials and Methods

Animals

Eighteen healthy male SD rats, weighing 250–350 g, were purchased from the laboratory animal center in the BioLASCO Taiwan Co. Animal experiments were conducted in the animal center of the China Medical University. The rats were placed in stainless steel cages at 25°C on a 12 h light-dark cycle. All experimental procedures followed *the Guideline Principles for the Care and Use of Laboratory Animals* of the China Medical University.

Experimental Procedure

Rats were anesthetized by intra-peritoneal injection of chloral hydrate (400 mg/kg), and placed in a supine position with the extremities fixed. A scalpel was used to incise the skin of the right inguinal region to expose the femoral artery, whereupon a PE-50 tube was inserted to monitor blood pressure, heart rate, arterial blood gas, and NO levels. In addition, a longitudinal incision in the central abdomen was made along the ventral median line (length approx. 5 cm), to expose the liver and the spleen.

Grouping and Intervention

Eighteen rats were randomly divided into three groups of six rats each as follows: (1) sham group (SG): the stainless steel needles (length 1.27 cm, #34 gauge, Chan Hui, Taiwan) were inserted into subcutaneous tissue of bilateral Yinlingquan (SP9) acupoints, and 2 cm above to the acupoints. The needles were not twisted and they were connected to an electrical stimulation device (Han's Healthronics Likon, Taipei, Taiwan); however, no electrical stimulation was delivered. The mean blood flow/perfusion in the liver and the spleen was recorded simultaneously for 10 min at pre-EA, during EA, and at post-EA; (2) Yinlingquan (SP9) group (YG): the methods were identical to those of SG; however, stainless steel needles were inserted into the muscle layer of bilateral Yinlingquan (SP9) acupoints, and 2 cm above the acupoints. The needles were connected to an electrical stimulation device set at 2 Hz. The intensity of the stimulation was just adequate to induce a visible muscle contraction; and (3) Ququan (LR8) group (QG): the methods were identical to those of YG; however, stainless steel needles were inserted into bilateral Ququan (LR8) acupoints, and 2 cm above the acupoints. These needles were also connected to an electrical stimulation device set at 2 Hz.

Measurement of Mean Blood Flow/Perfusion and NO

Two probes from a laser Doppler blood flow detector (DRT4, Moor Instruments Inc. Wilmington, USA) were placed 5 mm above the right lobe of the liver and the middle of the spleen to measure the mean blood flow/perfusion in the liver and the spleen. The data were stored in a personal computer prior to analysis. The mean blood flow/perfusion was

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determined prior to EA (representing the baseline), during EA, and post-EA, and each measurement period lasted for 10 min.

The blood (0.6 ml) was collected from the right femoral artery at the end of each series of mean blood flow/perfusion recordings. Blood (0.4 ml) was used for blood gas analysis and the rest (0.2 ml) was used to determine NO levels. Arterial blood gas analysis was measured using an automatic analyzer (Instrumentation Laboratory, Synthesis 25, USA), including the parameters of pH, pCO_2, pO_2, and HCO_3. The blood (0.2 ml) was placed in a 1.5 ml tube followed by 0.4 ml cold ethanol (0°C) and homogenized using a vibrator for 1 min. The sample stood for 30 min, and centrifuged at 14,000 g for 5 min. The supernatant was used to determine NO levels. NO levels were determined using a gas-phase chemiluminescent NO analyzer (NOA 280i, Sievers Instrument Inc., USA), in which NO reacted with oxyhemoglobin and superoxide anions to form a nitrate. Vanadium (III) chloride in hydrochloric acid was used to convert the nitrate to NO. NO levels were measured according to a standard curve of sodium nitrate of known concentration.

Statistical Analysis

The data were analyzed using SPSS and expressed as mean ± standard deviation (SD). The Kruskal-Wallis test was followed by least significant difference (LSD) for comparisons between series of measurements with $p < 0.05$ regarded as significant.

Results

The blood gas analysis including pH, pCO_2, pO_2, and HCO_3, showed no significant difference between the measurements taken at pre-EA and EA, pre-EA and post-EA, or between EA and post-EA in the SG, YG, and QG ($p > 0.05$, Table 1).

NO levels showed no significant difference between measurements taken at pre-EA and EA, pre-EA and post-EA, or between EA and post-EA in the SG, YG, and QG ($p > 0.05$, Table 2).

The mean blood flow/perfusion in the spleen and the liver showed no significant difference between measurements taken at pre-EA and EA, EA and post-EA, or between pre-EA and post-EA (all $p > 0.05$, Table 3) in the SG.

The mean blood flow/perfusion in the spleen was greater during EA than pre-EA, or post-EA (both $p < 0.05$, Table 3); whereas, the mean blood flow/perfusion in the spleen in the post-EA period was similar to that measured at pre-EA (all $p > 0.05$, Table 3) in the YG. The mean blood flow/perfusion of the liver showed no significant difference between pre-EA and EA, EA and post-EA, or between pre-EA and post-EA (all $p > 0.05$, Table 3) in the YG.

The mean blood flow/perfusion in the liver was greater during EA than at pre-EA ($p < 0.05$), or post-EA ($p < 0.01$, Table 3) in the QG; whereas, the mean blood flow/perfusion in the liver post-EA was similar to the measurements taken pre-EA ($p > 0.05$, Table 3) in the QG. The mean blood flow/perfusion in the spleen showed no significant difference between the measurements taken pre-EA and EA, EA and post-EA, or between pre-EA and post-EA ($p > 0.05$, Table 3) in the QG.

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Table 1. Arterial Blood Gas Analysis

Group	pH			pCO_2			pO_2			HCO_3		
	B	EA	Post-EA	B	EA	Post-EA	B	EA	Post-EA	B	EA	Post-EA
SG	7.28 ± 0.06	7.28 ± 0.04	7.24 ± 0.04	40.70 ± 4.31	42.49 ± 1.00	43.1 ± 3.24	138.94 ± 17.35	124.25 ± 19.36	128.97 ± 20.48	19.45 ± 2.14	20.21 ± 2.12	18.88 ± 2.85
YG	7.31 ± 0.01	7.28 ± 0.03	7.27 ± 0.03	43.70 ± 2.86	44.77 ± 2.63	45.70 ± 3.79	106.17 ± 14.66	105.67 ± 14.40	106.67 ± 17.05	22.23 ± 1.06	21.48 ± 1.12	21.18 ± 2.37
QG	7.23 ± 0.03	7.20 ± 0.02	7.24 ± 0.03	45.75 ± 1.47	48.72 ± 2.99	44.32 ± 5.60	88.33 ± 4.72	97.00 ± 12.54	110.00 ± 38.73	19.55 ± 1.53	19.30 ± 1.22	19.38 ± 2.43

Note: mean ± standard deviation. SG: sham group; YG: Yinlingquan (SP9) group; QG: Ququan (LR8) group. Baseline: before electro-acupuncture (pre-EA); during electro-acupuncture (EA); post-electro-acupuncture period (post-EA).

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Table 2. Nitric Oxide (NO) Levels (μM)

Group	B	EA	Post-EA
SG	35.06 ± 11.55	35.34 ± 13.63	33.61 ± 14.01
YG	39.20 ± 14.54	37.15 ± 11.07	39.09 ± 10.05
QG	29.46 ± 3.88	29.45 ± 3.50	28.79 ± 5.23

Note: mean $\pm$ standard deviation. SG: sham group; YG: Yinlingquan (SP9) group; QG: Ququan (LR8) group. Baseline: before electro-acupuncture (pre-EA); during electro-acupuncture (EA); post-electro-acupuncture period (post-EA).

Table 3. Effects of Electro-Acupuncture on Mean Blood Flow/Perfusion in the Spleen and the Liver

Group	Spleen			Liver		
	B	EA	Post-EA	B	EA	Post-EA
SG	352.3 ± 110.7	322.0 ± 86.7	300.4 ± 89.3	273.1 ± 72.1	257.6 ± 74.4	267.5 ± 109.5
YG	200.0 ± 58.0	271.6 ± 75.9*	$212.6 \pm 91.6^{\#}$	125.1 ± 74.1	129.7 ± 91.1	100.8 ± 92.7
QG	191.4 ± 35.0	238.7 ± 48.5	185.7 ± 57.4	88.4 ± 26.0	139.6 ± 30.6*	$106.3 \pm 21.6^{\#\#}$

Note: mean $\pm$ standard deviation. SG: sham group; YG: Yinlingquan (SP9) group; QG: Ququan (LR8) group. Baseline: before electro-acupuncture (pre-EA); during electro-acupuncture (EA); post-electro-acupuncture period (post-EA); $^{*}p < 0.05$ compare with the values of B; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compare with the values of EA.

Discussion

Based on the theory of channels and collaterals, a sea point means that when qi and blood is running in the meridians, as throughout the confluence of rivers into the sea. It mostly located near the elbows or knees. Yinlingquan (SP9) and Ququan (LR8) acupoints represent the sea point of the spleen meridian (SP) and the liver meridian (LR), respectively. In this study, we examined 2 Hz EA at Yinlingquan (SP9) and Ququan (LR8) acupoints to assess the influence of blood flow in the liver and the spleen. Our results indicate that the mean blood flow/perfusion of spleen and liver did not change in the SG, whereas the 2 Hz EA applied to bilateral Yinlingquan (SP9) acupoints increased the mean blood flow/perfusion in the spleen, but not in the liver. In contrast, the 2 Hz EA applied to bilateral Ququan (LR8) acupoints increased the mean blood flow/perfusion in the liver, but not in the spleen. These results indicate that the sea points of the spleen and the liver meridians are specific to their affiliated organs — the spleen or liver. These results partly support the meridian theory of traditional Chinese medicine in which the qi and blood pool at the sea point within a meridian, whereupon they are transferred to specific organ(s) (Wang, 2007).

Xu *et al.* (2007) found that 1 Hz EA stimulation at the five Shu points of the liver and lung meridians can activate regions of the brain, as demonstrated in functional magnetic resonance imaging (fMRI). These findings explain the existence of both correlations and independent characteristics between the liver and lung meridians (Xu *et al.*, 2007), and also partly supports our results. Jin *et al.* (2001) reported that the flow of bile and the content of Na^+, K^+, Cl^- were increased only in the Ququan (LR8) acupoint, delivering EA to various

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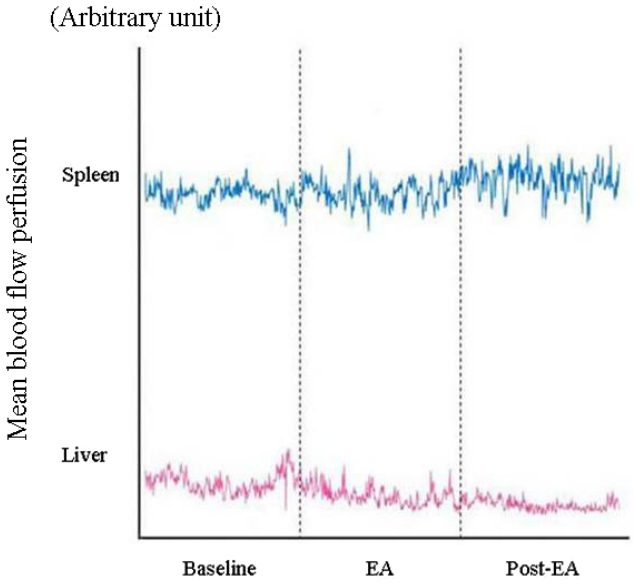

Figure 1. The mean changes of blood flow/perfusion induced by sham electro-acupuncture. Sham electro-acupuncture did not induce changes in mean blood flow/perfusion in the liver or the spleen. Baseline: before electro-acupuncture (pre-EA); during electro-acupuncture (EA); post-electro-acupuncture period (post-EA).

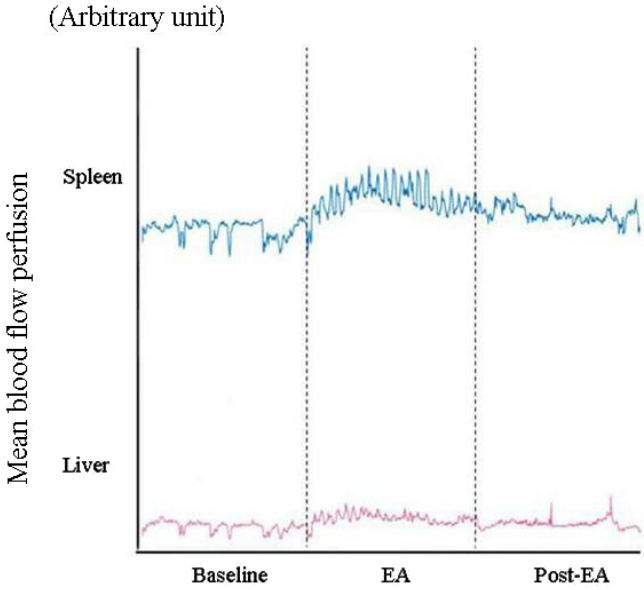

Figure 2. The mean changes of blood flow/perfusion induced by 2 Hz electro-acupuncture at Yinlingquan (SP9) acupoints. The 2 Hz electro-acupuncture applied at the Yinlingquan (SP9) acupoints induced an increase in the mean blood flow/perfusion in the spleen, but not in the liver. Baseline: before electro-acupuncture (pre-EA); during electro-acupuncture (EA); post-electro-acupuncture period (post-EA).

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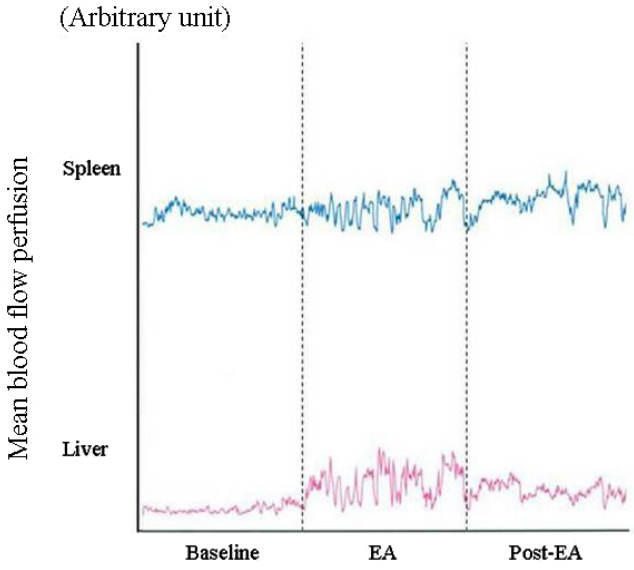

Figure 3. The mean changes of blood flow/perfusion induced by 2 Hz electro-acupuncture at Ququan (LR8) acupoints. The 2 Hz electro-acupuncture applied at the Ququan (LR8) acupoints induced an increase in the mean blood flow/perfusion in the liver, but not in the spleen. Baseline: before electro-acupuncture (pre-EA); during electro-acupuncture (EA); post-electro-acupuncture period (post-EA).

points of the liver, gallbladder, heart, and stomach meridians (Jin *et al.*, 2001). Zhang *et al.* (2009) found that both acupuncture and EA (1 Hz) at Zusanli (ST36) acupoints increased the blood flow in the stomach of rats (Zhang *et al.*, 2009). Acupuncture at Xiaguan (ST7) also increased the blood flow in the muscles, kidneys, brain, and heart of rats (Tsuru and Kawakita, 2009). Taken together, these results are consistent with our results, indicating that acupuncture or EA can increase blood flow in internal visceral organs, and each meridian is connected to a specific organ. In fact, the selection of acupoints in the clinical treatment of disease is performed in accordance with the traditional theory of meridians (Yu *et al.*, 2010).

Liver hemodynamics is regulated by many different factors, including the portal system, the input of the hepatic artery (Charbon and Anderson, 1989), and the release of neuropeptides from the autonomic nervous system (Uyama *et al.*, 2004). A number of studies have suggested that acupuncture influences the autonomic nervous system and increases the release of neuropeptides (Han, 2003; Lim *et al.*, 2003). Thus, it is clear that acupuncture influences local blood flow and the systemic blood circulation, including cardiac output, peripheral resistance, and the volume of the blood (Kubo *et al.*, 2010; Meng *et al.*, 1986). The pathways of signal transduction in EA or acupuncture are as follows: (1) via the afferent nerve from the receptor to the brain, and then down to the internal organs such as the liver or the spleen; (2) via bodily fluids, hormones, peptides, or neurotransmitters to spread to the internal organs. The circulation of blood in the liver is influenced by noradrenaline and acetylcholine, and neuropeptides including substance P, calcitonin

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gene-related peptides (CGRP), neuropeptide Y (NPY), vasoactive intestinal polypeptides (VIP), somatostatin, glucagon, glucagon-like peptide, neurotensin, such as serotonin and galanin (Uyama *et al.*, 2004; Yoneda, 1998); and (3) the results of this study indicate that the meridians correspond to specific organ(s), such as the relationship between Ququan (LR8) points and increased blood flow in the liver, and that of Yinlingquan (SP9) points and increased blood flow in the spleen. This suggests that nerve segments play at least a partial role in these processes; however, the underlying mechanisms require a further study. In addition, the results of the present study indicated no significant difference between the arterial blood gas (including pH, pCO_2, pO_2, and HCO_3, and NO levels) at pre-EA, EA, or post-EA in the SG, YG, or QG. Based on these results, we conclude that the study is credible; however, the role of NO requires further study. In addition, as the limitation of this study, the control point was only used in the spleen meridian and no control point for the liver meridian. Therefore, this will be considered in the future study.

In conclusion, EA at the Yinlingquan (SP9) and Ququan (LR8) acupoints can increase the blood flow in the liver and the spleen. These results provide scientific evidence of the specificity of meridians.

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