

Research Article

Effects of GV14 Acupuncture on Cerebral Blood Flow Velocity in the Basilar and Middle Cerebral Arteries and CO₂ Reactivity during Hypercapnia in Normal Individuals

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The Governing Vessel 14 (GV14) (Dazhui) is one of the acupuncture points referred to as “seven acupoints for stroke.” Nevertheless, there is a scarcity of research on the effects of acupuncture treatment at GV14. This study investigated the effects of acupuncture at GV14 on cerebral blood flow (CBF), especially that in the basilar artery (BA) and the middle cerebral arteries (MCA). Sixteen healthy men aged 20 to 29 years were enrolled in this study. CBF velocity and cerebrovascular reactivity (CVR) were measured using transcranial Doppler sonography (TCD). The following were assessed: closed circuit rebreathing- (CCR-) induced carbon dioxide (CO₂) reactivity, modified blood flow velocity at 40 mmHg (CV40) on BA and MCAs, blood pressure (BP), and heart rate (HR). Observed results were obtained after comparison with the baseline evaluation. Statistically significant elevations in CO₂ reactivity were recorded in the BA (3.28 to 4.70, $p < 0.001$) and MCAs (right: 3.81 to 5.25, $p = 0.001$; left: 3.84 to 5.12, $p = 0.005$) after acupuncture at GV14. The CV40 increased statistically significantly only in the BA (45.49 to 50.41, $p = 0.003$). No change was observed in BP (106.83 to 107.08 (mmHg), $p = 0.335$) and HR (77 to 75 (bpm), $p = 0.431$). Acupuncture at GV14 improved CBF velocity. These results could be explained by the regulation of endothelium-dependent vessel dilation effected by acupuncture. This trial is registered with Korean Clinical Trial Registry (<http://cris.nih.go.kr>; registration number: KCT0004787).

1. Introduction

Acupuncture has been used for the treatment of a variety of diseases, including stroke, for thousands of years. Several recent trials have shown that acupuncture has the potential to become valuable for the treatment [1–3], rehabilitation [4], and prevention of stroke [5, 6].

Several acupuncture mechanisms have been discovered to improve stroke, one of which involves increase in the cerebral blood flow (CBF) as demonstrated by positron emission tomography (PET), single-photon emission computed tomography (SPECT), and transcranial Doppler (TCD) [7–12].

TCD is highly advantageous because of the ease of the accessibility, i.e., the test is less time-consuming and

portable, cost-effective, low risk, and noninvasive [13, 14]. Moreover, it enables real-time flow monitoring, continuous measurement, and assessment of cerebral autoregulation in the laboratory or bedside using a dynamic approach [15]. TCD has been used to support the evidence of the prognostication and initiation of preventive strategies in diseases caused by impairment of the cerebrovascular autoregulation, vasoreactivity, and cerebral hyperperfusion such as sickle cell disease [16], subarachnoid hemorrhage [17], stroke [18], dementia [19], vascular depression [20], and Fabry disease [21]. Several previous studies using TCD have found CBF changes after acupuncture stimulation at specific acupuncture points, such as GV20 [22], GB20 [23], ST36 [24], GB34 [25], LI11 [7], and GB39 [8].

GV14 is the 14th acupoint on the Governing Vessel meridian. This meridian lies along the posterior median line and leads yang meridians throughout the body. Traditionally, GV14 has been included in acupuncture points known as the “seven acupoints for stroke,” which are used to treat the symptoms of stroke. Some clinical trials evaluated the effect of GV14 acupuncture on CBF. Litscher et al. [26] and Wang et al. [27] used violet laser acupuncture at GV14 in healthy individuals and found the CBF velocity increased in the BA along with improvements in the microcirculation of blood flow. Kim et al. [28] attempted electroacupuncture stimulation on GV20 and GV14 in ischemic mice and found that it increased blood perfusion to the cerebral cortex. Jeong [29] used pharmacopuncture therapy using Carthami Flos at GV14 in healthy and ischemia rats and found that the CBF was increased in healthy rats, while it enhanced the stability of CBF in the ischemic state. Zhang et al. [30] attempted acupuncture at GV14 in the cerebral ischemia rabbit model and used functional magnetic resonance imaging (fMRI) to examine the sites of brain activation. They found increased blood flow to the site of brain ischemia and peripheral tissues.

However, despite these various clinical uses, there was no study that has focused solely on the role of GV14 acupuncture for the CBF in humans. Therefore, this study aimed to evaluate the effect of acupuncture at GV14 on CO₂ reactivity in the cerebral arteries using TCD. We hypothesized that acupuncture at GV14 could improve CBF in BA and both MCAs.

2. Methods

The present study was designed as a single-center, before-and-after study. This study was approved by the Institutional Review Board at the Hospital of Korean Medicine, Kyung Hee Medical Center (KOMCIRB 2019-11-002-001), and registered with Clinical Research Information Service, a service of the Korea Centers for Disease Control and Prevention (KCT0004787).

2.1. Participants. Participants were recruited via in-hospital advertisement. Sixteen healthy male volunteers aged 20 to 29 years were enrolled in this study. All participants were informed of the procedures and signed a written informed consent form before enrollment. None of the participants had a history of cerebrovascular disease, cardiovascular disease, diabetes mellitus, hypertension, endocrinologic disease, or psychiatric problems. Moreover, the participants did not have any diseases during the study period. They were prohibited from consuming caffeine, alcohol, and drugs for 24 hours before the study. Participant recruitment was conducted between November 2019 and October 2020.

2.2. Sample Size Calculation. The sample size was calculated to enhance the reliability of the results. Sample size calculation was performed using G*power, version 3.1.9.4. No previous study has used GV14 acupuncture in humans. Thus, the sample size was calculated by referring to the

results of a previous study using GB20 [23]. The main outcome was the change in the CO₂ reactivity of the BA before and after GV14 acupuncture. According to the previous study [23], the CO₂ reactivity of the BA was $1.8 \pm 0.75\%$ /minute in the before acupuncture group and $2.5 \pm 0.89\%$ /minute in the after acupuncture group. Taking into consideration a two-sided significance level of 5% (α) and a test power of 80% (β), the required sample size was 14. Moreover, the required sample size was 16, considering a 10% dropout rate.

2.3. Acupuncture Treatment at GV14. All procedures of acupuncture treatment were performed by an experienced Korean medical doctor with more than 3 years of experience. The acupuncture point, GV14, is located on the posterior region of the neck, in the depression inferior to the spinous process of the 7th cervical vertebra (C7), along the posterior median line. The location of the GV14 was determined according to the WHO standard acupuncture point locations [31]. A stainless steel acupuncture needle (diameter 0.25 mm, length 40 mm; DongBang Acupuncture, Seoul, Korea) was used for GV14 acupuncture. The GV14 was stimulated manually with a twisting needle until the participant felt the De-Qi sensation (aching, soreness, pressure, fullness, heaviness, numbness, tingling, warmth, coolness, and dullness sensation) [32, 33]. The needle was inserted into the skin approximately 10 mm deep and the needle was removed after 20 minutes. All procedures were conducted in accordance with the Revised Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA [34]; Table 1) guidelines.

Each participant received acupuncture treatment twice, once at each visit.

2.4. Measurements. The research protocol was based on previous studies that used TCD to identify the relationship between blood flow in the cerebral arteries and acupuncture points [7, 8, 22–25, 35]. For each process, CBF velocity and the CO₂ reactivity of the BA and both MCAs were measured using a Multi-Dop X4 system TCD device (Compumedics DWL, Singen, Germany).

Variables that can affect CBF, i.e., blood pressure, heart rate, and end-tidal carbon dioxide (P_{ETCO_2}), were measured using various modules on the Cardiocap S/5 monitor (Datex-Ohmeda, Helsinki, Finland). The mean blood pressure was determined. The heart rate was continuously recorded via an oximetry device attached to the participant's finger. Further, P_{ETCO_2} was continuously obtained via a Cardiocap S/5 monitor-connected nasal prong placed in the participant's nostril, and each participant was instructed to breathe only through the nose during the procedure. A snapshot function in the Cardiocap S/5 monitor program was used to obtain the mean heart rate and P_{ETCO_2} at specific time points during the procedure. These variables were monitored and recorded on a computer that was connected to the Cardiocap S/5 monitor program.

Each participant visited the study center twice with the interval of 1 week and received acupuncture treatment at each visit.

TABLE 1: Acupuncture rationale and needling details according to STRICTA guideline.

1. Acupuncture rationale	(i) According to the meridian theory of Traditional Korean medicine (ii) Based on historical context, Seven acupoints of stroke
2. Details of needling	(i) 1 needle (ii) Dazhui (GV14) (iii) 10 mm (iv) De-Qi sensation (aching, soreness, pressure, fullness, heaviness, numbness, tingling, warmth, coolness, and dullness sensation) (v) Manual stimulation (vi) 20 minutes (vii) Stainless steel acupuncture (diameter 0.25 mm, length 40 mm, DongBang Acupuncture, Seoul, Korea)
3. Treatment regimen	(i) 2 sessions (once a week) (ii) Duration, 1 week
4. Other components of treatment	None
5. Practitioner background	All acupuncture treatments were performed by one experienced Korean medical doctor (Hyun Ku Lee).

2.4.1. Visit 1. At the first visit, each participant was asked to sit in a comfortable position. The Cardiacap S/5 monitor and probe-holding device were positioned (refer to Figure 1) to ascertain the CBF velocities of BA through the suboccipital window using a 2 MHz pulsed-Doppler probe. The strongest wave pattern was captured at depths ranging from 75 to 110 mm for BA. All measurements were initiated after making the participant rest for 5 minutes. Each participant was asked to sit in a comfortable position. The Cardiacap S/5 monitor and probe-holding device was positioned (refer to Figure 1.) to ascertain the CBF velocities through the suboccipital window using a 2 MHz pulsed-Doppler probe. The blood pressure was checked 3 times every 2 minutes. After starting the first measurement of CBF velocity, the participant was allowed to take rest for 2 minutes and CCR was performed over 1 minute (see Figure 2). The CCR method entailed that participants inhaled their own exhaled air again using a 5-liter reservoir bag. After performing CCR, the participants were given a 1-minute break and the first TCD measurement was stopped. Then, acupuncture was performed at GV14 for 20 minutes. Subsequently, the same procedure was repeated for the postacupuncture measurements (see Figure 3 for the entire process). For TCD measurement, the sample and gain values were corrected and saved if the CBF wave patterns remained constant. The mean blood flow velocity was calculated continuously as the time-averaged maximum velocity over the cardiac cycle, as computed from the envelope of maximum frequencies. The mean blood flow velocities were obtained at rest under stable normocapnic conditions and near the end of the CCR period under hypercapnic conditions. All TCD spectra were recorded for subsequent review.

2.4.2. Visit 2. On the second visit, bilateral probe holder (LAM-Rack; Compumedics DWL) was attached at both temporal windows for each participant (see Figures 4 and 5) and CBF velocities of both MCAs were collected. The strongest wave pattern was captured at depths ranging from 40 to 60 mm for MCAs. For the measurement of all variables, the same processes as used at the first visit were repeated.



FIGURE 1: Suboccipital probe holder.

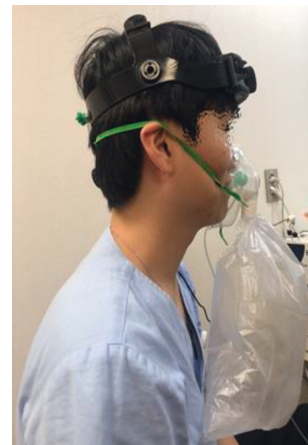


FIGURE 2: Suboccipital probe holder during closed circuit rebreathing.

2.5. Calculations. CO_2 reactivity refers to the percent change in mean blood flow velocity per millimeter of mercury change in P_{ETCO_2} , as calculated by the following formula [35]:

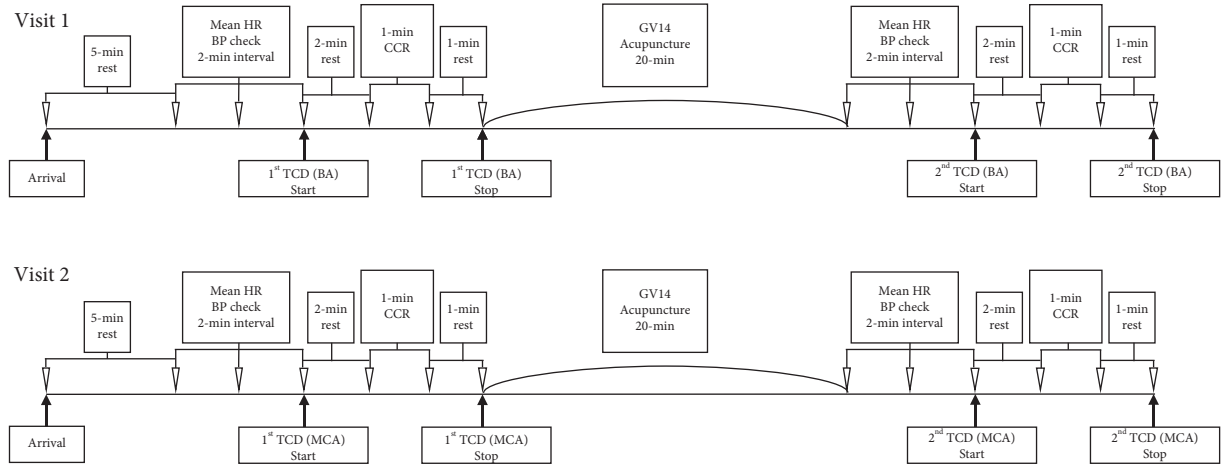


FIGURE 3: Timeline of study procedure. HR, heart rate; BP, blood pressure; TCD, transcranial Doppler; CCR, closed circuit rebreathing.

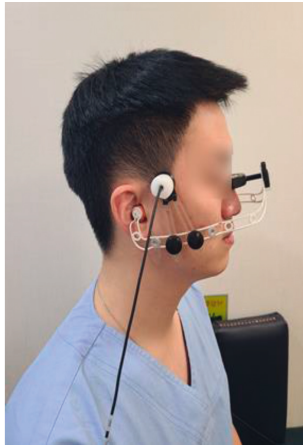


FIGURE 4: Bilateral probe holder.



FIGURE 5: Bilateral probe holder during closed circuit rebreathing.

$$\text{CO}_2 \text{ reactivity} = 100 \times \frac{[(V_{\text{hypercapnia}} - V_{\text{rest}})/V_{\text{rest}}]}{\Delta P_{\text{ETCO}_2}}, \quad (1)$$

where V_{rest} is the blood flow velocity at rest, obtained during the most stable period under stable normocapnic conditions; $V_{\text{hypercapnia}}$ is the blood flow velocity in the latter half of the 1-minute CCR period; and ΔP_{ETCO_2} is the change in P_{ETCO_2} between baseline and maximal CCR.

CBF velocity is dependent on the arterial CO_2 tension, and the corrected blood flow velocity was calculated at 40 mmHg of CO_2 tension (CV40, cm/s) using the following formula [36]:

$$\begin{aligned} & \text{CV40 (corrected velocity at } P_{\text{ETCO}_2} \text{ 40 mmHg)} \\ & = V_1 \cdot e^{b(P_{\text{CO}_2 \text{ 40mmHg}} - P_1 \text{CO}_2)}, \end{aligned} \quad (2)$$

where b represents CO_2 reactivity; V_1 represents velocity at $P_1 \text{CO}_2$; and P_{ETCO_2} represents the end-tidal CO_2 partial pressure.

2.6. Statistical Analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences version

25.0 for Windows (SPSS, Chicago, Illinois, United States). The data were summarized as the median (range). Statistical comparisons between the study parameters before and after GV14 acupuncture treatment were made using the Wilcoxon signed-rank test. p values under 0.05 were considered statistically significant. The data were summarized as the median (range).

3. Result

3.1. Changes of the CO_2 Reactivity of the BA and Both MCAs after GV14 Acupuncture. There was significant increase in the CO_2 reactivity of the BA and both MCAs after GV14 acupuncture treatment compared with baseline (Table 2; Figure 6).

3.2. Changes of the CV40 of the BA and Both MCAs after GV14 Acupuncture. There was a significant increase in the CV40 of the BA ($p = 0.003$) after GV14 acupuncture. However, there were no significant changes in the CV40 between before and after GV14 acupuncture in both the MCAs (Table 3; Figure 7).

TABLE 2: Comparing the CO₂ reactivity before and after GV14 acupuncture.

	GV14 acupuncture		<i>p</i> value*
	Before	After	
BA	3.28 (2.18–4.75)	4.70 (3.23–7.19)	<0.001
Right MCA	3.81 (1.83–8.05)	5.25 (2.81–11.58)	0.001
Left MCA	3.84 (2.38–9.21)	5.12 (2.95–8.29)	0.005

Values are median (range). BA, basilar artery; MCA, middle cerebral artery. **p* values were calculated by Wilcoxon signed-rank test.

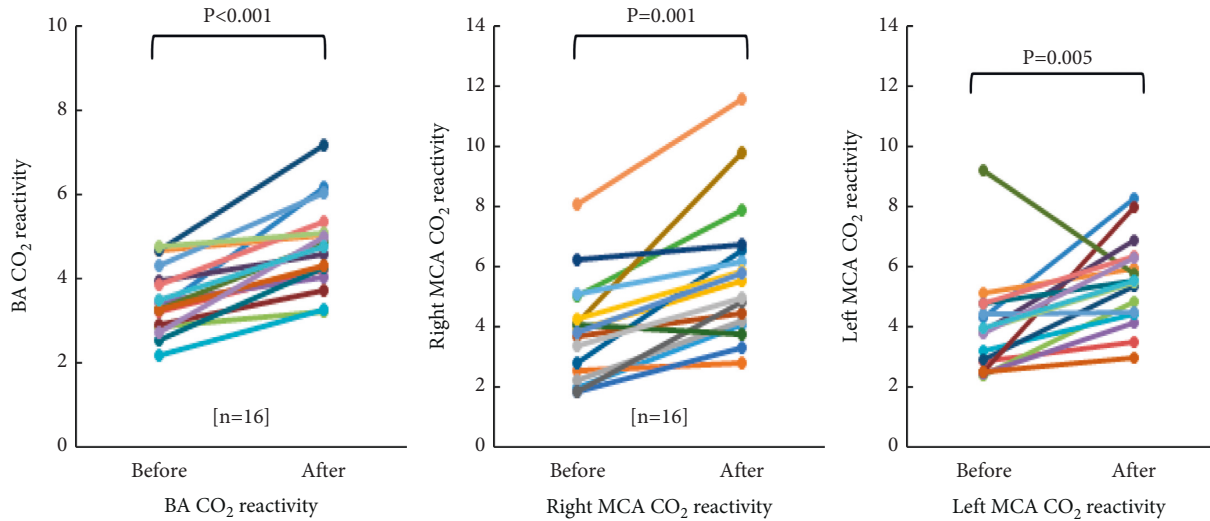


FIGURE 6: Changes in CO₂ reactivity of BA and MCAs after the GV14 acupuncture. CO₂ reactivity is significantly increased in BA and both MCAs after the GV14 acupuncture. BA, basilar artery; MCA, middle cerebral artery.

TABLE 3: Comparing the CV40 before and after GV14 acupuncture.

	GV14 acupuncture		<i>p</i> value*
	Before	After	
BA (cm/s)	45.49 (32.70–69.44)	50.41 (33.97–77.19)	0.003
Right MCA (cm/s)	74.08 (39.85–134.34)	78.07 (34.24–173.08)	0.408
Left MCA (cm/s)	68.15 (40.90–120.70)	69.78 (39.63–145.08)	0.234

Values are median (range). BA, basilar artery; MCA, middle cerebral artery; CV40, modified blood flow velocity at *P*_{ETCO₂} = 40 mmHg. **p* values were calculated by Wilcoxon signed-rank test.

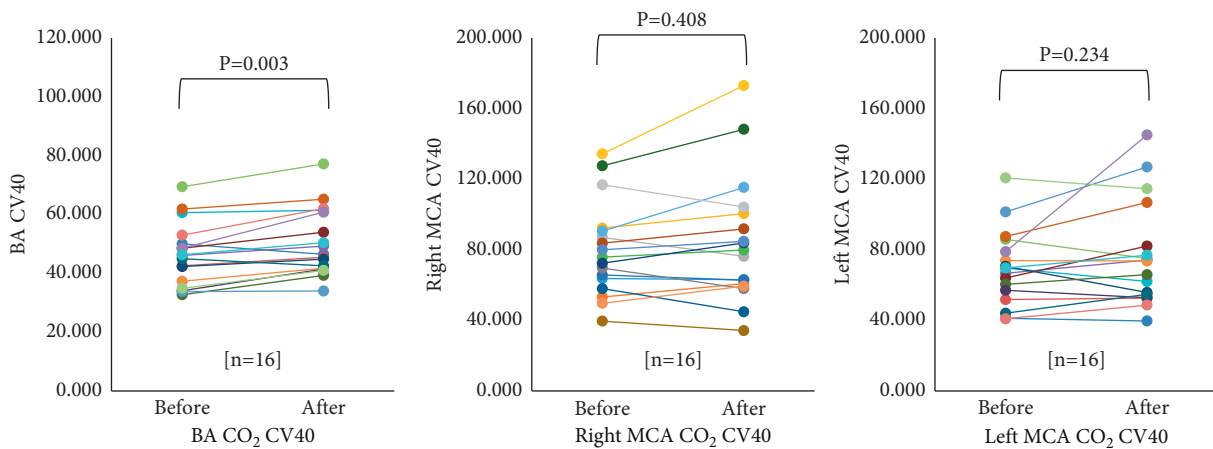


FIGURE 7: Changes in CV40 velocity (cm/s) of BA and MCAs after the GV14 acupuncture. CV40 velocity is significantly increased only in BA after the GV14 acupuncture (*p* = 0.003). BA, basilar artery; MCA, middle cerebral artery.

3.3. *Changes of the Mean Blood Pressure and Heart Rate after GV14 Acupuncture.* There was no significant difference in the mean blood pressure and heart rate before and after GV14 acupuncture (Table 4).

4. Discussion

The purpose of the present study was to determine whether GV14 acupuncture treatment would cause responses to CBF velocity and CO₂ reactivity of the BA and MCA during hypercapnia in normal subjects. The result showed that GV14 acupuncture treatment significantly increased the CO₂ reactivity of the BA from 3.28 to 4.70 ($p < 0.001$), Rt. MCA from 3.81 to 5.25 ($p = 0.001$), and Lt. MCA from 3.84 to 5.12 ($p = 0.005$). In addition, CV40 of the BA also increased significantly after GV14 acupuncture treatment from 45.49 to 50.41 ($p = 0.003$), while those of both MCAs remarkably increased but not reaching statistical significance. These results indicate that GV14 acupuncture is strongly associated with improvement of cerebral blood flow in both posterior and anterior circulations.

Cerebral vasomotor reactivity (CVR) is the compensatory potential of the vessels regulating blood flow to the brain and is represented as the percentage change in response to an arteriolar-dilating stimulus such as carbon dioxide (CO₂) or acetazolamide [37, 38]. In this study, the CCR-induced hypercapnia method was applied to measure CVR, namely, CO₂ reactivity. For CCR, participants inhaled their own exhaled air again to increase the partial pressure of CO₂ in the inhaled air. By repeatedly breathing in a 5-liter reservoir bag, the end-expiratory CO₂ concentration is gradually increased. Subsequently, the CBF velocity gradually increased and was stabilized after approximately 60 seconds [39] rebreathing. CBF change by and adjustment of the vessel diameter can be divided into endothelium-dependent and -nondependent. In previous studies, CBF and CVR were reported to be associated with endothelium dependence [40, 41]. Acupuncture is considered to have a beneficial effect on blood flow by treating endothelial dysfunction [42, 43]. A previous study reported that acupuncture enhanced endothelial function and vascular reactivity by regulating vasoconstrictors and vasodilators [43]. In addition, acupuncture treatments on certain acupuncture points were reported to have effects on the specific brain regions and cerebral arteries. When electroacupuncture was applied to ST36–ST41, GB34–GB39, and LI4–LI11, respectively, there was a distinct difference between the increased and decreased areas of CBF on SPECT and statistical parametric mapping (SPM) [44–46]. In studies using TCD, when different acupoints were stimulated, the velocities of different cerebral blood vessels were changed. It shows specific acupuncture produces specific reproducible quantifiable effects on blood flow velocity in brain arteries [47–49]. The defined acupoints are considered important because the classical theory of traditional oriental medicine claims that targeting an acupoint results in clinical effect. Further, stimulation of different acupuncture points has different effects [50–53].

There have been several previous studies, which measure the effect of acupuncture on cerebral blood flow using TCD. A study of acupuncture at GV20 [22] reported that CVR and CV40 of both MCAs and anterior cerebral arteries (ACAs) were significantly increased. A study of acupuncture at GB20 [23] reported that the CVR of the BA was significantly increased. A study of acupuncture at ST36 [24] reported that CVR of both the MCA and BA was significantly increased. A study of acupuncture at GB34 [25] reported that CVR of the ipsilateral MCA was significantly increased. A study of acupuncture at LI11 [7] reported that CVR of the contralateral MCA was significantly increased. A study of acupuncture at GB39 [8] reported that CVR of both the ACA and contralateral MCA and CV40 of the contralateral ACA was significantly increased. In these studies [7, 8, 22–25], the hyperventilation-induced hypocapnia method was applied to measure CO₂ reactivity. However, when hyperventilation is performed during TCD monitoring, even though it is a suitable method, the probe attached on the holder can be shaken, which might make it difficult to measure the blood flow. In addition, some participants appealed that rapid hyperventilation was not easy to do. Therefore, in this study, the CCR was used to try to improve these problems and the results indicate that it is easier and produce more stable measure values than hyperventilation method.

Previous studies showed that CBF velocity of the BA was increased and microcirculation of blood flow was improved after violet laser acupuncture on GV14 in normal individuals [26, 27]. Electroacupuncture stimulation on GV20 and GV14 increased blood flow perfusion in the cerebral cortex [28]. Pharmacopuncture therapy using Carthami Flos at GV14 could increase CBF in the normal state and improve the stability of CBF in ischemic state [29]. Acupuncture stimulation at GV14 in rabbits with cerebral ischemia could increase blood flow to the brain ischemia site and peripheral tissues on fMRI [30]. However, there have been no studies regarding the effect of GV14 single acupuncture on cerebral blood flow in human participants. In this study, GV14 single acupuncture was conducted in humans. Therefore, the CVR of BA and both MCAs and CV40 of BA significantly increased.

A number of studies showed that impairment of brain perfusion and CBF is one of the mechanisms by which vascular disease may contribute to neurodegeneration such as dementia and vascular depression [20, 21, 54, 55]. Recently, many studies have been conducted to improve these impairments using complementary and alternative treatments [56]. Through this study, it is thought that GV14 acupuncture can be used for various cerebral blood flow disorders other than just stroke.

The limitation of this study is that there was an absence of a control group, which may have caused placebo bias. All participants were male. Females were excluded because the menstrual cycle could affect CBF [57]. Furthermore, calculating the sample size increased the reliability of the study. In addition, it is meaningful to objectively prove the single effect of acupuncture on GV14 in humans.

TABLE 4: Comparing the mean blood pressure and heart rate before and after GV14 acupuncture.

	GV14 acupuncture		<i>p</i> value*
	Before	After	
Mean blood pressure (mmHg)	106.83 (94.33–120.33)	107.08 (91.67–118.67)	0.355
Heart rate (bpm)	77 (55–101)	75 (52–94)	0.431

Values are median (range). bpm, beats per minute. **p* values were calculated by Wilcoxon signed-rank test.

5. Conclusions

This study demonstrated that GV14 acupuncture treatment increased CO₂ reactivity in the BA and both MCAs and increased corrected velocity at P_{ETCO2} 40 mmHg in the BA during hypercapnia in healthy participants.

These results may clinically support the use of GV14 to treat disorders of BA and MCA circulation, such as ischemic stroke and cerebrovascular insufficiency.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

This paper is based on Hyun Ku Lee's theses for Master's degree.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Hyun Ku Lee and Sang-Kwan Moon contributed equally to this work as first authors.

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References

- [1] L. Chen, J. Fang, R. Ma et al., "Additional effects of acupuncture on early comprehensive rehabilitation in patients with mild to moderate acute ischemic stroke: a multicenter randomized controlled trial," *BMC Complementary and Alternative Medicine*, vol. 16, no. 1, p. 226, 2016.
- [2] Y. Cai, C. S. Zhang, S. Liu et al., "Electroacupuncture for poststroke spasticity: a systematic review and meta-analysis," *Archives of Physical Medicine and Rehabilitation*, vol. 98, no. 12, p. 2578.e4, 2017.
- [3] Y.-B. Long and X.-P. Wu, "A meta-analysis of the efficacy of acupuncture in treating dysphagia in patients with a stroke," *Acupuncture in Medicine*, vol. 30, no. 4, pp. 291–297, 2012.
- [4] E. Ernst and A. White, *Acupuncture: A Scientific Appraisal*, Science and Education Publishing, USA, 1999.
- [5] M. S. Dhamoon, R. R. Sciacca, T. Rundek, R. L. Sacco, and M. S. V. Elkind, "Recurrent stroke and cardiac risks after first ischemic stroke: the Northern Manhattan study," *Neurology*, vol. 66, no. 5, pp. 641–646, 2006.
- [6] C. C. Shih, C. C. Liao, M. F. Sun et al., "A retrospective cohort study comparing stroke recurrence rate in ischemic stroke patients with and without acupuncture treatment," *Medicine*, vol. 94, no. 39, Article ID e1572, 2015.
- [7] G. Kim, *Effects of Acupuncture at LI11 on CO₂ Reactivity in the Anterior and Middle Cerebral Arteries during Hypocapnia in Normal Subjects*, Kyung Hee University, Seoul, Republic of Korea, 2019.
- [8] S. Kim, *Effects of Acupuncture at GB39 on CO₂ Reactivity in the Anterior and Middle Cerebral Arteries during Hypocapnia in Normal Subjects*, Kyung Hee University, Seoul, Republic of Korea, 2020.
- [9] J. D. Lee, J. S. Chon, H. K. Jeong et al., "The cerebrovascular response to traditional acupuncture after stroke," *Neuroradiology*, vol. 45, no. 11, pp. 780–784, 2003.
- [10] R. E. Harris, J.-K. Zubieta, D. J. Scott, V. Napadow, R. H. Gracely, and D. J. Clauw, "Traditional Chinese acupuncture and placebo (sham) acupuncture are differentiated by their effects on μ -opioid receptors (MORs)," *NeuroImage*, vol. 47, no. 3, pp. 1077–1085, 2009.
- [11] J. D. Schaechter, B. D. Connell, W. B. Stason et al., "Correlated change in upper limb function and motor cortex activation after verum and sham acupuncture in patients with chronic stroke," *Journal of Alternative & Complementary Medicine*, vol. 13, no. 5, pp. 527–532, 2007.
- [12] A. C. M. Chau, R. T. Fai Cheung, X. Jiang, P. K. M. Au-Yeung, and L. S. W. Li, "An fMRI study showing the effect of acupuncture in chronic stage stroke patients with aphasia," *Journal of Acupuncture and Meridian Studies*, vol. 3, no. 1, pp. 53–57, 2010.
- [13] J. M. Hong, I. S. Joo, K. Huh, and S. S. Sheen, "Simultaneous vasomotor reactivity testing in the middle cerebral and basilar artery with suboccipital probe fixation device," *Journal of Neuroimaging*, vol. 20, no. 1, pp. 83–86, 2010.
- [14] J. Y. Lee, S. Yu, S. I. Lee, and K. H. Jung, "Transcranial doppler ultrasound: practice standards part I," *Test Performance and Interpretation*, vol. 8, pp. 1–13, 2016.
- [15] R. B. Panerai, "Transcranial Doppler for evaluation of cerebral autoregulation," *Clinical Autonomic Research*, vol. 19, no. 4, pp. 197–211, 2009.
- [16] R. J. Adams, V. C. McKie, E. M. Carl et al., "Long-term stroke risk in children with sickle cell disease screened with transcranial Doppler," *Annals of Neurology*, vol. 42, no. 5, pp. 699–704, 1997.
- [17] C. Lysakowski, B. Walder, M. C. Costanza, and M. R. Tramèr, "Transcranial Doppler versus angiography in patients with vasospasm due to a ruptured cerebral aneurysm," *Stroke*, vol. 32, no. 10, pp. 2292–2298, 2001.
- [18] A. M. Demchuk, I. Christou, T. H. Wein et al., "Accuracy and criteria for localizing arterial occlusion with transcranial Doppler," *Journal of Neuroimaging*, vol. 10, no. 1, pp. 1–12, 2000.

- [19] L. Vinciguerra, G. Lanza, V. Puglisi et al., "Transcranial Doppler ultrasound in vascular cognitive impairment-no dementia," *PLoS One*, vol. 14, Article ID e0216162, 2019.
- [20] V. Puglisi, A. Bramanti, G. Lanza et al., "Impaired cerebral haemodynamics in vascular depression: insights from transcranial Doppler ultrasonography," *Frontiers in Psychiatry*, vol. 9, pp. 1–9, 2018.
- [21] C. Vagli, F. Fiscicaro, L. Vinciguerra et al., "Cerebral hemodynamic changes to transcranial Doppler in asymptomatic patients with Fabry's disease," *Brain Sciences*, vol. 10, no. 8, p. 546, 2020.
- [22] H. S. Byeon, S. Moon, P. Kwan et al., "Effects of GV20 acupuncture on cerebral blood flow velocity of middle cerebral artery and anterior cerebral artery territories, and CO₂ reactivity during hypocapnia in normal subjects," *Journal of Alternative & Complementary Medicine*, vol. 17, no. 3, pp. 219–224, 2011.
- [23] J. W. Im, S. K. Moon, W. S. Jung et al., "Effects of acupuncture at GB20 on CO₂ reactivity in the basilar and middle cerebral arteries during hypocapnia in healthy participants," *Journal of Alternative & Complementary Medicine*, vol. 20, no. 10, pp. 764–770, 2014.
- [24] S. H. Hyun, J. W. Im, W. S. Jung et al., "Effect of ST36 acupuncture on hyperventilation-induced CO₂ reactivity of the basilar and middle cerebral arteries and heart rate variability in normal subjects," *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 574986, 2014.
- [25] S. K. Moon, S. Kwon, S. Y. Cho et al., "Effects of GB34 acupuncture on hyperventilation-induced carbon dioxide reactivity and cerebral blood flow velocity in the anterior and middle cerebral arteries of normal subjects," *Acupuncture in Medicine*, vol. 37, no. 5, pp. 277–282, 2019.
- [26] G. Litscher, T. Huang, L. Wang, and W. Zhang, "Violet laser acupuncture-part 1: effects on brain circulation," *Journal of Acupuncture and Meridian Studies*, vol. 3, no. 4, pp. 255–259, 2010.
- [27] L. Wang, T. Huang, W. Zhang, and G. Litscher, "Violet laser acupuncture-part 2: effects on peripheral microcirculation," *Journal of Acupuncture and Meridian Studies*, vol. 4, no. 1, pp. 24–28, 2011.
- [28] J. H. Kim, K. H. Choi, Y. J. Jang et al., "Electroacupuncture acutely improves cerebral blood flow and attenuates moderate ischemic injury via an endothelial mechanism in mice," *PLoS One*, vol. 8, pp. 1–9, 2013.
- [29] Y. P. Jeoung, *Effects of Phamacopuncture Therapy Using Carthami Flos at GV14 on the rCBF in Rats*, Dongshin University, Naju, Republic of Korea, 2009.
- [30] Y.-W. Zhang, "fMRI study on the intervention of brain ischemia with puncturing on Dazhui (GV14) and Baihui (GV20) in rabbits," *Journal of Cerebral Blood Flow and Metabolism*, vol. 38, pp. 948–950, 2018.
- [31] WHO, *WHO Standard Acupuncture Point Locations in the Western Pacific Region*, WHO, Geneva, Switzerland, 2008.
- [32] K. K. S. Hui, O. Marina, J. Liu, B. R. Rosen, and K. K. Kwong, "Acupuncture, the limbic system, and the anticorrelated networks of the brain," *Autonomic Neuroscience*, vol. 157, no. 1–2, pp. 81–90, 2010.
- [33] S. P. Zhu, L. Z. Luo, L. S. Zhang et al., "Acupuncture De-qi: from characterization to underlying mechanism," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 518784, 2013.
- [34] H. MacPherson, D. G. Altman, R. Hammerschlag et al., "Revised standards for reporting Interventions in clinical trials of acupuncture (STRICTA): extending the CONSORT statement," *Acupuncture in Medicine*, vol. 28, no. 2, pp. 83–93, 2010.
- [35] Y. Izumi, Y. Tsuda, S.-I. Ichihara, T. Takahashi, and H. Matsuo, "Effects of Defibrination on hemorheology, cerebral blood flow velocity, and CO₂ reactivity during hypocapnia in normal subjects," *Stroke*, vol. 27, no. 8, pp. 1328–1332, 1996.
- [36] T.-M. Markwalder, P. Grolimund, R. W. Seiler, F. Roth, and R. Aaslid, "Dependency of blood flow velocity in the middle cerebral artery on end-tidal carbon dioxide partial pressure—a transcranial ultrasound Doppler study," *Journal of Cerebral Blood Flow and Metabolism*, vol. 4, no. 3, pp. 368–372, 1984.
- [37] E. B. Ringelstein, C. Sievers, S. Ecker, P. A. Schneider, and S. M. Otis, "Noninvasive assessment of CO₂-induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions," *Stroke*, vol. 19, no. 8, pp. 963–969, 1988.
- [38] Y. Sakashita, M. Kanai, T. Sugimoto, S. Taki, and M. Takamori, "Changes in cerebral blood flow and vaso-reactivity in response to acetazolamide in patients with transient global amnesia," *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 63, no. 5, pp. 605–610, 1997.
- [39] J.-W. Park, "Cerebral CO_2 vasoreactivity by transcranial Doppler with rebreathing method: comparison between ischemic stroke and normal control," *Journal of the Korean Neurological Association*, vol. 22, pp. 440–446, 2004.
- [40] R. P. White, C. Deane, P. Vallance, and H. S. Markus, "Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia," *Stroke*, vol. 29, no. 2, pp. 467–472, 1998.
- [41] R. P. White, C. Deane, C. Hindley et al., "The effect of the nitric oxide donor glyceryl trinitrate on global and regional cerebral blood flow in man," *Journal of the Neurological Sciences*, vol. 178, no. 1, pp. 23–28, 2000.
- [42] M. Tsuchiya, E. F. Sato, M. Inoue, and A. Asada, "Acupuncture enhances generation of nitric oxide and increases local circulation," *Anesthesia & Analgesia*, vol. 104, no. 2, pp. 301–307, 2007.
- [43] S. B. Leung, H. Zhang, C. W. Lau, and Z. X. Lin, "Attenuation of blood pressure in spontaneously hypertensive rats by acupuncture was associated with reduction oxidative stress and improvement from endothelial dysfunction," *Chinese Medicine*, vol. 11, no. 1, p. 38, 2016.
- [44] R. Jong-Man, "Effect of LI4-LI11 electro-acupuncture on regional cerebral blood flow in healthy human - evaluated by 99mTc-ECD brain SPECT," *Journal of Korean Oriental medicine*, vol. 27, pp. 36–43, 2006.
- [45] H. Jin-An, "Effect of GB 34-GB 39 electro-acupuncture on regional cerebral blood flow in stroke patients and normal volunteers evaluated by 99mTc-ECD SPECT," *Journal of Korean Medical Science*, vol. 27, pp. 187–200, 2006.
- [46] S. Moon, "Effect of ST 36-ST 41 electro-acupuncture on regional cerebral blood flow in normal volunteers evaluated by 99mTc-ECD SPECT," *Journal of Korean Oriental Medicine*, vol. 31, no. 1, pp. 130–137, 2010.
- [47] G. Litscher, L. Wang, N.-H. Yang, and G. Schwarz, "Ultrasound-monitored effects of acupuncture on brain and eye," *Neurological Research*, vol. 21, no. 4, pp. 373–377, 1999.
- [48] G. Litscher, "Computer-based quantification of traditional Chinese-, ear- and Korean hand acupuncture: needle-induced changes of regional cerebral blood flow velocity," *Neurological Research*, vol. 24, no. 4, pp. 377–380, 2002.
- [49] G. Litscher, L. Wang, N. H. Yang, and G. Schwarz, "Computer-controlled acupuncture. Quantification and separation

- of specific effects,” *Neurological Research*, vol. 21, no. 6, pp. 530–534, 1999.
- [50] Y. Chae, D. Chang, L. Seon et al., “Inserting needles into the body: a meta-analysis of brain activity associated with acupuncture needle stimulation,” *The Journal of Pain*, vol. 14, no. 3, pp. 215–222, 2013.
- [51] E. M. Choi, F. Jiang, and J. C. Longhurst, “Point specificity in acupuncture,” *Chinese Medicine*, vol. 7, pp. 4–5, 2012.
- [52] S. C. Tjen-A-Looi, P. Li, and J. C. Longhurst, “Medullary substrate and differential cardiovascular responses during stimulation of specific acupoints,” *American Journal of Physiology—Regulatory, Integrative and Comparative Physiology*, vol. 287, no. 4, pp. 852–862, 2004.
- [53] A. Campbell, “Point specificity of acupuncture in the light of recent clinical and imaging studies,” *Acupuncture in Medicine*, vol. 24, no. 3, pp. 118–122, 2006.
- [54] C. Luckhaus, M. O. Flüß, H.-J. Wittsack et al., “Detection of changed regional cerebral blood flow in mild cognitive impairment and early Alzheimer’s dementia by perfusion-weighted magnetic resonance imaging,” *NeuroImage*, vol. 40, no. 2, pp. 495–503, 2008.
- [55] G. C. Román, “Brain hypoperfusion: a critical factor in vascular dementia,” *Neurological Research*, vol. 26, no. 5, pp. 454–458, 2004.
- [56] G. Lanza, S. S. Centonze, G. Destro et al., “Shiatsu as an adjuvant therapy for depression in patients with Alzheimer’s disease: a pilot study,” *Complementary Therapies in Medicine*, vol. 38, pp. 74–78, 2018.
- [57] K. J. Brackley, M. M. Ramsay, F. Broughton Pipkin, and P. C. Rubin, “The effect of the menstrual cycle on human cerebral blood flow: studies using Doppler ultrasound,” *Ultrasound in Obstetrics and Gynecology*, vol. 14, no. 1, pp. 52–57, 1999.