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Research Report

Neuroprotective effects of magnesium-sulfate on ischemic injury mediated by modulating the release of glutamate and reduced of hyperreperfusion

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ABSTRACT

This study examined the neuroprotective effects of magnesium-sulfate ($MgSO_4$) on the cerebral blood flow (CBF) and extracellular glutamate concentration in an eleven vessel occlusion (11VO) rat model. Twenty-one male Sprague–Dawley rats (250–350 g) were used for the 11VO ischemic model, which was induced by a 10-min transient occlusion. The animals were divided into 3 groups, including ischemic-induced animals (ischemia group), ischemic-induced and $MgSO_4$ treated animals ($MgSO_4$ group), and sham animals for comparison. The real-time extracellular glutamate concentration was measured using a microdialysis biosensor, and the CBF was monitored by laser Doppler flowmetry. Neuronal cell death in the hippocampal region was observed 72 h after ischemia by several stains (Nissl, DAPI, NeuN, and cleaved caspase3). A significant decrease in %CBF was observed in both the ischemia and $MgSO_4$ groups, such as ~10% during the ischemic period. However, the $MgSO_4$ group showed a significant decrease in the initial reperfusion %CBF compared to the ischemia group. A significantly lower level of glutamate release was observed in the $MgSO_4$ group than in the ischemia group during the ischemic and reperfusion episode. Our staining results revealed a significant decrease in neuronal cell death in the hippocampus in the $MgSO_4$ group compared to the ischemia group. These results suggest that $MgSO_4$ is responsible for the protection of neuronal cells by suppressing the release of extracellular glutamate under ischemic conditions and the CBF response during the initial reperfusion period.

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Abbreviations: 11VO, eleven vessel occlusion; CBF, cerebral blood flow; NMDA, *N*-methyl-D-aspartate; $MgSO_4$, magnesium-sulfate

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1. Introduction

Ischemic stroke is one of the leading causes of death and long-lasting disability, resulting from a transient or permanent decrease in cerebral blood flow (CBF) (Todd et al., 1986) caused by cardiac arrest, cerebral arterial occlusion, or severe vasospasm after subarachnoid ischemia (Taoufik and Probert, 2008). Cerebral ischemic stroke is one of the most common neurodegenerative diseases with severe brain damage, including infarction, death of brain tissue, and loss of brain function, particularly in the aged population (Takagi, 2009). The brain damage by ischemic stroke facilitates the extracellular concentrations of activated excitatory amino acids (Chi et al., 2009).

Glutamate is a major excitatory neurotransmitter and an important neurotoxin that promotes neuronal cell death (Kirino, 1989), and its excessive release amplifies the impact of the neuronal injury (Park et al., 2010). The extracellular glutamate concentration was measured precisely to understand the excitotoxic processes under clinical situations (Lee et al., 2009a). The sensor system that combined with microdialysis sampling has been developed to directly measure the glutamate release *in vivo*. Real-time *in vivo* monitoring of glutamate has been investigated using amperometirc biosensor technology with successful applications in research (Yao et al., 2001).Previous studies performed real-time monitoring of the release of extracellular glutamate in an 11 vessel occlusion (11VO) model using either a microdialysis biosensor (Lee et al., 2008) or enzyme-immobilized carbon nanotube-field effect transistor (CNT-FET) (Lee et al., 2009b). The level of glutamate release in the 5- and 10-min 11VO ischemia groups was reported (Lee et al., 2009a), which revealed that the excessive extracellular glutamate release correlated with neuronal cell death (Park et al., 2010).

The 11VO model introduced in 1998 has been suggested to better mimic complete ischemia by measuring the glutamate levels in real-time (Caragine et al., 1998; Choi et al., 2008). These studies have reported that the 11VO model can easily induce an infarction because of the reproducibility of the CBF plateau to ischemic levels and the faster decrease in CBF compared to other ischemic models(Park et al., 2010).

Excitotoxic damage is affected by the glutamate release through over activation of its receptors (Taghibiglou et al., 2009). For example, exposure to agonists of *N*-methyl-D-aspartate (NMDA) receptors, a main subtype of glutamate receptors, leads to increased cell death, whereas antagonists offer some protection against brain damage (Camara-Lemarroy et al., 2009).

It was reported that magnesium stimulated the blockade of NMDA receptors, resulting in a decrease in glutamate release during the ischemic period (Belfort et al., 2003). They showed that magnesium could have an antieclamptic effect as cerebral vasodilator. Although the specific mechanisms remains still unclear, another study has reported that magnesium-sulfate may act as a vasodilator and protect the blood–brain barrier, including minimizing cerebral edema formation (Euser and Cipolla, 2005). Many studies have suggested that the clinical effects of magnesium offer pharmacological advances that protect against ischemic brain injury without major side effects (Chacon et al., 2006; Gormus et al., 2009). It have been reported that magnesium could have positive effects not only in ischemia but also in other diseases such as subarachnoid hemorrhage (Rabinstein et al., 2010), neonatal brain (Pazaiti et al., 2009), birth asphyxia (Gathwala et al., 2006), spinal cord trauma (Ozdemir et al., 2005), and preeclampsia (Rogers et al., 2010). However, the role of magnesium-sulfate ($MgSO_4$) on the CBF and glutamate release in an 11VO ischemia model is unclear.

This study examined the effects of $MgSO_4$ on the CBF and extracellular glutamate concentration by inducing ischemic and reperfusion conditions, using an 11VO model and real-time measurement methods.

2. Results

2.1. *Change in the CBF levels between ischemia and $MgSO_4$ treated groups*

Since the CBF plays an important role in cerebral brain ischemia, we monitored the %CBF levels simultaneously with extracellular glutamate release. Then we examined the effects by magnesium-sulfate during the ischemic and reperfusion periods. The pattern of the %CBF responses in the ischemia and $MgSO_4$ groups in an 11VO model was different. After the onset of ischemia, there was a rapid decrease in the %CBF to 10.07 ± 2.37% and 6.46 ± 2.55% for the ischemia and $MgSO_4$ group, respectively (Table 1). These changes were maintained successfully during the ischemic period. However, after initiating reperfusion, the peak level and time of %CBF upon reperfusion were different in each group. The ischemia group showed an increasing %CBF pattern for 681.94 ± 122.66 s, whereas the $MgSO_4$ group showed a slow increase in the %CBF pattern for 433.28 ± 89.58 s ($p = 0.0002$). Furthermore, the maximum %CBF was 212.30 ± 54.36% in the ischemia group, which then decreased significantly to the pre-ischemic levels. However, in the $MgSO_4$ group, the peak level of %CBF decreased to 81.23 ± 23.64% during the initial reperfusion period, $p < 0.0001$ (Fig. 1).

2.2. *Change in the glutamate levels between the ischemia and $MgSO_4$ treated groups*

The changes in extracellular glutamate release were analyzed using a microdialysis electrode to confirm the changes in glutamate release between the ischemia and $MgSO_4$ groups.

Table 1 – Changes in ischemic-evoked cerebral blood flow (CBF) for ischemia and magnesium-sulfate ($MgSO_4$) treated groups.

Variable	Ischemia group	$MgSO_4$ group
Ischemic plateau (%)	10.07 ± 2.37	6.46 ± 2.55
Peak level on reperfusion %CBF (%)	212.30 ± 54.36	81.23 ± 23.64
Time interval between the onset of reperfusion and the peak level of CBF (seconds)	681.94 ± 122.66	433.28 ± 89.58

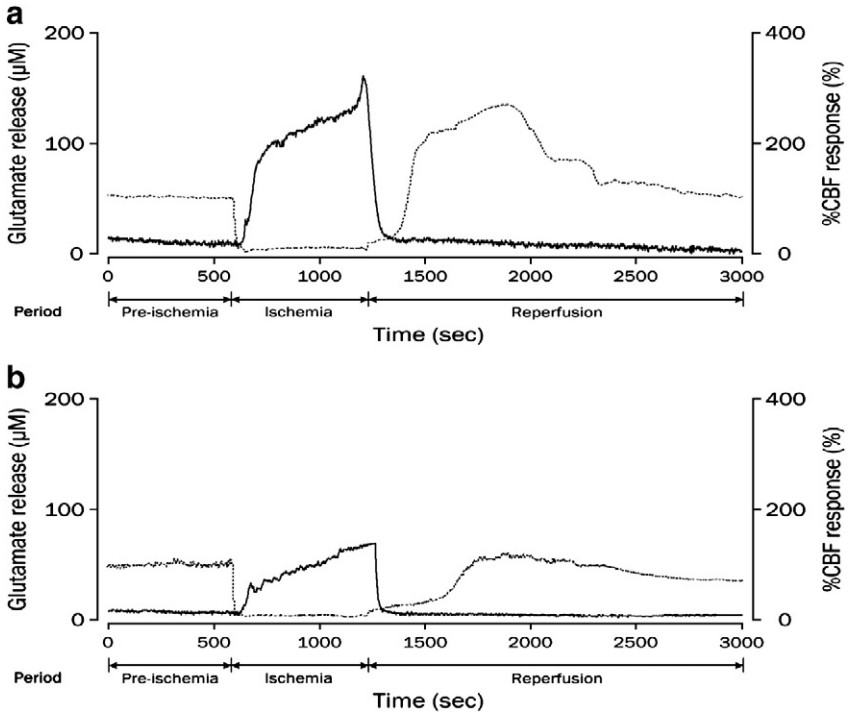

Fig. 1 – Real-time changes in ischemia cerebral blood flow (CBF) and extracellular glutamate release during an 11VO occlusion model between the ischemia and magnesium-sulfate ($MgSO_4$) treated groups, measured using a biosensor.

The rapid increase in glutamate release was observed from 96.15 ± 19.54 s after the onset of ischemia and continued to rise throughout the ischemic period in both groups (Table 2). The level of glutamate decreased rapidly to the pre-ischemic levels within 5 min after reperfusion (Fig. 1). The maximum change in the glutamate concentration in the ischemia and $MgSO_4$ groups during the ischemic period was 182.9 ± 64.0 μM and 53.46 ± 12.64 μM, respectively. After reperfusion, the peak glutamate concentration in the ischemia and $MgSO_4$ groups was 197.21 ± 67.21 μM and 62.69 ± 19.63 μM, respectively. The amount of glutamate released in the two groups was significantly different, $p < 0.05$. Fig. 1 shows the difference in glutamate dynamics between the $MgSO_4$ and ischemia groups.

2.3. *Histological analysis*

2.3.1. *Nissl staining*

To evaluate the neuroprotective effects of $MgSO_4$ in the 11VO model, this study examined whether $MgSO_4$ was associated with an increase in neuronal cell survival in the hippocampal region after ischemia. The CA1 pyramidal cells from four hemispherical sections, which showed stained nuclei under cresyl violet staining, were counted and averaged. The percentage of visible cells in the hippocampus in the ischemia and $MgSO_4$ groups was 18.5 ± 5.8% and 77.2 ± 5.7%, respectively, compared to the sham group, $p < 0.05$ (Fig. 2). The mean number of stained cells was determined by three researchers who were blinded to the experimental conditions. The cell viability from each group was presented as a percentage of the mean number of viable cells from the sham animals.

2.3.2. *Cleaved caspase-3 and NeuN*

The CA1 pyramidal cells from four hemispherical sections, which showed stained nuclei under DAPI, NeuN, and cleaved caspase 3, were counted and averaged. The NeuN stain percentage of visible cells in the hippocampus in the ischemia and $MgSO_4$ groups was 39.5 ± 5.2% and 79.4 ± 4.9%, respectively, compared to the sham group, $p < 0.05$ (Fig. 3). Cleaved caspase 3 stain percentage in the ischemia and $MgSO_4$ groups was 90.2 ± 6.3% and 21.1 ± 2.2%, respectively, compared to the sham group, $p < 0.05$ (Fig. 3). The mean number of stained cells was determined by three researchers who were blinded to the experimental conditions. The cell viability from each group was presented as a percentage of the mean number of viable cells from the sham animals.

Table 2 – Levels of ischemic-evoked neurotransmitter glutamate for ischemia and $MgSO_4$ treated groups.

Variable	Ischemia group	$MgSO_4$ group
Peak level of ischemic glutamate release (μM)	182.99 ± 64.05	53.46 ± 12.64
Peak level of reperfusion glutamate release (μM)	197.21 ± 67.21	62.69 ± 19.63
Time delay of the beginning of glutamate release after the onset of ischemia (seconds)	96.15 ± 19.54	101.69 ± 17.96

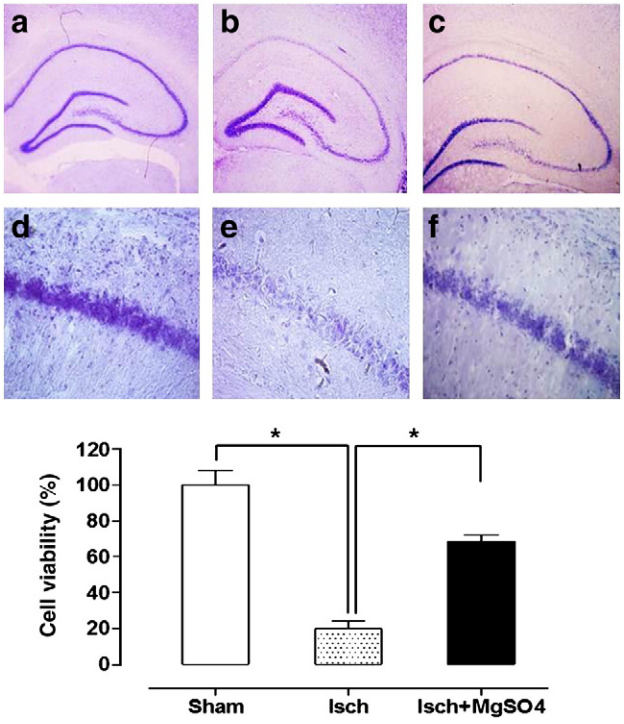

Fig. 2 – Representative morphology stained with cresyl violet in the hippocampal region from the sham group (a and d), ischemia group (b and e), and $MgSO_4$ treated group (c and f) at 72 h of reperfusion. The graph shows the quantitative results of the CA1 neuronal cell counts.

3. Discussion

This study examined the neuroprotective effects of $MgSO_4$ in an 11VO animal model induced by modulating the release of glutamate. In this study, the level of glutamate release increased during the occlusion period. During the ischemic period, the %CBF was significantly lower in the $MgSO_4$ group than in the control group. The glutamate concentration was significantly higher in the ischemia group than in the $MgSO_4$ group. Three days after reperfusion, ischemic cell death was observed in the hippocampal region of the 11VO model.

Brain ischemia, induced by insufficient CBF, leads to a poor oxygen supply due to cerebral hypoxia, which can cause death in brain tissue, cerebral infarction, and loss of brain function (Bramlett and Dietrich, 2004). Therefore, the CBF plays a key role in the development of brain ischemia including defects in the cerebral metabolic rate with the energy state, significant death during the perfusion period (Pulsinelli and Brierley, 1979), low survival rate for a successful ischemia study (Todd et al., 1986), and high mortality rate (Sugio et al., 1988). Hyperreperfusion can also lead to brain damage, as mentioned previously (Takamatsu et al., 2000).

Magnesium can have an effect, including enhanced CBF to ischemic areas, inhibition of calcium flow into the cells through leakage, noncompetitive blockade of the NMDA receptors, and favorable recovery of the cellular energy metabolism after the restoration of reperfusion (Lin et al., 2002).

In this study, a decrease in the %CBF after 11VO was observed during the ischemic period, with a consistent increase in glutamate release. And the cell death in hippocampus in the $MgSO_4$ group reduced compared to control group. This finding suggests that an 11VO model may show profound reversible ischemia to complete forebrain ischemia. Furthermore, this model highlights the reproducibility of peracute excitotoxic damage with elevated glutamate levels, which is in agreement with previous studies (Caragine et al., 1998; Lee et al., 2009a,b). Decline of glutamate release could suppress apoptosis, which led to neuronal cell death.

An excessive extracellular concentration of glutamate release, which is associated with a range of neuronal disorders, may play an important role in neuronal death and apoptosis (Kirino, 1989; Taoufik and Probert, 2008; Zhao et al., 1998). Therefore, it is essential to have a clear understanding of the excitotoxic processes and use accurate real-time measurements of the extracellular glutamate concentrations during and after an ischemic injury (Lee et al., 2009b). The microdialysis biosensor was initially used to measure the level of neurotransmitters in the rat brain, suggesting that an increase in extracellular glutamate release is a key factor in the development of brain damage after ischemia (Benveniste et al., 1984). It was reported that (1) ischemic-induced rats increased the release of extracellular glutamate during the ischemic period (Ogawa et al., 2007), (2) release of glutamate induced apoptosis, and (3) resulting in neuronal cell death (Dennis, 1996).

The first application of a microdialysis biosensor was to detect the interstitial glucose concentrations in humans (Lonnroth et al., 1987).

The NMDA receptor is a glutamate-binding molecule that regulates the extracellular glutamate concentration in the brain. During normal physiological conditions, magnesium is a non-competitive inhibitor of the NMDA receptor, which regulates the calcium influx (Poleszak et al., 2008). The $MgSO_4$ has the ability to dilate blood vessels (Euser and Cipolla, 2005).

The application of magnesium in clinical trials was aimed at treating acute cerebral ischemia. Therefore, recent studies of $MgSO_4$ focused on the neuroprotective effects of ischemia (Chacon et al., 2006; Meloni et al., 2009). For example, the administration of $MgSO_4$ in brain ischemia reduced the infarct size, ischemic brain tissue, and energy failure and acidosis (Gee et al., 2004). Furthermore, it was demonstrated that various neuroprotective functions of magnesium in brain ischemia included a decrease in the presynaptic release of glutamate and a blockade of the NMDA receptors with the calcium entry via the voltage-gated channels (Meloni et al., 2009). In this study, the glutamate concentration was increased after 11VO in the ischemia group. Three days after ischemic induction, the higher levels of glutamate correlated with neuronal cell damage in the hippocampal region of the ischemia group, which concurred with previous report (Park et al., 2010). However, the administration of $MgSO_4$ inhibited the activation of the extracellular glutamate concentration, resulting in a decrease in neuronal cell death due to inhibition of apoptosis in the hippocampal region of the 11VO animal model.

In conclusion, the suppression of the extracellular glutamate levels and CBF during the initial reperfusion period by

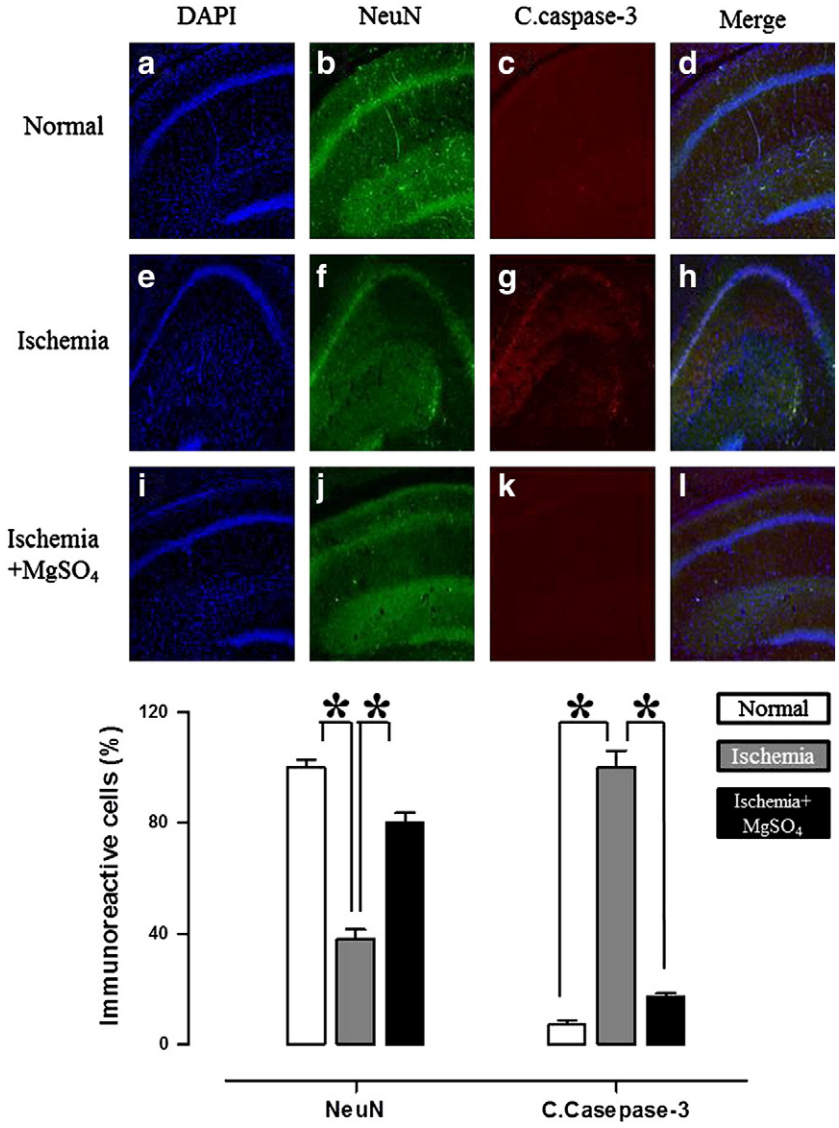

Fig. 3 – Representative morphology stained with DAPI (a, e, and i), NeuN (b, f, and j), and C.casp3 (c, g, and k) in the hippocampal region from the sham group, ischemia group, and $MgSO_4$ treated group at 72 h of reperfusion. The graph shows the quantitative results of the CA1 neuronal cell counts.

$MgSO_4$ may have neuroprotective effects on neuronal cells in an 11VO model.

4. Experimental procedures

4.1. *Animal preparation*

Twenty-one of male Sprague–Dawley rats, weighing 200–250 g, were housed at 22–24 °C and 12-h light/dark cycle in a controlled environment. The animals were fed commercial rat chow and water *ad libitum*. An adequate injection and anesthesia were planned as a measure to minimize the level of pain or discomfort. All animal experiments were approved by the Ethical Committee of the Kyung Hee University School of Medicine and were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The animals were divided into three groups; ischemic-induced animals (ischemia group, $n=7$), ischemic-induced and $MgSO_4$ treated animals ($MgSO_4$ group, $n=7$), and sham animals (sham group, $n=7$) for a histological based comparison. During the experimental procedure, the body temperature was maintained at 37.1±0.10 °C using a homoeothermic blanket control unit (Harvard Apparatus, Holliston, MA, USA), and SpO_2 was maintained at least 85% using Surgivet Pulseoximeter (Smiths Medicals PM Inc., WI, USA). The rats were anesthetized with an intraperitoneal injection of 300 mg/kg chloral hydrate. Additional chloral hydrate doses were given where needed to maintain the level of anesthesia during the experiment. We directly dripped the $MgSO_4$ solution in the vicinity of the burr hole for the CBF probe during the 10-min

ischemic period, which was diluted 1 mmol/kg with saline according to reference (Kopal et al., 2007).

4.2. Eleven vessel occlusion model preparation

Rats were placed in the supine position. After a midline vertical incision from the cricoids cartilage to the sternal border with a platysma incision, the common carotid artery was defined between the sternocleidomastoid and sternohyoid muscles. The occipital artery, ascending pharyngeal artery, and superior thyroid artery of the external carotid artery were occluded with bipolar electrocautery. The pterygopalatine artery of the internal carotid artery was detected just medial of the tympanic bulla and coagulated. Before occlusion of the basilar artery, a tracheostomy was made at the level of the third or fourth tracheal ring, and then a silastic tube (inner diameter: 1.8–2.1 mm) was inserted and tied up with 4–0 black silk. After sequential retraction of the trachea, esophagus, and both longus colli muscles, we ascertained the clivus and drilled it out with a 3-mm diameter. The basilar artery was selected at the mid pontine groove at the caudal level of the anterior cerebellar artery after the dura incision and arachnoid dissection, and then the basilar artery was permanently coagulated. We sealed the pore with bone wax to prevent leakage of cerebrovascular fluid. Finally, the carotid arteries were prepared for the induction of ischemia. For safe exposure, the carotid artery was dissected with caution so as not to injure the vagus nerve and autonomic nervous chains. On ischemia induction, we hung a rubber ring on the common carotid artery and internal carotid artery on both sides to make blood flow decrease over time, which enabled vascular patency upon subsequent reperfusion.

4.3. Real-time monitoring setup and induction of ischemia

On the prone position with a stereotactic frame, the skin was incised with midline along the superior sagittal suture. Four burr holes were made for real-time measurements of the brain temperature, glutamate and two channels CBF. The CBF was measured using a BLF21D laser Doppler flow meter (Transonic Systems Inc., USA). The glutamate level was measured using a general 20–10–4–4 type dialysis electrode (Sycopel International Ltd., USA).The dialysis electrode displayed a 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 sensor calibration, the microdialysis electrode was inserted into the motor cortex at coordinates A 1: L 4: V 4 mm (from the bregma and the dura) through a small incision in the dura.

The data were collected from the analog output ports of the laser Doppler flowmeter, glutamate potentiostat, and temperature controller (Harvard Apparatus, USA). The signal was digitized using a DT21-EZ analog-to-digital (AD) converter with a 12-bit resolution (Data Translation Inc., Marlboro, MA, USA). The digitized signal, which was sampled 256 times per second using the same AD converter, was transferred in real-time to a personal computer and saved for further analysis.

11VO cerebral ischemia was initiated by pulling the snares on the common carotid artery and extemal carotid artery. The snares were released and withdrawn after 10 min.

4.4. Nissl staining

At 72 h after brain ischemia, the animals were sacrificed with chloral hydrate and fixed with a transcardiac perfusion of ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS) (0.1 M, pH 7.4). The brains were postfixed overnight at 4 °C in the same solution and soaked in 0.5 M PBS, containing 30% sucrose for cryoprotection. The coronal sections (40 μm) were cut at the level of the dorsal hippocampus using a freezing microtome (Leica, Nussloch, Germany).

To identify viable and nonviable stained cells, the sections were mounted on gelatin-coated slides and stained with cresyl violet for a histological assessment of neuronal cell damage. The viable neurons were defined as cells with a normal morphology, exhibiting round nuclei stained with cresyl violet. The number of viable neuronal cells in a $500 \times 500\ \mu m^2$ area of the hippocampal CA1 regions, approximately 300 μm from the hillus of the three coronal sections (approximately 1.4 to 1.8 mm posterior to bregma), was counted using Image Plus 2.0 (Motic, Xiamen, China). The mean number of stained cells was determined by three researchers who were blinded to the experimental group.

4.5. Immunohistochemistry

Immunostaining was performed using antibodies recognizing neurons (mouse anti-neuronal nuclei (NeuN), Chemicon, 1:1000), an apoptotic effector (rabbit antihuman cleaved caspase-3, Cell Signaling Technology,1:100). For each animal, immediately adjacent sections were used for each of the primary antibodies. Briefly, fresh frozen sections were postfixed in buffered formalin and then defatted and dehydrated in a graded series of ethanol washes. Sections were then incubated in a humidifying chamber with 1% hydrogen peroxide to quench endogenous peroxidase enzyme activity and were subsequently blocked with universal blocking serum (Dako) or normal horse serum mixed with Triton X-100 (0.2% for NeuN and caspase-3) to reduce nonspecific background staining. Sections were allowed to incubate with the primary antibodies for 1 h (NeuN) or overnight (caspase-3). After incubation with the species appropriate secondary IgG antibody (all Dako, 1:200) for 30 min, the sections were carefully washed and incubated for 30 min with anavidin–biotin complex (ABC 1:100, Vector Laboratories),washed three times and immunoreactivity visualized with 3,3′-diaminobenzedine tetrahydrochloride (DAB, Sigma).The number of viable neuronal cells in a $500 \times 500\ \mu m^2$ area of the hippocampal CA1 regions, approximately 300 μm from the hillus of the three coronal sections (approximately 1.4 to 1.8 mm posterior to bregma), was counted using Image Plus 2.0 (Motic, Xiamen, China). The mean number of stained cells was determined by three researchers who were blinded to the experimental group.

4.6. Statistical analysis

The data for the %CBF and glutamate changes were expressed as the mean ± the standard error. Statistical analysis was performed to compare the levels of CBF and glutamate changes between the two groups using a two-tailed Student's

t-test. The significant differences in neuronal cell viability were assessed using a one-way analysis of the variance, followed by a Tukey's post hoc test using SPSS statistical software (version 18.0 for Windows, SPSS Inc., Chicago, IL). *p*-Values <0.05 were considered significant.

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REFERENCES

Belfort, M.A., Anthony, J., Saade, G.R., Allen Jr., J.C., 2003. A comparison of magnesium sulfate and nimodipine for the prevention of eclampsia. N. Engl. J. Med. 348, 304–311.

Benveniste, H., Drejer, J., Schousboe, A., Diemer, N.H., 1984. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. 43, 1369–1374.

Bramlett, H.M., Dietrich, W.D., 2004. Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. J. Cereb. Blood Flow Metab. 24, 133–150.

Camara-Lemarroy, C.R., Guzman-de la Garza, F.J., Alarcon-Galvan, G., Cordero-Perez, P., Fernandez-Garza, N.E., 2009. The effects of NMDA receptor antagonists over intestinal ischemia/reperfusion injury in rats. Eur. J. Pharmacol. 621, 78–85.

Caragine, L.P., Park, H.K., Diaz, F.G., Phillis, J.W., 1998. Real-time measurement of ischemia-evoked glutamate release in the cerebral cortex of four and eleven vessel rat occlusion models. Brain Res. 793, 255–264.

Chacon, A., Lisott, E., Eblen-Zajjur, A., 2006. Magnesium sulphate reduces cell volume in physiological conditions but not in the cytotoxic oedema during global brain ischemia. Brain Inj. 20, 1087–1091.

Euser, A.G., Cipolla, M.J., 2005. Resistance artery vasodilation to magnesium sulfate during pregnancy and the postpartum state. Am. J. Physiol. Heart Circ. Physiol. 288, 1521–1525.

Chi, O.Z., Hunter, C., Liu, X., Weiss, H.R., 2009. Effects of exogenous excitatory amino acid neurotransmitters on blood–brain barrier disruption in focal cerebral ischemia. Neurochem. Res. 34, 1249–1254.

Choi, S., Lim, G.l.J., Eo, Y., Kang, S., Han, J., Kim, B., Oh, B., Park, H., 2008. Development of animal model for cerebral ischemia. Korean J. Integra. Med. 3, 11–16.

Dennis, W.C., 1996. Ischemia-induced neuronal apoptosis. Curr. Opin. Neurobiol. 6, 667–672.

Gathwala, G., Khera, A., Singh, I., 2006. Magnesium therapy in birth asphyxia. Indian J. Pediatr. 73, 209–212.

Gee, J.B., Corbett, R.J.T., Perlman, J., Laptook, A.R., 2004. The effects of systemic magnesium sulfate infusion on brain magnesium concentrations and energy state during hypoxia–ischemia in newborn miniswine. Pediatr. Res. 55, 93–100.

Gormus, Z.I., Ergene, N., Toy, H., Baltaci, A.K., Mogulkoc, R., 2009. Preventive role of magnesium on skeletal muscle ischemia–reperfusion injury—an experimental study. Biol. Trace Elem. Res. 127, 183–189.

Kirino, T., 1989. Neuronal degeneration and glutamate. Rinsho Shinkeigaku 29, 1522–1525.

Kopal, C., Deveci, M., Oztürk, S., Sengezer, M., 2007. Effects of topical glutathione treatment in rat ischemic wound model. Ann. Plast. Surg. 58, 449–455.

Lee, G.J., Choi, S.K., Eo, Y.H., Kang, S.W., Choi, S., Park, J.H., Lim, J.E., Hong, K.W., Jin, H.S., Oh, B.S., Park, H.K., 2009a. The effect of extracellular glutamate release on repetitive transient ischemic injury in global ischemia model. Korean J. Physiol. Pharmacol. 13, 23–26.

Lee, G.J., Lim, J.E., Park, J.H., Choi, S.K., Hong, S., Park, H.K., 2009b. Neurotransmitter detection by enzyme-immobilized CNT-FET. Curr. Appl Phys. 9, S25–S28.

Lee, G.J., Park, J.H., Park, H.K., 2008. Microdialysis applications in neuroscience. Neurol. Res. 30, 661–668.

Lin, J.Y., Chung, S.Y., Lin, M.C., Cheng, F.C., 2002. Effects of magnesium sulfate on energy metabolites and glutamate in the cortex during focal cerebral ischemia and reperfusion in the gerbil monitored by a dual-probe microdialysis technique. Life Sci. 71, 803–811.

Lonnroth, P., Jansson, P.A., Smith, U., 1987. A microdialysis method allowing characterization of intercellular water space in humans. Am. J. Physiol. 253, E228–231.

Meloni, B.P., Campbell, K., Zhu, H.D., Knuckey, N.W., 2009. In search of clinical neuroprotection after brain ischemia the case for mild hypothermia (35 degrees C) and magnesium. Stroke 40, 2236–2240.

Ogawa, S., Kitao, Y., Hori, O., 2007. Ischemia-induced neuronal cell death and stress response. Antioxid. Redox Signal. 9, 573–587.

Ozdemir, M., Cengiz, S.L., Gürbilek, M., Oğün, T.C., Ustün, M.E., 2005. Effects of magnesium sulfate on spinal cord tissue lactate and malondialdehyde levels after spinal cord trauma. Magnes. Res. 18, 170–174.

Pazaiti, A., Soubasi, V., Spandou, E., Karkavelas, G., Georgiou, T., Karalis, P., Guiba-Tziampiri, O., 2009. Evaluation of long-lasting sensorimotor consequences following neonatal hypoxic–ischemic brain injury in rats: the neuroprotective role of $MgSO_4$. Neonatology 95, 33–40.

Rabinstein, A.A., Lanzino, G., Wijdicks, E.F., 2010. Multidisciplinary management and emerging therapeutic strategies in aneurysmal subarachnoid haemorrhage. Lancet Neurol. 9, 504–519.

Rogers, D.T., Colon, M., Gambala, C., Wilkins, I., Hibbard, J.U., 2010. Effects of magnesium on central arterial compliance in preeclampsia. Am. J. Obstet. Gynecol. 202, 448.

Park, E., Lee, G.J., Choi, S., Choi, S.K., Chae, S.J., Kang, S.W., Park, H.K., 2010. Correlation between extracellular glutamate release and neuronal cell death in an eleven vessel occlusion model in rat. Brain Res. 1342, 160–166.

Poleszak, E., Wlaz, P., Wrobel, A., Fidecka, S., Nowak, G., 2008. NMDA/glutamate mechanism of magnesium-induced anxiolytic-like behavior in mice. Pharmacol. Rep. 60, 655–663.

Pulsinelli, W.A., Brierley, J.B., 1979. A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 10, 267–272.

Sugio, K., Horigome, N., Sakaguchi, T., Goto, M., 1988. A model of bilateral hemispheric ischemia-modified four-vessel occlusion in rats. Stroke 19, 922.

Taghibiglou, C., Martin, H.G., Lai, T.W., Cho, T., Prasad, S., Kojic, L., Lu, J., Liu, Y., Lo, E., Zhang, S., Wu, J.Z., Li, Y.P., Wen, Y.H., Imm, J.H., Cynader, M.S., Wang, Y.T., 2009. Role of NMDA receptor-dependent activation of SREBP1 in excitotoxic and ischemic neuronal injuries. Nat. Med. 15, 1399–1406.

Takagi, N., 2009. Pathology and strategies for the treatment of ischemic brain injury. Yakugaku Zasshi-Journal of the Pharmaceutical Society of Japan 129, 1215–1219.

Takamatsu, H., Tsukada, H., Kakiuchi, T., Nishiyama, S., Noda, A., Umemura, K., 2000. Detection of reperfusion injury using PET in a monkey model of cerebral ischemia. J. Nucl. Med. 41, 1409–1416.

Taoufik, E., Probert, L., 2008. Ischemic neuronal damage. Curr. Pharm. Des. 14, 3565–3573.

Todd, N.V., Picozzi, P., Crockard, H.A., Ross, Russell, R.W., 1986. Recirculation after cerebral ischemia. Simultaneous measurement of cerebral bloodflow, brain edema, cerebrovascular permeability and cortical EEG in the rat Acta Neurol. Scand. 74, 269–278.

Yao, T., Nanjyo, Y., Nishino, H., 2001. Micro-flow in vivo analysis of L-glutamate with an on-line enzyme amplifier based on substrate recycling. Anal. Sci. 17, 703–708.

Zhao, H., Asai, S., Kohno, T., Ishikawa, K., 1998. Effects of brain temperature on CBF thresholds for extracellular glutamate release and reuptake in the striatum in a rat model of graded global ischemia. NeuroReport 9, 3183–3188.