Acupuncture attenuates extracellular glutamate level in global ischemia model of rat

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Objectives: *Acupuncture has been known to be effective in ischemia, and glutamate excitotoxicity is an important factor for the neuronal cell death. We examined the effect of acupuncture on glutamate level in the ischemic stroke model.*
Methods: *A global ischemia was induced using the method of 11-vessel occlusion in rat. Rats were randomly divided into two groups: the control group (*n*=5, with 11-vessel occlusion) and the acupuncture group (*n*=5, with 11-vessel occlusion + acupuncture). The extracellular glutamate level was assessed using an intracerebral biosensor system measuring 256 samples per second. Simultaneously, the cerebral blood flow was measured and the electroencephalogram data were recorded. The time schedule of the experiment was as follows: 10 minutes of baseline measurement, 10 minutes of occlusion and 30 minutes of follow-up monitoring. Acupuncture stimulation was applied to the acupuncture points GB34 (Yangneungcheon) and GB39 (Hyeonjong) during the occlusion period.*
Results: *In the control group, the extracellular glutamate level was changed as 135.19 ± 23.76 μM (mean ± standard deviation) from the baseline level. However, this increase was suppressed in the acupuncture group (72.20 ± 27.15 μM,* $p<0.01$ *versus the control group). The changes of cerebral blood flow and electroencephalogram were not significantly different between the groups.*
Discussion: *This result suggests that the effect of acupuncture might be closely associated with modulation of the brain glutamate release in the ischemic condition.* [Neurol Res 2010; **32**: 79–83]

Keywords: Acupuncture; cerebral blood flow; 11-vessel occlusion model; glutamate; ischemia

INTRODUCTION

Ischemic stroke, which takes ~80% of all stroke cases[1], is one of the leading causes of death[2]. The prevalence of stroke is increasing and more attention is called for in an era of global aging[3]. Acupuncture, an ancient art of healing in East Asia, has been one of the main modalities applied to stroke patients[4]. A recent review recommended further acupuncture research for stroke rehabilitation, based on its widespread use, promising results, relatively less severe adverse effects and lower cost[2].

In ischemia, glutamate excitotoxicity is an important factor of neuronal cell death[5]. Accumulated extracellular glutamate aggravates and amplifies the ischemic injury[6]. Glutamate level in the brain has been studied using conventional HPLC analysis methods with microdialysis probe or cortical cup. However, these methods show disadvantages that collected samples are diluted and averaged over the sampling period time[7]. Technological development enabled a real-time monitoring of glutamate level *in vivo*, using amperometric biosensor technology[8]. Amperometric biosensor method is known to reflect the real-time changes in glutamate level more faithfully than conventional methods[7]. Electroacupuncture, a form of acupuncture using electrical stimulation instead of manual manipulation of a needle, has been reported to attenuate excessive glutamate in ischemic injury in common carotid artery occlusion model when two acupuncture points, GV11 (Sindo) and GV16 (Pungbu), were stimulated[9]. Effects on aspartate and taurin level have also been reported using the middle cerebral artery occlusion model when two acupuncture points, GV8 (Geunchuk) and GV16, were stimulated[10]. Eleven-vessel occlusion, known as 11VO, a model of global cerebral ischemia, is a relatively new model. It is known to produce a reliable drop of cerebral blood flow and dampening of electroencephalography, when compared to more conventional models like four-vessel occlusion[7]. However, real-time monitoring was not adopted in previous studies. No study has tried to evaluate the effect of acupuncture in 11-vessel occlusion model.

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In this study, we observed the effect of manual acupuncture on real-time changes of extracellular glutamate level using the 11-vessel occlusion model, simultaneously with cerebral blood flow and electroencephalogram.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (250–350 g) were used for the experiment after 1 week acclimation under standard laboratory conditions at 22 ± 2°C, constant humidity and photoperiod (12 hour light–dark cycle). Commercial rat chow and water were provided *ad libitum*. The experiment was performed in accordance with the animal experimentation guidelines of Kyung Hee University and that of the government regulation.

Experimental procedure

Eleven-vessel occlusion global ischemia model[7] was established in a partially modified way[11]. Briefly, rats were anesthetized with chloral hydrate (0.1 ml/100 g, i.p.), with additional intermittent injection (0.1 ml/300 g) for maintenance when necessary. The body temperature was maintained at 36.5 ± 0.5°C using the homeostatic blanket control unit (Harvard apparatus, Holliston, MA, USA) throughout the experimental procedure. Four burr holes were made on the cranium to measure the glutamate level, brain temperature and cerebral blood flow. Sensor for the real-time monitoring system was set up through the burr holes. Brain temperature was maintained at 37.5 ± 1.0°C using an epidural thermocouple connected controller. Basilar artery, pterygopalatine arteries, occipital arteries and superior thyroid arteries were coagulated. External carotid arteries and common carotid arteries were exposed. Rubber rings were put around the exposed arteries for later transient compression of the arteries.

After a baseline measurement for 10 minutes, 11-vessel occlusion cerebral ischemia was initiated by hanging weights on the rubber rings put around the external carotid and common carotid arteries. Ischemic period lasted for 10 minutes. Then, weights were removed and reperfusion period lasted for 60 minutes. Rats were randomly divided into two groups: the control group (n=5, with 11-vessel occlusion) and the acupuncture group (n=5, with 11-vessel occlusion + acupuncture).

Acupuncture stimulation

In the acupuncture group, acupuncture stimulation was applied throughout the ischemic period (10 minutes). Four acupuncture needles, 0.25 mm in diameter and 40 mm long (Dongbang Acupuncture Inc., Chungnam, Korea), were manually inserted ~5 mm deep to the acupuncture points GB34 (Yangneungcheon) and GB39 (Hyeonjong) on both sides of the body. GB34 was located just anterior and inferior to the fibular head and GB39 was located distal four-fifths points on the imaginary line connecting the lateral side of the knee and the lateral malleolus of the tibiofibula (*Figure 1*)[12]. Each needle was twirled (1 Hz, clockwise and counterclockwise 180°) for 10 seconds. When a needle was twirled, the other three needles remained inserted. All four needles were twirled in turn until the occlusion period ended.

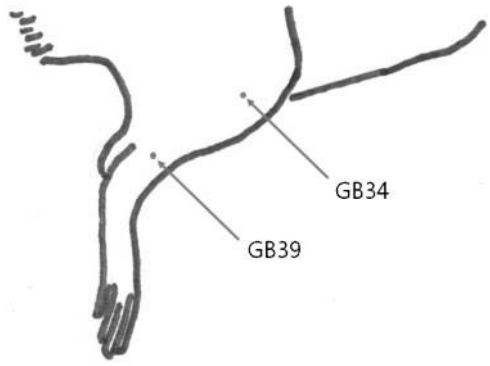

Figure 1: The acupuncture points GB34 and GB39. GB34 is located just anterior and inferior to the fibular head and GB39 is located distal four-fifths points on the imaginary line connecting the lateral side of the knee and the lateral malleolus of the tibiofibula

Real-time monitoring system

Blood flow of cerebral cortex was monitored with laser Doppler flowmetry (Transonic Systems Inc., Ithaca, NY, USA). The microdialysis electrode (Sycopel International Ltd, Tyne & Wear, UK) for glutamate monitoring (type: general 20-10-4-4) was filled with phosphate-buffered saline (PBS) to electropolymerize the *o*-phenylenediamine on the platinum electrode at 0.65 V for 20 minutes with Sycopel BD2000 potentiostat (Sycopel International Ltd). Then, PBS-containing glutamate oxidase was perfused in the dialysis electrode with a flow rate of 0.5 μl/min. The dialysis electrode showed linear response in the concentration range of 50–450 μM standard glutamate solution with a sensitivity of 0.22 nA/μM (R^2, coefficient of regression of 0.998). After the calibration of the sensor, the microdialysis electrode was inserted into motor cortex at coordinates A1 : L4 : V4 mm (from bregma and the dura) through a small incision made in the dura. Electroencephalogram was recorded through the electrodes attached to the parietal bones using a 511 AC amplifier (Grass-Telefactor Product Group, Astro-Med, Inc., West Warwick, RI, USA). Signals from potentiostat for glutamate laser, Doppler flowmetry and electroencephalogram were obtained and displayed with DASYLab (Data Acquisition System, National Instruments, Austin, TX, USA) and analysed by DADiSP 2002 (DSP Development Co., Newton, MA, USA).

Statistical analysis

The analysis was performed using SPSS Statistical Software (version 17.0 for Windows). The data were expressed as mean ± standard deviation. Statistical analysis was performed to compare two groups for glutamate release and cerebral blood flow changes,

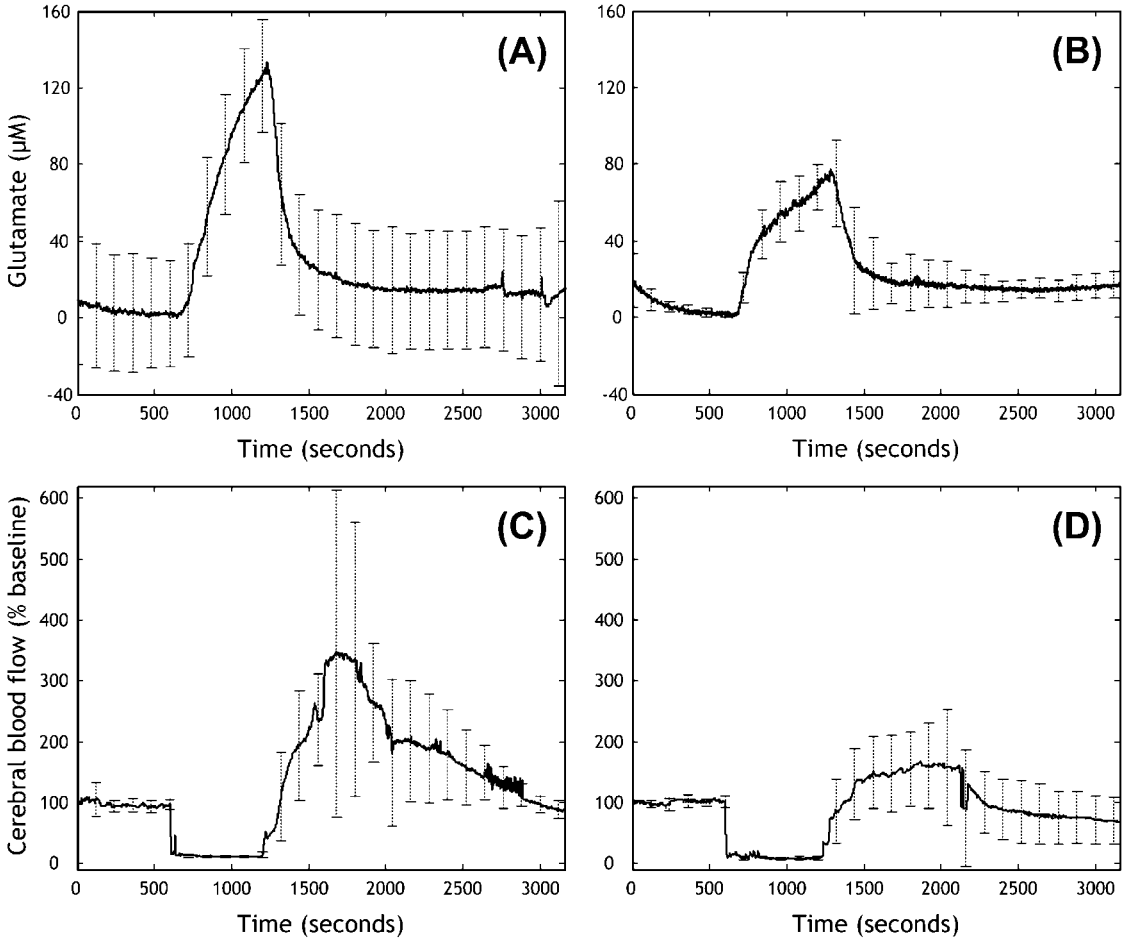

Figure 2: Real-time measurement of extracellular glutamate level (μM) in the control group (**A**) and in the acupuncture group (**B**). Cerebral blood flow changes (% baseline) in the control group (**C**) and in the acupuncture group (**D**). Eleven-vessel occlusion model of global ischemia in a rat was used with or without acupuncture stimulation at acupuncture points GB34 and GB39 during 10 minutes of the occlusion periods. Rats were randomly divided into two groups: the control group (n=5, with 11-vessel occlusion) and the acupuncture group (n=5, with 11-vessel occlusion + acupuncture). Values are presented as mean ± standard deviation

using two-tailed Student's *t*-test with a significance level of 0.05.

RESULTS

Changes of glutamate release

In the control group, the glutamate release level began to increase after the onset of occlusion and continued to rise throughout the entire ischemic period, as shown in *Figure 2A*. Soon after the reperfusion, the glutamate level declined rapidly to the pre-ischemic level. In the acupuncture group, the pattern of glutamate release was similar to the control group, as shown in *Figure 2B*. However, the increase in glutamate release was suppressed. The change of glutamate concentration was 135.19 ± 23.76 μM in the control group and 72.20 ± 27.15 μM in the acupuncture group during the ischemic period, as shown in *Table 1*. The change of glutamate concentration was 138.63 ± 20.00 μM in the control group and 72.30 ± 30.22 μM in the acupuncture group

Table 1: Change of glutamate release during 10 minutes of ischemia and 30 minutes of reperfusion

	Glutamate value (μM)		
Measurement	Control (n=5)	Acupuncture (n=5)	p
Peak change during ischemia	135.19 ± 23.76	72.20 ± 27.15	<0.01*
Peak change during reperfusion	138.63 ± 20.00	72.30 ± 30.22	<0.01*

Eleven-vessel occlusion model of global ischemia in a rat was used with or without acupuncture stimulation at the acupuncture points GB34 and GB39 during 10 minutes of the occlusion periods. Rats were randomly divided into two groups: the control group (with 11-vessel occlusion) and the acupuncture group (with 11-vessel occlusion + acupuncture). Values are presented as mean ± standard deviation.
*Two-tailed Student's *t*-test.

during the reperfusion period. The parameters for glutamate release in *Table 1* showed statistically significant differences between two groups.

Changes of cerebral blood flow

The pattern of ischemia-evoked response was similar in both groups, as shown in *Figure 2C,D*. After the onset of ischemia, cerebral blood flow declined rapidly to near to zero level, concurrently with the development of flat electroencephalogram signal. After the initiation of reperfusion, the level of cerebral blood flow showed a huge increase, followed by a decline to the pre-ischemic levels. The change of electroencephalogram activity was in accordance with the cerebral blood flow (data not shown). Cerebral blood flow changes during the ischemic and reperfusion period were not significantly different between the control and acupuncture groups, as shown in *Table 2*.

DISCUSSION

This is the first study investigating the effect of acupuncture stimulation in the brain acute global ischemic condition using an 11-vessel occlusion model. Glutamate level was observed to be attenuated with acupuncture stimulation during occlusion and reperfusion period, using a real-time *in vivo* monitoring system of extracellular glutamate, cerebral blood flow and electroencephalogram. This monitoring system is well established[7] and known to offer fast and precise measurement data.

Acupuncture is generally known to be an effective adjunctive therapy for stroke rehabilitation[4,13]. It has been shown in previous reports that acupuncture stimulation helps neuronal survival[14] and plasticity[15], in addition to improvement of spasticity[16] in stroke conditions, although there still continues a controversy on acupuncture effect[17] and strong evidence still remains to be sought[2]. These effects might be possible through the modulation of glutamate excitotoxicity and reperfusion hyperemia[9], reduced lipid peroxidation[18], enhanced dopamine level[14], facilitated neurogenesis[19] and increased blood flow[20].

We observed acupuncture effect under a condition more acute and severe than previously reported model such as a middle cerebral artery occlusion[10] or carotid artery occlusion method[9]. Ischemia model using the method of 11-vessel occlusion in this study is well established as a severe global ischemia[7]. Eleven-vessel occlusion model has advantages of immediate drop in cerebral blood flow to near zero levels and maximized glutamate release with maintaining self-respiration[11,21], which shows mimicking physiological forebrain ischemia compared to the traditional four-vessel occlusion technique. In line with preliminary observation[22], the acupuncture and the control groups showed a significant difference in the extracellular glutamate level, while the reduction of cerebral blood flow and reperfusion hyperemia were not significantly different between two groups. Commonly, glutamate is an excitatory neurotransmitter that plays an important role in the human brain. The excessive release of glutamate is believed to be a cause of the neuronal death associated with various neurological disorders. Therefore, the extracellular levels of glutamate, which may be neurotoxic to the brain, are considered to be a possible factor contributing to the increased neural damage in ischemia. Therefore, the modulation of glutamate release could be an important mechanism explaining acupuncture effect on ischemic stroke, in that glutamate excitotoxicity is a major mechanism of neuronal cell death in ischemic conditions and a probable target of therapeutic intervention[23].

In the paradigm of acupuncture, it has been known that selections of the acupuncture point and stimulation method have different physiological effects. For example, when using electroacupuncture stimulation, it is observed that low frequency (2 Hz) is more efficient than high frequency (120 Hz)[24]. Acupuncture points were selected by certified oriental medical doctors referred to the major classic textbook of acupuncture[25]. It is traditionally known that GB34 and GB39, both belonging to the category of eight meeting points, show prominent therapeutic effects for motor function disorders in bone marrow and tendon[26]. These points are located on the lateral aspect of both hindlimbs of a rat according to the anatomical correspondence to human acupuncture points[12]. And this study employed a manual manipulation method, which was frequently applied in clinical settings. In further study, it is necessary to investigate the effects of diverse points and stimulation modes.

This study showed a glutamate controlling effect of acupuncture stimulation under acute global ischemic condition using a real-time monitoring system.

Table 2: Blood flow change during 10 minutes of ischemia for 10 minutes of ischemia and 30 minutes of reperfusion

	Cerebral blood flow change (%)		
Measurement	Control (n=5)	Acupuncture (n=5)	p
Mean baseline value	100.00 ± 10.94	100.00 ± 6.81	NS*
Mean value during ischemia	12.27 ± 2.89	6.85 ± 2.30	NS*
Peak value during reperfusion	390.72 ± 265.99	177.40 ± 45.86	NS*

Eleven-vessel occlusion model of global ischemia in a rat was used with or without acupuncture stimulation at the acupuncture points GB34 and GB39 during 10 minutes of the occlusion periods. Rats were randomly divided into two groups: the control group (with 11-vessel occlusion) and the acupuncture group (with 11-vessel occlusion + acupuncture). *n*, number. Values are presented as mean ± standard deviation.
*NS, not significant by two-tailed Student's *t*-test.

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