Novel antiepileptic drug lacosamide exerts neuroprotective effects by decreasing glial activation in the hippocampus of a gerbil model of ischemic stroke

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Abstract. Lacosamide, which is a novel antiepileptic drug, has been reported to exert various additional therapeutic effects. The present study investigated the neuroprotective effects of lacosamide against transient cerebral ischemia-induced neuronal cell damage in the hippocampal cornu ammonis (CA)-1 region of a gerbil model. Neuronal Nuclei immunohistochemistry demonstrated that pre- and post-surgical treatment (5 min ischemia) with 25 mg/kg lacosamide protected CA1 pyramidal neurons in the lacosamide-treated-ischemia-operated group from ischemic injury 5 days post-ischemia, as compared with gerbils in the vehicle-treated-ischemia-operated group. Furthermore, treatment with 25 mg/kg lacosamide markedly attenuated the activation of astrocytes and microglia in the ischemic CA1 region at 5 days post-ischemia. The results of the present study suggested that pre- and post-surgical treatment of the gerbils with lacosamide was able to protect against transient cerebral ischemic injury-induced CA1 pyramidal neuronal cell death in the hippocampus. In addition, the neuroprotective effects of lacosamide may be associated with decreased activation of glial cells in the ischemic CA1 region.

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Introduction

Acute ischemic stroke has been recognized as the third leading cause of mortality (1). *In vivo* and *in vitro* models of ischemic stroke have previously been used to investigate the mechanisms underlying ischemic damage, and to analyze the effectiveness of various therapeutic compounds for treating ischemic stroke (2-6). Experimental transient cerebral ischemia, which is typically induced by middle cerebral artery occlusion in rats/mice, and bilateral common carotid artery occlusion in gerbils, has previously been associated with selective neuronal cell damage/death in vulnerable regions of the brain, including the neocortex and hippocampus (7,8). In addition, experimental transient cerebral ischemia-induced neuronal cell damage has been associated with free radical-associated damage and oxidative stress (7,9,10). Therefore, regulating oxidative stress levels and the production of antioxidants may be considered a potential strategy in the prevention and treatment of transient cerebral ischemia-induced neuronal cell damage/death (11,12).

Antiepileptic drugs act on one or more target molecules in the brain, including ion channels, neurotransmitter

transporters and neurotransmitter metabolic enzymes, which are involved in major physiological processes, and learning and memory functions (13,14). Lacosamide (R-2-acetamido-N-benzyl-3-methoxypropionamide), which was formerly known as harkoseride, belongs to a group of functionalized amino acids and is a novel antiepileptic drug (15,16). Lacosamide was initially shown to exert activity in various animal models of epilepsy (17,18), and has since been evaluated in phase III clinical trials for the treatment of human patients with epilepsy (19). As well as analgesic properties, lacosamide has demonstrated therapeutic potential in experimental animal models of neuropathic and inflammatory pain (20-22). Furthermore, lacosamide has been reported to alleviate neuropathic pain-like behaviors in the central nervous system of a rat model of spinal cord injury (16). It has previously been suggested that antiepileptic drugs may affect various signaling pathways associated with neuronal plasticity and survival, thus suggesting a potential application for antiepileptic drugs in the treatment of nonepileptic conditions (23).

Various drugs have been reported to show efficacy in the treatment of symptoms other than what they were designed for. Therefore, broadening the activity profile of existing clinical drugs has emerged as a novel strategy in the drug developmental process. In our previous study, we demonstrated that the antipsychotic drug, risperidone, was able to exert neuroprotective effects against transient cerebral ischemia-induced neuronal cell death (12). To the best of our knowledge, there is no study regarding the effects of lacosamide on experimentally-induced ischemic stroke; therefore, the present study aimed to analyze the protective effects of lacosamide against neuronal cell damage/death in the hippocampus of a gerbil model of transient cerebral ischemia. Gerbils have previously demonstrated efficacy for investigating the effects of transient cerebral ischemia (24-26).

Materials and methods

Experimental animals. A total number of 42 male Mongolian Gerbils (age, 6 months; weight, 65-75 g) were obtained from the Experimental Animal Center of Kangwon National University (Chuncheon, South Korea) and maintained in a controlled environment (temperature, 23˚C; humidity, 60%), under a 12 h light/12 h dark cycle, with *ad libitum* access to food and water throughout the experimental period. All experimental procedures were conducted in accordance with the National Institutes of Health guidelines for the Care And Use of Laboratory Animals (27), and the experimental protocol was approved by the Institutional Animal Care and Use Committee of Kangwon National University (Chuncheon, South Korea; approval no. KW-130424-1). All of the experiments were designed to minimize the number of gerbils used and their suffering.

Induction of transient cerebral ischemia. The gerbils were anesthetized using a mixture of 2.5% isoflurane (Baxter, Deerfield, IL, USA), 33% oxygen and 67% nitrous oxide. The bilateral common carotid arteries were isolated and occluded using non-traumatic aneurysm clips, after which gauze soaked in 0.9% saline was placed onto the necks of the gerbils in order to prevent dehydration of the arteries and the surrounding tissue. Complete interruption of blood flow was confirmed by observing the central artery in the retinae of the gerbils using an ophthalmoscope. Following occlusion for 5 min, the aneurysm clips were removed from the common carotid arteries. The body temperatures under normothermic conditions (37±0.5˚C) were monitored using rectal temperature probes (TR-100; Fine Science Tools, Foster City, CA, USA), and were maintained using a thermometric blanket prior to, during and following the surgery, until the gerbils recovered from the anesthesia. Thereafter, animals were maintained in a thermal incubator (Mirae Medical Industry, Seoul, South Korea), prior to sacrifice. The sham-operated gerbils were subjected to an identical surgical protocol; however the common carotid arteries were not occluded.

Treatment with lacosamide. In order to investigate the protective effects of lacosamide against ischemic damage 5 days post-surgery, the gerbils were distributed into the following groups (n=7 at each time in each group): i) Vehicle (saline)-treated-sham-operated-group (sham-group); ii) vehicle-treated-ischemia-operated-group (ischemia-group); iii) 10 mg/kg lacosamide-pretreated-ischemia-operated-group; iv) 25 mg/kg lacosamide-pretreated-ischemia-operated-group; v) 10 mg/kg lacosamide-posttreated-ischemia-operated-group; and vi) 25 mg/kg lacosamide-posttreated-ischemia-operated-group. Lacosamide (UCB Pharma SA, Brussels, Belgium) was dissolved in saline and intraperitoneally administered to the gerbils once daily for 3 days prior to or following the ischemic surgery. The final gerbil to be treated in the lacosamide-pretreated-ischemia-groups, and the first in the lacosamide-posttreated-ischemia-groups, were treated at 30 min prior to and following surgery, respectively.

Spontaneous motor activity (SMA) analysis. In order to investigate the effects of lacosamide on ischemia-induced hyperactivity, the SMA of the gerbils was measured 1 day following ischemia-reperfusion. The gerbils, which were not exposed to the open field prior to ischemia, were individually placed in a Plexiglas cage (25x20x12 cm) located within a soundproof chamber. The locomotor activity was recorded using the Photobeam Activity System-Home Cage (San Diego Instruments, San Diego, CA, USA), according to our previous study, with modifications (28). The cage was fitted with two parallel horizontal infrared beams 2 cm off the floor. Movement was detected via the interruption of an array of 32 infrared beams produced by photocells. The SMA of all the gerbils was monitored simultaneously for 60 min, and locomotor activity data were acquired using an AMB analyzer (IPC Electronics, Cumbria, UK). Data collection was initiated 15 min following habituation in the Plexiglas cage. The results were evaluated in terms of the distance (meters) traveled in the 60 min test period.

Tissue processing for histology. For histological analysis, the gerbils were anesthetized using sodium pentobarbital (30 mg/kg; JW Pharm. Co., Ltd., Seoul, South Korea), after which they were treated with 0.1 M phosphate-buffered saline (PBS; pH 7.4), and then 4% paraformaldehyde in 0.1 M phosphate-buffer (pH 7.4), via a transcardial perfusion. Subsequently, the brains were removed and postfixed in paraformaldehyde for 6 h, after which the brain tissues were cryoprotected via infiltration with 30% sucrose overnight. Thereafter, frozen tissues were

serially sectioned using a cryostat (Leica Microsystems GmbH, Wetzlar, Germany) into 30 μm coronal sections, which were subsequently distributed into 6-well plates containing PBS.

Staining with Neuronal Nuclei (NeuN). In order to examine the neuronal damage in the hippocampus following ischemia-reperfusion, NeuN (a marker for neurons) immunohistochemistry was conducted, as outlined in previous studies (29,30). Briefly, the tissue sections were treated with 0.3% hydrogen peroxide in PBS for 30 min, followed by 10% normal horse or normal rabbit serum (Vector Laboratories, Inc., Burlingame, CA, USA) in 0.05 M PBS for 30 min. Subsequently, the tissue sections were incubated with diluted mouse anti-NeuN (a neuron-specific soluble nuclear antigen; cat. no., MAB377; dilution, 1:1,000; Chemicon International, Inc., Temecula, CA, USA) overnight at 4˚C. Thereafter, the tissues were exposed to streptavidin peroxidase-conjugated biotinylated goat anti-mouse immunoglobulin G (cat. no., BA-9200; dilution, 1:250; Vector Laboratories, Inc.), after which they were visualized using 3,3'-diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M Tris HCl buffer, and mounted onto gelatin-coated slides. Following dehydration, the tissue sections were mounted with Canada balsam (Kato Chemical, Co., Tokyo, Japan).

In order to quantitatively analyze NeuN immunoreactivity, digital images of the hippocampus were captured using an AxioM1 light microscope (Carl Zeiss AG, Oberkochen, Germany), equipped with a digital camera (Axiocam; Carl Zeiss AG) connected to a PC monitor. NeuN immunoreactive neurons were counted in a 250x250 μm square applied at the approximate center of the cornu ammonis (CA)-1 region using the Optimas 6.5 Image Analyzing software (CyberMetrics, Co., Scottsdale, AZ, USA). The studied tissue sections were selected with 300 μm intervals, according to anatomical landmarks corresponding to AP -1.4 to -1.9 mm of the gerbil brain atlas (31). Cell counts were obtained by averaging the counts from each gerbil.

Immunohistochemical glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule-1 (Iba-1) staining. In order to investigate glial activation and oxidative stress in the ischemic hippocampus of the gerbils following transient cerebral ischemia, immunohistochemical staining using rabbit anti-GFAP (cat. no., AB5804; dilution, 1:1,000; Chemicon International, Inc.) for astrocytes and rabbit anti-Iba (cat. no., 019-19741; dilution, 1:1,000, Wako Pure Chemical Industries, Ltd., Osaka, Japan) for microglia, was conducted, as outlined previously (32). In addition, a negative control test was conducted using pre-immune serum as a substitute for primary antibody, in order to confirm the specificity of the immunostaining procedure. The negative control test resulted in the absence of immunoreactivity in all structures.

A total of 15 sections per gerbil were selected to quantitatively analyze GFAP and Iba-1 immunoreactivity. GFAP and Iba-1 immunoreactivity were graded as follows: Digital images of the hippocampal CA1 region were captured using an AxioM1 light microscope (Carl Zeiss AG), equipped with a digital camera (Axiocam; Carl Zeiss AG) connected to a PC monitor. Semi-quantification of the immunostaining intensities of GFAP and Iba-1 was conducted using MetaMorph 4.01 digital image analysis software (Universal Imaging, Bedford Hills, NY, USA). The mean intensity of GFAP and Iba-1 immunostaining in each immunoreactive structure was measured using a 0-255 gray scale system (white to dark signal corresponded to 255 to 0). Using this approach, the level of immunoreactivity was scaled as one of the following: -, ±, + or ++, representing no staining (gray scale value, ≥200), weakly positive (gray scale value, 150–199), moderately positive (gray scale value, 100–149), or strongly positive (gray scale value, ≤99) staining, respectively.

Statistical analysis. The data are presented as the mean ± standard error of the mean. Statistical differences were analyzed using one-way analysis of variance, followed by post-hoc Bonferroni's multiple comparison test with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

SMA analysis. The SMA of the gerbils was examined 1 day following ischemia-reperfusion. The SMA of the gerbils in the ischemia-group was significantly increased 1 day following ischemia-reperfusion, as compared with the sham-group gerbils ($P<0.05$; Fig. 1). Furthermore, the SMA of the gerbils in the lacosamide-pretreated-ischemia-groups (10 and 25 mg/kg) was significantly decreased, as compared with the ischemia-group gerbils, although the reduction in SMA was more significant for the gerbils pretreated with 25 mg/kg lacosamide, as compared with the 10 mg/kg lacosamide pretreated group ($P<0.05$; Fig. 1). In addition, the alterations in the SMA of the lacosamide-posttreated-ischemia-group gerbils were consistent with those observed for the lacosamide-pretreated-ischemia-groups.

Neuroprotective effects. NeuN-positive neurons were predominantly observed in the stratum pyramidale (SP) of the hippocampal CA1 region of the sham-group gerbils (Fig. 2A and B), whereas very few NeuN-positive neurons were detected in the SP of the ischemia-group gerbils (~8% of the sham-group; Fig. 2C and D), 5 days following ischemia-reperfusion.

The mean number of NeuN-positive neurons was significantly increased (~60% of the sham-group) in the 10 mg/kg lacosamide-pretreated-ischemia-group, as compared with the ischemia-group ($P<0.05$; Fig. 2E and F). Conversely, the mean number of NeuN-positive neurons in the SP of the 25 mg/kg lacosamide-pretreated-ischemia-group resembled that in the sham-group ($P>0.05$; Fig. 2G, H and M).

The mean number of NeuN-positive neurons in the 10 mg/kg lacosamide-posttreated-ischemia-group resembled (~9% of the sham-group) that in the ischemia-group ($P>0.05$; Fig. 2I, J and M). Conversely, in the 25 mg/kg lacosamide-posttreated-ischemia-group, a high number of NeuN-positive neurons (~75% of the sham-group) was detected in the SP 5 days following ischemia-reperfusion ($P>0.05$; Fig. 2K-M).

Glial activation

Astrocytes. GFAP-immunoreactive astrocytes in the sham-group gerbils were at resting potential, corresponding to a small body with thread-like processes, and were detected in all the layers of the CA1 hippocampal region (Fig. 3Aa). Conversely,

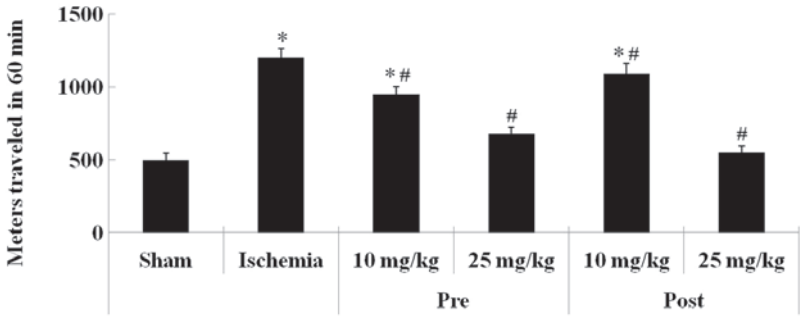

Figure 1. SMA 1 day following ischemia-reperfusion in the vehicle-sham-, vehicle-ischemia-, and lacosamide-treated-ischemia-groups. SMA was significantly decreased in the 25 mg/kg lacosamide-treated-ischemia-groups (n=7/group). Data are presented as the mean ± standard error of the mean. *P<0.05, vs. the vehicle-sham-group; #P<0.05, vs. the vehicle-ischemia-group. SMA, spontaneous motor activity.

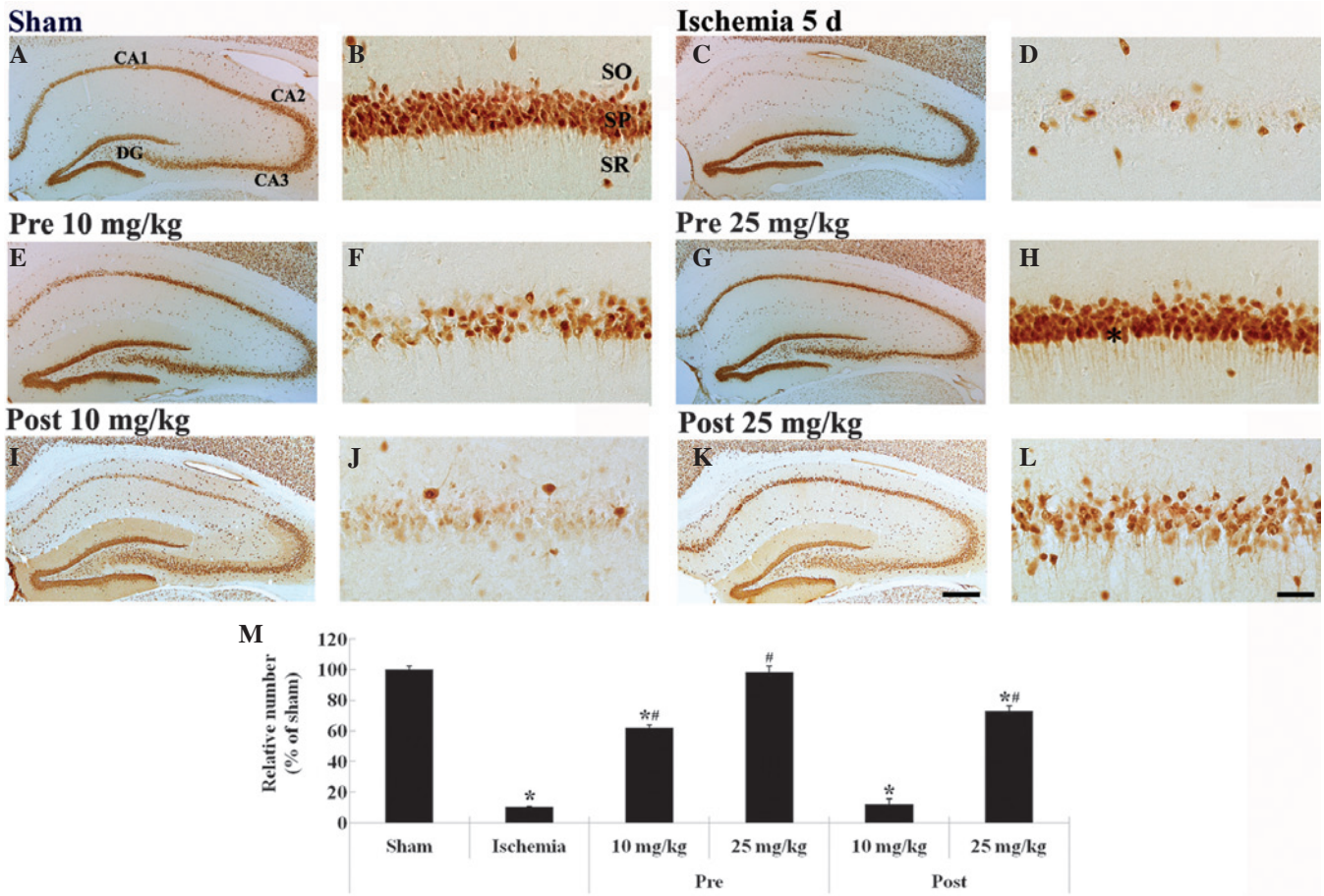

Figure 2. NeuN immunohistochemistry in the hippocampus of the (A and B) sham-, (C and D) ischemia-, (E-H) lacosamide-pretreated-ischemia- and (I-L) lacosamide-posttreated-ischemia-groups (n=7 gerbils/group) 5 days following ischemia-reperfusion. In the 25 mg/kg lacosamide-pretreated-ischemia-group, the distribution pattern of NeuN-positive neurons in the SP (asterisk) resembled that in the sham-group (Scale bar=400 μm for A, C, E, G, I and K; scale bar=50 μm for B, D, F, H, J and L). (M) Relative percentage of NeuN-immunoreactive neurons in the hippocampal CA1 region of the various groups. Data are presented as the mean ± standard error of the mean. *P<0.05, vs. the sham-group; #P<0.05, vs. the ischemia-group. NeuN, neuronal nuclei; CA, cornu ammonis; DG, dentate gyrus; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum.

GFAP-immunoreactive astrocytes in the ischemia-group were activated, and contained a bulky cytoplasm. Furthermore, GFAP immunoreactivity was markedly increased in all layers of the CA1 region of the ischemia-group rats (Table I and Fig. 3Ab).

GFAP-immunoreactive astrocytes were activated in the 10 mg/kg lacosamide-pretreated-ischemia-group gerbils, although to a lesser extent than in the ischemia-group gerbils (Table I and Fig. 3Ac). Conversely, GFAP-immunoreactive astrocytes were not markedly activated in the 25 mg/kg lacosamide-pretreated-ischemia-group, as compared with in the 10 mg/kg lacosamide-pretreated-ischemia-group (Table I and Fig. 3Ad). The morphology of GFAP-immunoreactive astrocytes in the 10 mg/kg lacosamide-posttreated-ischemia-group resembled those in the ischemia-group (Table I and Fig. 3Ae), whereas GFAP-immunoreactive astrocytes in the 25 mg/kg

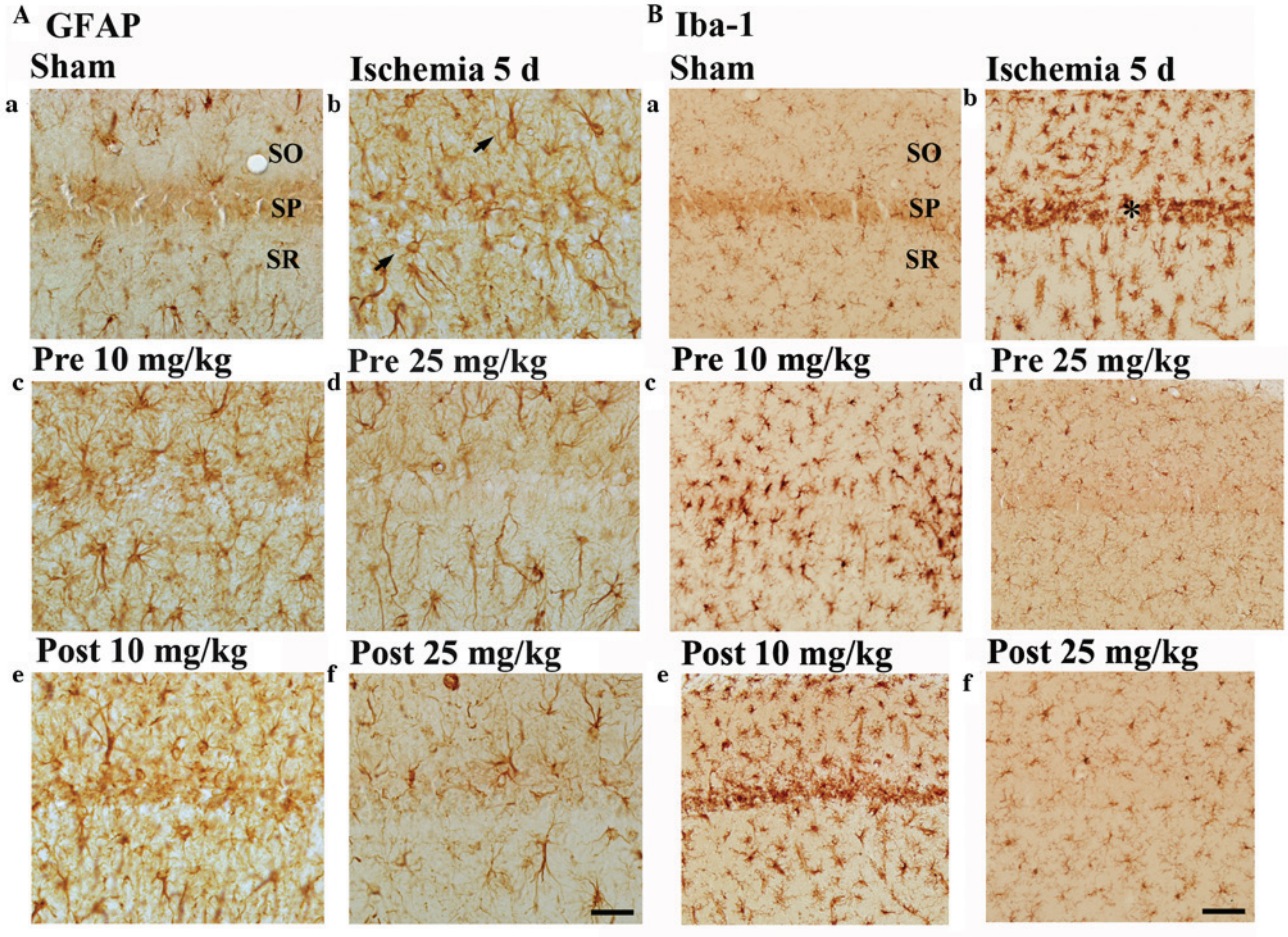

Figure 3. (A) GFAP immunohistochemistry in the CA1 region of the (a) sham-, (b) ischemia-, (c and d) lacosamide-pretreated-ischemia- and (e and f) lacosamide-posttreated-ischemia- groups 5 days following ischemia-reperfusion. GFAP-immunoreactive astrocytes (arrows) were markedly activated in the ischemia-group gerbils. In the 10 mg/kg lacosamide-treated-ischemia-groups, the distribution pattern of GFAP-immunoreactive astrocytes resembled that in the ischemia-group. Conversely, in the 25 mg/kg lacosamide-treated-ischemia-groups, the activation of GFAP-immunoreactive astrocytes was markedly reduced, as compared with that in the ischemia group. (B) Iba-1 immunohistochemistry in the CA1 region of the (a) sham-, (b) ischemia-, (c and d) lacosamide-pretreated-ischemia- and (e and f) lacosamide-posttreated-ischemia- groups 5 days following ischemia-reperfusion. In the ischemia-group, Iba-1-immunoreactive microglia in the CA1 region were activated and aggregated in the SP (asterisk). In the 25 mg/kg lacosamide-treated-ischemia-groups, the pattern of Iba-1-immunoreactive microglia resembled that in the sham group. Scale bar=50 μm. CA, cornu ammonis; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium-binding adapter molecule.

lacosamide-posttreated-ischemia-group were not markedly activated, as compared with the 10 mg/kg lacosamide-posttreated-ischemia-group (Table I and Fig. 3Af).

Microglia. Iba-1-immunoreactive microglia were distributed throughout all layers of the CA1 region of the sham-group gerbils, and were observed in a rest form that was ramified in appearance (Fig. 3Ba). Conversely, the Iba-1-immunoreactive microglia in the ischemia-group were activated (hypertrophied in appearance) and aggregated in the SP layer of the CA1 region. In addition, the Iba-1 immunoreactivity of the ischemia-group microglia was markedly increased (Table II and Fig. 3Bb), as compared with the sham-group.

Iba-1-immunoreactive microglia in the 10 mg/kg lacosamide-pretreated-ischemia-group were activated and exhibited strongly positive Iba-1 immunoreactivity; however, they did not appear to be aggregated in the SP layer of the CA1 region (Table II and Fig. 3Bc). Conversely, the distribution pattern of Iba-1 immunoreactive microglia in the 25 mg/kg lacosamide-pretreated-ischemia-group resembled that in the sham-group (Table II and Fig. 3Bd). Iba-1-immunoreactive microglia in the 10 mg/kg lacosamide-posttreated-ischemia-group were aggregated in the SP, similar to those in the ischemia-group (Table II and Fig. 3Be); whereas, the distribution pattern of Iba-1-immunoreactive microglia in the 25 mg/kg lacosamide-posttreated-ischemia-group resembled that in the sham-group, although their Iba-1-immunoreactivity was increased (Table II and Fig. 3Df), as compared with the sham group.

Discussion

The results of the present study suggested that lacosamide may exert additional beneficial therapeutic effects beyond its use as an antiepileptic drug. Lacosamide effectively protected against ischemia-induced neuronal cell damage in the hippocampal CA1 region of a gerbil model of transient cerebral ischemia. Pretreatment of the gerbils with 10 or 25 mg/kg lacosamide protected against ischemia-induced neuronal cell

Table I. Semi-quantification of the immunostaining intensity of GFAP in the CA1 region of the sham-, ischemia- and lacosamide-treated-groups, 5 days following ischemia-reperfusion.

Group	CA1 subregion	Immunostaining intensity
Sham	SO	+
	SP	±
	SR	+
Ischemia	SO	++
	SP	+++
	SR	++
Pretreatment		
10 mg/kg	SO	++
	SP	+
	SR	++
25 mg/kg	SO	+
	SP	±
	SR	+
Posttreatment		
10 mg/kg	SO	++
	SP	++
	SR	++
25 mg/kg	SO	++
	SP	+
	SR	++

The intensity of immunostaining is scaled as −, ±, +, or ++, representing no staining or weakly, moderately, or strongly positive staining, respectively. GFAP, glial fibrillary acidic protein; CA, cornu ammonis; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum.

Table II. Semi-quantification of the immunostaining intensity of Iba-1 in the CA1 region of the sham-, ischemia- and lacosamide-treated groups, 5 days following ischemia-reperfusion.

Group	CA1 subregion	Immunostaining intensity
Sham	SO	±
	SP	±
	SR	±
Ischemia	SO	++
	SP	++
	SR	++
Pre-treatment		
10 mg/kg	SO	++
	SP	+
	SR	++
25 mg/kg	SO	±
	SP	±
	SR	±
Post-treatment		
10 mg/kg	SO	++
	SP	++
	SR	++
25 mg/kg	SO	+
	SP	±
	SR	+

The intensity of immunostaining is scaled as −, ±, +, or ++, representing no staining or weakly, moderately, or strongly positive staining, respectively. Iba-1, ionized calcium-binding adapter molecule-1; CA, cornu ammonis; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum.

damage, whereas posttreatment with 25 mg/kg lacosamide protected against ischemic damage in the gerbil hippocampal CA1 region 5 days following ischemia-reperfusion.

To the best of our knowledge, the present study is the first to investigate the protective effects of lacosamide against ischemia-induced neuronal cell damage/death; however, Licko *et al* (33) previously reported that the long-term treatment of a rat model of electrical status epilepticus with lacosamide attenuated neuronal cell loss and alterations in hippocampal neurogenesis. The present study demonstrated that neuroprotective strategies may be effective in the treatment of patients with ischemic insults, and found that novel antiepileptic drugs may exert additional activities that protect against ischemia-induced neuronal cell damage.

In the present study, treatment with lacosamide attenuated the ischemia-induced activation of astrocytes and microglia in the gerbil ischemic hippocampal CA1 region in a dose-dependent manner. Previous studies have detected an association between ischemic stroke and glial cells: Glial cells were activated by an ischemic stroke and were shown to be associated with ischemia-induced neuronal cell death via the release of inflammatory cytokines/mediators (34,35). Furthermore, the production of inflammatory cytokines/mediators has previously been associated with inflammation and neuropathic pain (36,37), and lacosamide has been shown to effectively inhibit pain, with minor adverse side effects, in an animal model of inflammation and neuropathic pain (33). In addition, lacosamide demonstrated antihyperalgesic activity in a rat model of tumor necrosis factor-α-induced muscle pain (38). Furthermore, lacosamide has been reported to decrease the activation of microglia and upregulation of glial migration factors, and delayed the downregulation of an interleukin-6 cytokine receptor subunit (39). Therefore, in the present study, the attenuation of ischemia-induced microglial activation in the hippocampal CA1 region of lacosamide-treated gerbils may have been associated with the neuroprotective effects of lacosamide against transient cerebral ischemic damage.

In conclusion, the results of the present study suggested that lacosamide was able to protect against ischemia-induced

neuronal cell death/damage, and that this neuroprotective activity may have been associated with attenuation of glial cell activation.

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References

1. Singh J and Nguyen TN: Endovascular and neurosurgical management of acute ischemic stroke. Emerg Med Clin North Am 30: 695-712, 2012.
2. Domoráková I, Mechírová E, Danková M, Danielisová V and Burda J: Effect of antioxidant treatment in global ischemia and ischemic postconditioning in the rat hippocampus. Cell Mol Neurobiol 29: 837-844, 2009.
3. Huang X, Li Q, Li H and Guo L: Neuroprotective and antioxidative effect of cactus polysaccharides in vivo and in vitro. Cell Mol Neurobiol 29: 1211-1221, 2009.
4. Mousavi SH, Tayarani-Najaran Z, Asghari M and Sadeghnia HR: Protective effect of *Nigella sativa* extract and thymoquinone on serum/glucose deprivation-induced PC12 cells death. Cell Mol Neurobiol 30: 591-598, 2010.
5. Nagy D, Marosi M, Kis Z, Farkas T, Rakos G, Vecsei L, Teichberg VI and Toldi J: Oxaloacetate decreases the infarct size and attenuates the reduction in evoked responses after photothrombotic focal ischemia in the rat cortex. Cell Mol Neurobiol 29: 827-835, 2009.
6. Oliveira IJ, Molz S, Souza DO and Tasca CI: Neuroprotective effect of GMP in hippocampal slices submitted to an in vitro model of ischemia. Cell Mol Neurobiol 22: 335-344, 2002.
7. Kataoka T, Etani R, Takata Y, Nishiyama Y, Kawabe A, Kumashiro M, Taguchi T and Yamaoka K: Radon inhalation protects against transient global cerebral ischemic injury in gerbils. Inflammation 37: 1675-1682, 2014.
8. Sakai N, Yanai K, Ryu JH, Nagasawa H, Hasegawa T, Sasaki T, Kogure K and Watanabe T: Behavioral studies on rats with transient cerebral ischemia induced by occlusion of the middle cerebral artery. Behav Brain Res 77: 181-188, 1996.
9. Candelario-Jalil E, Alvarez D, Merino N and León OS: Delayed treatment with nimesulide reduces measures of oxidative stress following global ischemic brain injury in gerbils. Neurosci Res 47: 245-253, 2003.
10. Oztanir MN, Ciftci O, Cetin A and Aladag MA: Hesperidin attenuates oxidative and neuronal damage caused by global cerebral ischemia/reperfusion in a C57BL/J6 mouse model. Neurol Sci 35: 1393-1399, 2014.
11. Pei H, Cao D, Guo Z, Liu G, Guo Y and Lu C: Bone morphogenetic protein-7 ameliorates cerebral ischemia and reperfusion injury via inhibiting oxidative stress and neuronal apoptosis. Int J Mol Sci 14: 23441-23453, 2013.
12. Yan BC, Park JH, Ahn JH, Kim IH, Park OK, Lee JC, Yoo KY, Choi JH, Lee CH, Hwang IK, *et al*: Neuroprotection of post-treatment with risperidone, an atypical antipsychotic drug, in rat and gerbil models of ischemic stroke and the maintenance of antioxidants in a gerbil model of ischemic stroke. J Neurosci Res 92: 795-807, 2014.
13. Chen J, Cai F, Cao J, Zhang X and Li S: Long-term antiepileptic drug administration during early life inhibits hippocampal neurogenesis in the developing brain. J Neurosci Res 87: 2898-2907, 2009.
14. Rogawski MA and Löscher W: The neurobiology of antiepileptic drugs. Nat Rev Neurosci 5: 553-564, 2004.
15. Geis C, Beyreuther BK, Stöhr T and Sommer C: Lacosamide has protective disease modifying properties in experimental vincristine neuropathy. Neuropharmacology 61: 600-607, 2011.
16. Hao JX, Stöhr T, Selve N, Wiesenfeld-Hallin Z and Xu XJ: Lacosamide, a new anti-epileptic, alleviates neuropathic pain-like behaviors in rat models of spinal cord or trigeminal nerve injury. Eur J Pharmacol 553: 135-140, 2006.
17. Wasterlain CG, Stöhr T and Matagne A: The acute and chronic effects of the novel anticonvulsant lacosamide in an experimental model of status epilepticus. Epilepsy Res 94: 10-17, 2011.
18. Shandra A, Shandra P, Kaschenko O, Matagne A and Stöhr T: Synergism of lacosamide with established antiepileptic drugs in the 6-Hz seizure model in mice. Epilepsia 54: 1167-1175, 2013.
19. Hovinga CA: SPM-927 (Schwarz Pharma). IDrugs 6: 479-485, 2003.
20. Beyreuther B, Callizot N and Stöhr T: Antinociceptive efficacy of lacosamide in a rat model for painful diabetic neuropathy. Eur J Pharmacol 539: 64-70, 2006.
21. Beyreuther B, Callizot N and Stöhr T: Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. Arthritis Res Ther 9: R14, 2007.
22. Stöhr T, Krause E and Selve N: Lacosamide displays potent antinociceptive effects in animal models for inflammatory pain. Eur J Pain 10: 241-249, 2006.
23. Rogawski MA and Löscher W: The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. Nat Med 10: 685-692, 2004.
24. Liu YR, Lei RY, Wang CE, Zhang BA, Lu H, Zhu HC and Zhang GB: Effects of catalpol on ATPase and amino acids in gerbils with cerebral ischemia/reperfusion injury. Neurol Sci 35: 1229-1233, 2014.
25. Liu YR, Li PW, Suo JJ, Sun Y, Zhang BA, Lu H, Zhu HC and Zhang GB: Catalpol provides protective effects against cerebral ischaemia/reperfusion injury in gerbils. J Pharm Pharmacol 66: 1265-1270, 2014.
26. Wang W, Wang T, Feng WY, Wang ZY, Cheng MS and Wang YJ: Ecdysterone protects gerbil brain from temporal global cerebral ischemia/reperfusion injury via preventing neuron apoptosis and deactivating astrocytes and microglia cells. Neurosci Res 81-82: 21-29, 2014.
27. Institute of Laboratory Animal Research, Committee for the Update of the Guide for the Care and Use of Laboratory Animals, National Research Council. Guide For The Care And Use Of Laboratory Animals. 8th edition. National Academy Press, Washington DC, p220, 2011.
28. Yan BC, Choi JH, Yoo KY, Lee CH, Hwang IK, You SG, Kang IJ, Kim JD, Kim DJ, Kim YM and Won MH: Leptin's neuroprotective action in experimental transient ischemic damage of the gerbil hippocampus is linked to altered leptin receptor immunoreactivity. J Neurol Sci 303: 100-108, 2011.
29. Park JH, Joo HS, Yoo KY, Shin BN, Kim IH, Lee CH, Choi JH, Byun K, Lee B, Lim SS, *et al*: Extract from *Terminalia chebula* seeds protect against experimental ischemic neuronal damage via maintaining SODs and BDNF levels. Neurochem Res 36: 2043-2050, 2011.
30. Schmued LC and Hopkins KJ: Fluoro-Jade B: A high affinity fluorescent marker for the localization of neuronal degeneration. Brain Res 874: 123-130, 2000.
31. Loskota WJ, Lomax P and Verity MA (Eds.): A stereotaxic atlas of the Mongolian gerbil brain (*Meriones unguiculatus*). Ann Arbor Science, Ann Arbor, MI, pp70-81, 1974.
32. Park JH, Joo HS, Yoo KY, Shin BN, Kim IH, Lee CH, Choi JH, Byun K, Lee B, Lim SS, *et al*: Extract from *Terminalia chebula* seeds protect against experimental ischemic neuronal damage via maintaining SODs and BDNF levels. Neurochem Res 36: 2043-2050, 2011.
33. Licko T, Seeger N, Zellinger C, Russmann V, Matagne A and Potschka H: Lacosamide treatment following status epilepticus attenuates neuronal cell loss and alterations in hippocampal neurogenesis in a rat electrical status epilepticus model. Epilepsia 54: 1176-1185, 2013.
34. Bell MT, Puskas F, Agoston VA, Cleveland JC Jr, Freeman KA, Gamboni F, Herson PS, Meng X, Smith PD, Weyant MJ, *et al*: Toll-like receptor 4-dependent microglial activation mediates spinal cord ischemia-reperfusion injury. Circulation 128 (Suppl 1): S152-S156, 2013.

35. Roy Choudhury G, Ryou MG, Poteet E, Wen Y, He R, Sun F, Yuan F, Jin K and Yang SH: Involvement of p38 MAPK in reactive astrogliosis induced by ischemic stroke. Brain Res 1551: 45-58, 2014.
36. Day YJ, Liou JT, Lee CM, Lin YC, Mao CC, Chou AH, Liao CC and Lee HC: Lack of interleukin-17 leads to a modulated micro-environment and amelioration of mechanical hypersensitivity after peripheral nerve injury in mice. Pain 155: 1293-1302, 2014.
37. Yan X, Yadav R, Gao M and Weng HR: Interleukin-1 beta enhances endocytosis of glial glutamate transporters in the spinal dorsal horn through activating protein kinase C. Glia 62: 1093-1109, 2014.
38. Beyreuther BK, Geis C, Stöhr T and Sommer C: Antihyperalgesic efficacy of lacosamide in a rat model for muscle pain induced by TNF. Neuropharmacology 52: 1312-1317, 2007.
39. Wang B, Dawson H, Wang H, Kernagis D, Kolls BJ, Yao L and Laskowitz DT: Lacosamide improves outcome in a murine model of traumatic brain injury. Neurocrit Care 19: 125-134, 2013.