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Effects of Acupuncture with a Combination of GV20 and EX-HN1 Acupoints on CO_2 Reactivity in the Anterior and Middle Cerebral Arteries during Hypercapnia in Normal Subjects: A Randomized Crossover Trial

Hojung Park[1*], Sang-Kwan Moon[1,2*], Han-Gyul Lee[1,2], Seungwon Kwon[1,2#], Seung-Yeon Cho[1,2]
Seong-Uk Park[1,2], Woo-sang Jung[1,2], Jung-Mi Park[1,2], Chang-Nam Ko[1,2], Ki-Ho Cho[1,2]

[1]Dept. of Clinical Korean Medicine, Graduate School, Kyung Hee University
[2]Dept. of Cardiology and Neurology, College of Korean Medicine, Kyung Hee University

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[2]Dept. of Cardiology and Neurology, College of Korean Medicine, Kyung Hee University

ABSTRACT

Objectives: To investigate the effects of acupuncture at GV20 and EX-HN1 on cerebral blood flow (CBF) velocity and cerebrovascular reactivity (CVR) in the middle cerebral arteries (MCA) and anterior cerebral arteries (ACA) and to compare the effects to acupuncture at GV20.

Methods: The study was a randomized, crossover trial that included 10 healthy men aged 20 to 29 years who underwent acupuncture treatment four times with a washout period of one week. The CBF velocity and CVR were measured by transcranial Doppler sonography (TCD) on both MCAs at the first and second visits, and both ACAs at the third and fourth visits. Participants were randomly assigned to one of two groups (A and B) before the first and third visits. Group A received two phases of acupuncture intervention at a single GV20 point and a combination of GV20 and EX-HN1 acupoints. Group B received the same interventions, but in the reverse order.

Results: The increase in CO_2 reactivity was significantly higher for the combination acupoints of GV20 and EX-HN1 than for the GV20 single acupoint in both MCAs (Right: 136 to 178, $p=0.017$; Left: 127 to 191, $p=0.017$) and ACAs (Right: 133 to 158, $p=0.013$; Left: 122 to 168, $p=0.025$). No significant change was noted in the corrected velocity at $PETCO_2$ 40 mmHg, blood pressure, or heart rate.

Conclusions: The findings suggest that improvement of the CBF in the MCA and ACA after GV20 acupuncture increases when acupuncture is also performed at EX-HN1. These results clinically support the combined use of EX-HN1 and GV20 to treat disorders of MCA and ACA circulation.

Key words: GV20, EX-HN1, anterior cerebral artery, middle cerebral artery, cerebrovascular reactivity

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· 교신저자: 권승원 서울시 동대문구 경희대로 23
경희대학교 한방병원 순환신경내과학교실
TEL: 02-958-9275 FAX: 02-958-9132
E-mail: kkokkottung@hanmail.net
· * These authors are contributed equally as first authors.
· # Corresponding author
· 이 논문은 2022년도 경희대학교 대학원 한의학 석사학위 논문임.

Ⅰ. Introduction

Baihui (GV20) is the 20th acupoint of the Du meridian (the government vessel) located on the

highest place of the head where all the yang meridians meet[1]. And GV20 is a principle acupoint of "*Seven Acupoints for Stroke*" which are used to treat the symptoms of stroke[1]. GV20 is used to treat neurological and psychiatric diseases such as headache, dizziness, anxiety and stroke[2]. In previous study using transcranial doppler (TCD), both Cerebrovascular Reactivity (CVR) and Corrected Velocity at $PETCO_2$ 40 mmHg (CV40) were significantly increased in both Middle Cerebral Arteries (MCA) and Anterior Cerebral Arteries (ACA) after GV20 acupuncture treatment[3]. It showed that GV20 acupuncture has the effect on improving CBF by reducing peripheral vascular resistance, increasing CBF velocity and CVR.

A previous study[4] that evaluate the effect of GV20 acupuncture on CBF of MCA in normal subjects using TCD showed the results of a signigicant increase in mean velocity, systolic velocity, diastolic velocity, and decrease in pulsatility index, which suggested the GV20 acupuncture have a specific effect on CBF. According to a systemic review and meta-analysis study[2], in an animal model of focal cerebral ischemia, GV20 based scalp acupuncture improved infarct volume and neurological function score, and it suggested potential neuroprotective role of GV20 acupuncture. Previous studies[2-4] showed that GV20 acupuncture has the effect on improving CBF by reducing peripheral vascular resistance, increasing CBF velocity and CVR.

Sishencong (EX-HN1) is composed of four acupoints located 1 B-cun(proportional methods) anterior, posterior and bilateral to GV20, which are often used together with GV20 for stroke, dementia, headache. Previous studies[5-9] showed that acupuncture at GV20 and EX-HN1 improves the cerebral blood flow, brain activity and alleviate the symptoms of cerebrovascular disease. Electroacupuncture treatment including GV20 and EX-HN1 in cervical vertigo patients improved blood supply of vertebro-basliar artery and its clinical effect is better than the effect of western medicine treatment[5]. Moreover, Acupuncture treatment including GV20 and EX-HN1 improved balance fuction in stroke patients[6] and have a significant effect on the improvement of cognitive function in vascular dementia[7,8]. It has been confirmed through functional infrared spectroscopy (fNIRS) imaging that the application of intradermal acupuncture at GV20 and EX-HN1 with Neurodevelopmental treatment to cerebral palsy children improves brain activity compared to applying Neurodevelopmental treatment alone[9].

In addition, acupuncture at certain acupoints has been reported to affect the specific brain regions and cerebral arteries[10]. Moreover, when comparing the functional brain areas stimulated by a single acupoint and acupoint combinations using fMRI, acupuncture treatment with acupoint combinations act on wider brain areas than a single acupoint. These studies[11,12] support a synergistic effect of acupoint combinations.

Therefore, this study aimed to compare the changes on CVR between GV20 and combination of GV20 and EX-HN1 in the cerebral arteries using TCD. Furthermore, this study is expected to provide a clinical evidence for the use of GV20 and EX-HN1 in clinical practice to improve cerebral blood flow.

Ⅱ. Materials and Methods

1. Study design

The study was designed as a randomized crossover trial. Participants visited the study center four

times with the interval of one-week to receive acupuncture treatment. TCD was performed on both MCAs at the first and second visits, and both ACAs at the third and fourth visits. Before the first and third visits, participants were randomly allocated to two groups (Group A and Group B, n=5 per group).

Group A sequentially received two phases of intervention of acupuncture at GV20 single point (the control group) and combination of GV20 and EX-HN1 acupoints(the experimental group), whereas Group B received interventions in the reverse order. The phases were separated by a one-week washout interval[13-15] to eliminate the residual effect by the former phase (Fig. 1). For outcome assessor blinding, the randomizer, acupuncturist, and TCD performer-statistician (assessor) were separated.

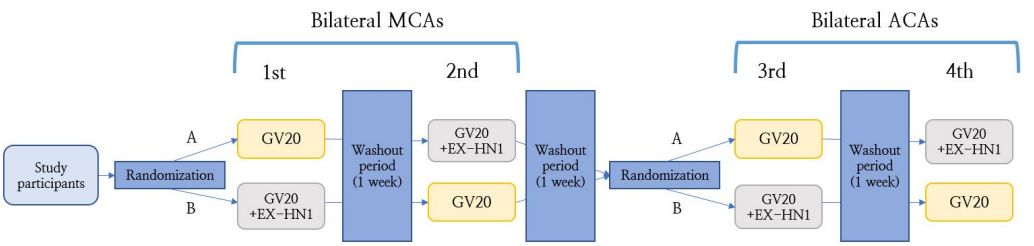

Fig. 1. Flowchart of study procedure.

As a randomization method, this study used block randomization to equalize the number of subjects between groups. Group A or B, 5 of each, were listed. Paired with the list, the random numbers were generated using Excel program and then listed in descending order. The randomizer determined the group allocations in this order according to the subjects' screening sequence numbers which is given in order of registration.

These randomization results were transferred from the randomizer to the acupuncturist as an allocation concealment using the envelope method. When the randomizer finished the randomization, the group allocation results put into opaqe sealed envelopes and was delivered to the acupuncturist. The acupuncturist opened the envelopes according to the subjects' screening sequence numbers and performed acupuncture according to the group allocation. And the allocation result was not informed to the TCD performer. Through this process, the TCD performer-statistician (assessor) was blinded to the group allocation until the analysis was finished.

The study was approved by the Institutional Review Board at the Hospital of Korean Medicine, KyungHee Medical Center (KOMCIRB 2021-03-001-001) and registered with Clinical Research Information Service, a service of the Korea Centers for Disease Control and Prevention (KCT0006324).

2. Participants

Ten healthy male volunteers aged 20 to 29 years were enrolled in this study. As age increases, the CBF velocity and CVR significantly decrease[16]. In addition, as estrogen decreases vascular resistance, the CBF velocity of women is significantly higher than that of men. And the CBF is affected by changes in estrogen secretion according to a woman's

menstrual cycle[17].

Because age and gender act as physiological determinants of cerebral blood flow rate, the participants were limited to males in 20s.

Participant were recruited from June 2021 to October 2021.

Participants were included only when they met the following inclusion criteria:

- Inclusion Criteria

1) Males aged 20 to 29 years

2) Physically and mentally healthy confirmed by a physician

3) Good protocol compliance and agreeing to sign an informed consent document

Participants would be excluded if they had one of the following conditions:

- Exclusion Criteria

1) History of cerebrovascular disease, cardiovascular disease, diabetes mellitus, hypertension, endocrinologic disease, or psychiatric problems.

2) Any diseases during the study period.

3) Consuming caffeine, alcohol and drugs for 24 hours before the study.

All participants were informed of the procedures and signed a written informed consent form prior to enrollment.

3. 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. The sample size was calculated by referring to the results of a previous study using GV20[3]. The main outcome was the change in the CO_2 reactivity of the Rt.MCA before and after GV20 acupuncture. According to the previous study[3], the CO_2 reactivity of the Rt.MCA was 1.98±0.41%/minute in the before acupuncture group and 3.08±0.74%/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 6. Based on the result, in order to consider the dropout rate of 10% and to verify the results of the previous study, the sample size was set to 10 as in the previous study.

4. Acupuncture Treatment

All procedures of acupuncture treatment were performed by an experienced Korean Medical Doctor with more than 3 years of clinical experience. The control group received acupuncture treatment at GV20, and the experimental group received acupuncture treatment at GV20 and EX-HN1. The acupuncture point, Baihui (GV20), is located on the head, 5 B-cun superior to the anterior median line. When the ears are folded, GV20 is located at the midpoint of the connecting line between the auricular apices. Sishencong (EX-HN1) are four points located 1 B-cun anterior, posterior and bilateral to GV20. The locations of the GV20 and EX-HN1 were determined according to the WHO standard Acupuncture Point Locations[18]. A stainless steel acupuncture needles (diameter 0.25 mm, length 40 mm; DongBang Acupuncture, Seoul, Korea) were used for GV20, EX-HN1 acupuncture. The acupoints were stimulated manually until the participant felt the De-Qi sensation (aching, dullness, heaviness, numbness, radiating, tingling and spreading sensations)[15]. The needles were inserted into the skin approximately 5mm deep and removed after 20 minutes. All procedures were conducted in accordance with the Revised Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA[19], Table 1) guidelines.

Table 1. Acupuncture Rationale and Needling Details according to STRICTA Guideline

1. Acupuncture rationale	1a) According to the meridian theory of Traditional East Asian medicine 1b) Based on historical context - *Seven acupoints of stroke*	
2. Details of needling	Control group	2a) 1 needle 2b) Baihui (GV20) 2c) 5mm 2d) De-qi sensation 2e) Manual stimulation 2f) 20 minutes 2g) Stainless steel acupuncture (diameter 0.25 mm, length 40 mm, DongBang Acupuncture, Seoul, Korea)
	Experimental group	2a) 5 needles 2b) Baihui (GV20), Sishencong (EX-HN1) 2c) 5 mm 2d) De-qi sensation 2e) Manual stimulation 2f) 20 minutes 2g) Stainless steel acupuncture (diameter 0.25 mm, length 40 mm, DongBang Acupuncture, Seoul, Korea)
3. Treatment regimen	3a) 1 session 3b) Once a week	
4. Other components of treatment	4a) None	
5. Practitioner background	5a) All acupuncture treatments were performed by one experienced Korean Medical Doctor.	

5. 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[3,20-22]. For each process, CBF velocity and the CO_2 reactivity of both MCAs and both ACAs were measured using the - a Multi-Dop X4 system TCD device (Compumedics DWL, Singen, Germany). All procedures of TCD measurement were performed by a trained TCD perfomer with more than 2 years of experience.

Variables that can affect CBF, i.e., blood pressure, heart rate, and end-tidal carbon dioxide ($PETCO_2$), were measured using various modules on the Cardiocap S/5 monitor (Datex-Ohmeda, Helsinki, Finland). The mean blood pressure was determined and the heart rate was continuously recorded via an oximetry device attached to the participant's finger to exclude the possibility that CBF changes were caused by BP, HR changes. Further, $PETCO_2$ 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 $PETCO_2$ 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.

When the visit, each participant was asked to sit in a comfortable position. The Cardiocap S/5 monitor and bilateral probe holder (LAM-Rack: Compumedics DWL) was positioned (Fig. 2.) to measure the CBF velocities of MCAs or ACAs through the temporal windows using a 2 MHz pulsed-Doppler probe. The strongest wave pattern was captured at depths ranging from 40 to 60 mm for MCAs, and from 65 to 80 mm for ACAs. All measurements were initiated after making the participant rest for 5 minutes. The blood pressure was checked 3 times every 2 minutes. After starting the first measurement of CBF velocity, the participant had rest for 2 minutes and performed CCR (Closed Circuit Rebreathing) for 1 minute. The CCR was the method to breath that participants inhaled their own exhaled air again using a 5 liter reservoir bag. After performing CCR, the participant was given a 1 minute break and the first TCD measurement was completed. Then, acupuncture was performed at GV20/GV20+EX-HN1 for 20 minutes. Subsequently, the same procedure was repeated for the post-acupuncture measurements (See Fig. 2. for the entire process).

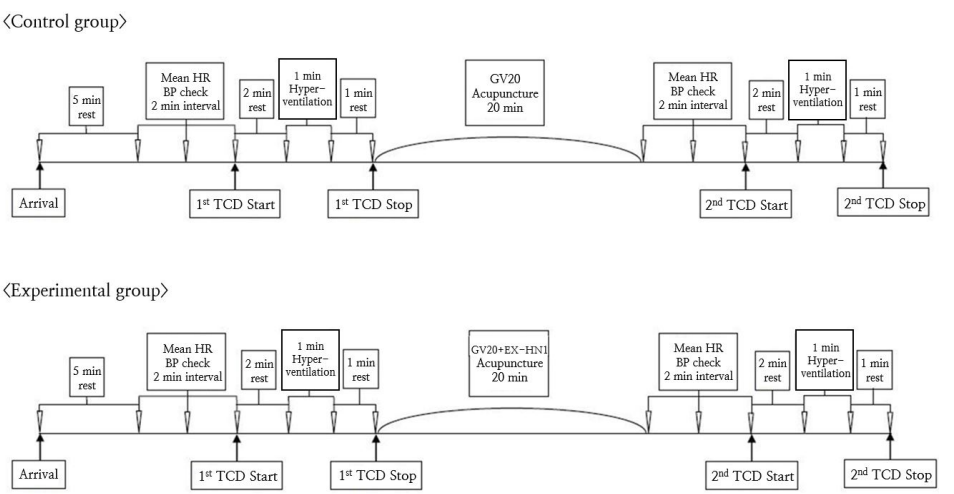

Fig. 2. Timeline of study procedure of the control, experimental group.

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 spectras were recorded for subsequent review.

6. Calculations

CO_2 reactivity refers to the percent change in mean blood flow velocity per millimeter of mercury change in $PETCO_2$, as calculated by the following formula[23]:

CO_2 reactivity $= 100 \times \frac{[V_{hypercapnia} - V_{rest}]}{V_{rest}} / \triangle P_{ETCO_2}$

Where V_{rest} is the blood flow velocity at rest, obtained during the most stable period under stable normocapnic conditions; $V_{hypercapnia}$ is the blood flow velocity in the latter half of the 1 minute CCR period; and $\Delta PETCO_2$ is the change in $PETCO_2$ between baseline and maximal CCR.

CBF velocity is dependent on the arterial CO_2 tension, and corrected the blood flow velocity was calculated at 40 mmHg of CO_2 tension (CV40, cm/s) using the following formula[24]:

CV40 (Corrected Velocity at $PETCO_2$ 40 mmHg) $= V_1 \cdot e^{b(PCO2\ \ 40mmHg-P1CO2)}$

Where b represents CO_2 reactivity; V1 represents velocity at P1CO2; and $PETCO_2$ represents the end-tidal CO_2 partial pressure.

The rate of change of CO_2 reactivity was calculated as the percentage(%) of CO_2 reactivity after acupuncture compared to CO_2 reactivity before acupuncture.

Rate of change of CO_2 reactivity $= 100 \times CO_2$ $reactivity_{after}/CO_2$ $reactivity_{before}$

CO_2 $reactivity_{after}$ is the CO_2 reactivity after acupuncture and CO_2 $reactivity_{before}$ is the CO_2 reactivity before acupuncture

The rate of change of CV40 was calculated as the percentage (%) of CV40 after acupuncture compared to CV40 before acupuncture.

Rate of change of $CV40 = 100 \times CV40_{after}/CV40_{before}$

$CV40_{after}$ is the CV40 after acupuncture and $CV40_{before}$ is the CV40 before acupuncture

7. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 25.0 for Windows (SPSS, Chicago, Illinois, United States). As a result of a normality test using Shapiro-Wilk, the normality was not satisfied in some data. Wilcoxon signed-rank test were used to made statistical comparisons of the study parameteres between before and after GV20, EX-HN1 acupuncture and between GV20 single point acupuncture and combination acupuncture of GV20, EX-HN1. P-values under 0.05 were considered statistically significant. Data was summarized as the mean±standard deviation.

Ⅲ. Results

1. Changes after GV20 Acupuncture

1) Changes of the CO_2 reactivity of both MCAs and ACAs after GV20 Acupuncture

There was significant increase in the CO_2 reactivity of both MCA and ACA after GV20 acupuncture treatment compared with baseline (Table 2).

2) Changes of the CV40 of both MCAs and ACAs after GV20 Acupuncture

There were no significant changes in the CV40 between before and after GV20 acupuncture in both MCAs and ACAs (Table 3).

3) Changes of the mean blood pressure and heart rate after GV20 Acupuncture

There was no significant difference in the mean blood pressure and heart rate before and after GV20 acupuncture (Table 4).

2. Changes after Acupuncture at GV20 and EX-HN1

1) Changes of the CO_2 reactivity of both MCAs and ACAs after Acupuncture at GV20 and EX-HN1

There was significant increase in the CO_2 reactivity of both MCAs and ACAs after acupuncture treatment at GV20 and EX-HN1 compared with baseline (Table 2).

2) Changes of the CV40 of both MCAs and ACAs after Acupuncture at GV20 and EX-HN1

There were no significant changes in the CV40 between before and after acupuncture treatment at GV20 and EX-HN1 in both MCAs and ACAs (Table 3).

3) Changes of the mean blood pressure and heart rate after Acupuncture at GV20 and EX-HN1

There was no significant difference in the mean blood pressure and heart rate before and after acupuncture treatment at GV20 and EX-HN1 (Table 4).

3. Comparison of the parameters before acupuncture between the Control group (GV20 single point) and the Experimental group (combination of GV20 and EX-HN1 acupoints)

1) Comparison of the CO_2 reactivity before acupuncture between the control group (GV20 single point) and the experimental group (combination of GV20 and EX-HN1 acupoints)

There was no significant difference in the CO_2 reactivity before acupuncture treatment between control group (GV20 single point) and experimental group (combination of GV20 and EX-HN1 acupoints) in both MCAs and ACAs. (Rt. MCA: 3.85 to 4.02, $p=0.721$; Lt.MCA: 3.57 to 3.52, $p=0.799$; Rt. ACA: 3.78 to 3.43, $p=0.074$; Lt.ACA: 4.23 to 3.71, $p=0.074$)

2) Comparison of the CV40 before acupuncture between control group (GV20 single point) and experimental group (combination of GV20 and EX-HN1 acupoints)

There was no significant difference in the CV40 before acupuncture treatment between control group (GV20 single point) and experimental group (combination of GV20 and EX-HN1 acupoints) in both MCAs and ACAs. (Rt. MCA: 65.89 to 66.45, $p=0.959$; Lt.MCA: 68.26 to 65.84, $p=0.508$; Rt. ACA: 63.00 to 62.30, $p=0.799$; Lt.ACA: 69.76 to 65.79, $p=0.285$)

3) Comparison of the mean blood pressure and heart rate before acupuncture between control group (GV20 single point) and experimental group (combination of GV20 and EX-HN1 acupoints)

There was no significant difference in the mean blood pressure and heart rate before acupuncture treatment between control group (GV20 single point) and experimental group (combination of GV20 and EX-HN1 acupoints). (Mean blood pressure: 103.98 to 104.22, $p=0.968$; Heart rate: 73.32 to 72.38, $p=0.614$)

4. Comparison between the Control group (GV20 single point) and the Experimental group (combination of GV20 and EX-HN1 acupoints)

1) Comparison of the rate of Change of the CO_2 reactivity between the control group (GV20 single point) and the experimental group (combination of GV20 and EX-HN1)

In both MCAs and ACAs, the increase rate of CO_2 reactivity before and after acupuncture at combination of GV20 and EX-HN1 was significantly higher than the increase rate of CO_2 reactivity before and after acupuncture at GV20 single point (Table 2).

2) Comparison of the rate of Change of the CV40 between the control group (GV20 single point) and the experimental group (combination of GV20 and EX-HN1)

There were no significant changes in the rate of change of CV40 between acupuncture at GV20 single point and combination of GV20 and EX-HN1 in both MCAs and ACAs (Table 3).

Table 2. Comparing the CO_2 Reactivity (%/minute) before and after GV20 Acupuncture or Acupuncture at GV20 and EX-HN1

	Acupuncture	Before	After	p-value*	The rate of CO_2 reactivity	p-value**
Right MCA	GV20	3.85±1.62	4.92±1.16	0.013	136.08±33.89	0.017
	GV20+EX-HN1	4.02±1.37	6.80±1.79	0.005	178.39±53.45	
Left MCA	GV20	3.57±1.10	4.27±0.65	0.037	127.45±32.78	0.017
	GV20+EX-HN1	3.52±0.97	6.46±2.14	0.005	191.38±67.68	
Right ACA	GV20	3.78±1.77	4.66±1.39	0.022	133.95±37.43	0.013
	GV20+EX-HN1	3.43±1.55	4.92±1.40	0.009	158.42±56.57	
Left ACA	GV20	4.23±1.91	5.08±2.20	0.037	122.80±24.93	0.028
	GV20+EX-HN1	3.71±1.77	6.21±3.56	0.005	168.00±35.74	

Values are mean±standard deviation.
MCA : middle cerebral artery, ACA : anterior cerebral artery
*p-value for comparing the CO_2 reactivity (%/minute) before and after acupuncture
**p-value for comparing the rate of CO_2 reactivity between the control group (GV20) and the experimental group (GV20+EX-HN1)
P-values are calculated by Wilcoxon signed-rank test.

Table 3. Comparing the CV40 (cm/s) before and after GV20 Acupuncture or Acupuncture at GV20 and EX-HN1

	Acupuncture	Before	After	p-value*	The rate of CV40	p-value**
Right MCA	GV20	65.89±17.95	66.07±14.59	0.959	102.40±11.24	0.878
	GV20+EX-HN1	66.45±21.24	66.48±19.47	0.878	101.20±7.63	
Left MCA	GV20	68.26±14.67	71.29±13.78	0.386	105.61±11.29	0.799
	GV20+EX-HN1	65.84±12.57	68.25±14.04	0.241	104.24±13.25	
Right ACA	GV20	63.00±11.93	65.66±15.24	0.646	104.63±15.92	0.508
	GV20+EX-HN1	62.30±9.82	64.06±12.77	0.285	102.34±5.42	
Left ACA	GV20	69.76±8.75	69.06±9.28	0.646	99.10±6.36	0.959
	GV20+EX-HN1	65.79±6.37	66.17±6.88	0.799	101.02±10.85	

Values are mean±standard deviation.
MCA : middle cerebral artery, ACA : anterior cerebral artery
*p-value for comparing the CV40 (cm/s) before and after acupuncture
**p-value for comparing the rate of CV40 between the control group (GV20) and the experimental group (GV20+EX-HN1)
P-values are calculated by Wilcoxon signed-rank test.

Table 4. Comparing the Mean Blood Pressure and Heart Rate before and after GV20 Acupuncture or Acupuncture at GV20 and EX-HN1

Acupuncture		Before	After	p-value*
GV20	Mean blood pressure (mmHg)	103.98±8.35	105.18±7.71	0.067
	Heart rate (bpm)	73.32±7.21	71.92±6.76	0.115
GV20+EX-HN1	Mean blood pressure (mmHg)	104.22±8.09	104.65±9.12	0.503
	Heart rate (bpm)	72.38±8.66	70.53±6.58	0.062

Values are mean±standard deviation.
bpm : beats per minute
*P-values are calculated by Wilcoxon signed-rank test.

Ⅳ. Discussion

Cerebrovascular reactivity (CVR) is the change in cerebral blood flow in response to a vasodilatory or vasoconstrictive stimulus[25], and it represents the compensatory potential of the vessels regulating blood flow to the brain. Several studies have reported decreased cerebrovascular reactivity in patients with cerebral ischemia[26]. Furthermore, the CVR of the ischemic side was more impaired than that of the unaffected side, which means that the arterioles are already chronically dilated and the range of automatic regulatory function is reduced[27].

In this study, the CO_2 reactivities of both MCA and ACA were significantly increased in both acupuncture treatment at GV20 single point and combination of GV20 and EX-HN1. And the increase rate of the CO_2 reactivity was significantly higher in both MCA and ACA when acupuncture treatment was performed at GV20 and EX-HN1 compared to GV20 alone. However the CV40 of both MCA and ACA has no significant change in both acupuncture treatment at GV20 single point and combination of GV20 and EX-HN1, and the change rate of CV40 also has no significant difference between the control group and the experimental group.

As the results of this study verified the increase rate of CVR of GV20 and EX-HN1 acupuncture was significantly higher than GV20 acupuncture, the study suggests that the effect of GV20 acupuncture increases when acupuncture along with EX-HN1. And it suggests the potential of a synergistic effect of EX-HN1 on the effect on GV20 acupuncture on CBF.

When compared with the results of the previous study[3], the CVR of both MCA and ACA increased significantly after GV20 acupuncture as in the previous study. However, while the CV40 of both MCA and ACA also increased significantly after GV20 acupuncture in the previous study, there was no significant change of CV40 in this study.

The difference between the progress of the previous study and this study is that the cerebral blood flow rate was increased through closed circuit respiration in this study, while the previous study applied hyperventilation-induced hypocapnia to measure the CVR. It is thought that since the degree of change in the partial pressure of carbon dioxide was different according to the change in the breathing method, the degree of change in the CBF velocity also had difference between the studies. Also, when hyperventilation is performed during TCD monitoring, 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. 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. Therefore, the results of this study that applied the CCR are considered to be more reliable. Furthermore, the sample size calculation was performed based on the results on the CO_2 reactivity in previous GV20 study[3], not base on the results of CV40. Therefore the calculated sample size may not be sufficient to confirm the effect of GV20 acupuncture on CV40.

Acupuncture is thought to improve blood flow by treating endothelial dysfunction[28,29]. A previous study reported that acupuncture enhanced endothelial function and vascular reactivity by regulating vasoconstrictors and vasodilators[29]. One of the most significant vasodilating substances secreted by the

endothelium is endothelium-derived nitric oxide (EDNO). Reduced NO in impaired endothelial functional status has been reported, which may be due to decreased activity of endothelial NO synthetase (eNOS) and decreased bioavailability of NO[29]. Several studies have shown that acupuncture increased the production of NO in arterioles, and activated the expression of eNOS[28]. These results suggest that the effect of acupuncture on endothelial dysfunction might result from inducing synthesis and activation of EDNO[30]. Also, CBF change by an adjustment of the vessel diameter can be divided into endothelium-dependent and non-dependent. In previous studies, CBF and CVR were reported to be associated with endothelium dependent[31,32]. Therefore, it can be estimated that acupuncture improves cerebral blood flow and further increases CVR by improving the endothelial function of cerebral blood vessels.

The limitation of this study is that there was an absence of subject blinding, which may have caused placebo bias. And the study is limited to males in 20s, so extended studies of different ages and genders can support the conclusion of this experiment. Also, since the study is limited to healty people with normal cerebral blood flow state, additional studies on patients with hemodynamic disorder such as arteriosclerosis or cerebral ischemia may be necessary.

This study confirmed the difference in cerebral blood flow before and after acupuncture between GV20 alone acupuncture treatment and combination acupuncture treatment of GV20 and EX-HN1, which has been widely used for stroke through an experimental method. Therefore, in clinical practice, this study may be the evidence to apply acupucture at combination of GV20 and EX-HN1 to improve cerebral blood flow. Furthermore, because the CCR method was applied, the results of this study are thought to be more objective and reliable than previous studies.

V. Conclusions

This study demonstrated that GV20 and EX-HN1 acupuncture treatment increased CO_2 reactivity in both MCA and both ACA and the increase rate of the CO_2 reactivity was significantly higher in GV20 and EX-HN1 than in GV20 acupuncture alone on both MCA and ACA.

These results may clinically support the use of EX-HN1 with GV20 to treat disorders of MCA and ACA circulation, such as ischemic stroke and cerebrovascular insufficiency.

References

1. Shen EY, Chen FJ, Chen YY, Lin MF. Locating the acupoint Baihui (GV20) beneath the cerebral cortex with MRI reconstructed 3D neuroimages. *Evidence-Based Complementary and Alternative Medicine* 2011:2011:362494.
2. Wang WW, Xie CL, Lu L, Zheng GQ. A systematic review and meta-analysis of Baihui (GV20)-based scalp acupuncture in experimental ischemic stroke. *Scientific reports* 2014:4:3981.
3. Byeon HS, Moon SK, Park SU, Jung WS, Park JM, Ko CN, et al. Effects of GV20 acupuncture on cerebral blood flow velocity of middle cerebral artery and anterior cerebral artery territories, and CO_2 reactivity during hypocapnia in normal subjects. *J Altern Complement Med* 2011:17(3):219-24.
4. Lee HM, Cheong BS, Yun HS, Cho SG, Lee SH, Jun HJ, et al. Effect of Paekoe (GV20)

acupuncture on cerebral blood flow. *Journal of Acupuncture Research* 2001;18(6):105-13.
5. Guocan LI. Clinical Observation of Electroacupuncture for Cervical Vertigo. *Journal of New Chinese Medicine* 2018:03.
6. Liu SY, Hsieh CL, Wei TS, Liu PT, Chang YJ, Li TC. Acupuncture stimulation improves balance function in stroke patients: a single-blinded controlled, randomized study. *The American journal of Chinese medicine* 2009;37(3):483-94.
7. Kim BM, Jo HG, Kang HW, Choi SY, Song MY, Su JU, et al. Proposal of evidence-based east-west integrative medicine manual for vascular dementia. *The Journal of Korean Medicine* 2019;40(1):46-62.
8. Liu Q, Wang XJ, Zhang ZC, Xue R, Li P, Li B. Neuroprotection against vascular dementia after acupuncture combined with donepezil hydrochloride: P300 event related potential. *Neural regeneration research* 2016;11(3):460-4.
9. Chang SJ, Nam YG, Kim JH, Ko MJ, Kwon BS, Lim CY, et al. Integrative medicine rehabilitation of simultaneous intra-dermal acupuncture (IDA) and neurodevelopmental treatment (NDT) for children with cerebral palsy: Pilot Study of Functional Near-Infrared Spectroscopy. *The Journal of Pediatrics of Korean Medicine* 2021;35(1):139-47.
10. Litscher G. Computer-based quantification of traditional chinese-, ear- and Korean hand acupuncture: needle-induced changes of regional cerebral blood flow velocity. *Neurol Res* 2002;24(4):377-80.
11. Zhang J, Zheng Y, Wang Y, Qu S, Zhang S, Wu C, et al. Evidence of a synergistic effect of acupoint combination: a resting-state functional magnetic resonance imaging study. *The Journal of Alternative and Complementary Medicine* 2016;22(10):800-9.
12. Jiping Zhang, Xiaowen Cai, Yanjie Wang, Yu Zheng, Shanshan Qu, Zhinan Zhang, et al. Different Brain Activation after Acupuncture at Combined Acupoints and Single Acupoint in Hypertension Patients: An Rs-fMRI Study Based on ReHo Analysis. *Evidence-Based Complementary and Alternative Medicine* 2019;Article ID 5262896 :1-10.
13. Park JM, Shin AS, Park SU, Sohn IS, Jung WS, Moon SK, et al. The acute effect of acupuncture on endothelial dysfunction in patients with hypertension: a pilot, randomized, double-blind, placebo-controlled crossover trial. *The Journal of Alternative and Complementary Medicine* 2010;16(8):883-8.
14. Tobbackx Y, Meeus M, Wauters L, De Vilder P, Roose J, Verhaeghe T, et al. Does acupuncture activate endogenous analgesia in chronic whiplash-associated disorders? A randomized crossover trial. *European Journal of Pain* 2013;17(2):279-89.
15. Liang ZH, Xie CC, Li ZP, Zhu XP, Lu AP, Fu WB. Deqi sensation in placebo acupuncture: a crossover study on Chinese medicine students. Evidence-Based Complementary and Alternative Medicine 2013;2013:620671.
16. Lee GT, Kim JG. Evaluation of Cerebrovascular Reactivity According to Age Using Transcranial Doppler. *Korean J Clin Lab Sci* 2004;36(1):43-8.
17. Vriens EM, Kraaier V, Musbach M, Wieneke GH, van Huffelen AC. Transcranial pulsed Doppler measurements of blood velocity in the middle cerebral artery: reference values at rest and during hyperventilation in healthy volunteers in relation to age and sex. *Ultrasound in medicine & biology* 1989;15(1):1-8.

18. WHO. WHO Standard Acupuncture Point Locations in the Western Pacific Region. Geneva: World Health Organization; 2008.
19. MacPherson H, Altman DG, Hammerschlag R, Youping L, Taixiang W, White A, Moher D; STRICTA Revision Group. Revised STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA): extending the CONSORT statement. *J Altern Complement Med* 2010 Oct;16(10):ST1-14.
20. Moon SK, Kwon SW, Cho SY, Park SU, Jung WS, Park JM, 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. *Acupunct Med* 2019;37(5):277-82.
21. Kim SW. Effects of acupuncture at GB39 on CO_2 reactivity in the anterior and middle cerebral arteries during hypocapnia in normal subjects. *Kyung Hee University* 2020.
22. Lee HK, Moon SK, Jin C, Cho SY, Park SU, Jung WS, et al. Effects of GV14 Acupuncture on Cerebral Blood Flow Velocity in the Basilar and Middle Cerebral Arteries and CO_2 Reactivity during Hypercapnia in Normal Individuals. *Evidence-Based Complementary and Alternative Medicine* 2021;2021:9319413.
23. Izumi Y, Tsuda Y, Ichihara S, Takahashi T, Matsuo H. Effects of Defibrination on Hemorheology, Cerebral Blood Flow Velocity, and CO Reactivity During Hypocapnia in Normal Subjects. *Stroke* 1996;27(8):1328-32.
24. Markwalder TM, Grolimund P, Seiler RW, Roth F, Aaslid R. 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 & Metabolism* 1984;4(3):368-72.
25. Park SM, Lee KS, Choi JY, Kim YI. Cerebral Vasoreactivity in Carotid Stenosis or Occlusion Cases: A Transcranial Doppler Study with Acetazolamide (Diamox) Test. *Journal of the Korean Neurological Association* 1998;16(4):439-43.
26. Ringelstein EB, Sievers C, Ecker S, Schneider PA, Otis SM. Noninvasive Assessment of CO_2-Induced Cerebral Vasomotor Response in Normal Individuals and Patients with Internal Carotid Artery Occlusions. *Stroke* 1988;19(8):963-9.
27. Hong KS, Roh JK. Evaluation of Cerebrovascular Reactivity in Normal and Atherosclerotic Subjects: using Transcranial Doppler. *Journal of the Korean Neurological Association* 1994;12(2):237-44.
28. Tsuchiya M, Sato EF, Inoue M, Asada A. Acupuncture enhances generation of nitric oxide and increases local circulation. *Anesth Analg* 2007;104(2):301-7.
29. Leung SB, Zhang H, Lau CW, Lin ZX. Attenuation of Blood Pressure in Spontaneously Hypertensive Rats by Acupuncture Was Associated with Reduction Oxidative Stress and Improvement from Endothelial Dysfunction. *Chinese medicine* 2016;11(1):38.
30. Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. *Journal of Biological Chemistry* 1994;269(19):13725-8.
31. White RP, Deane C, Vallance P, Markus HS. Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia. *Stroke* 1998;29(2):467-72.
32. White RP, Deane C, Hindley C, Bloomfield PM, Cunningham VJ, Vallance P, 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* 2000;178(1):23-8.

Disclosure

This paper is based on Hojung Park's theses for Master's Degree.