Inhibitory Effects of Isoquinoline Alkaloid Berberine on Ischemia-Induced Apoptosis via Activation of Phosphoinositide 3-Kinase/Protein Kinase B Signaling Pathway

Mia Kim, Mal Soon Shin, Jae Min Lee, Han Sam Cho, Chang Ju Kim, Young Joon Kim, Hey Ran Choi, Jung Won Jeon

Department of Cardiovascular and Neurologic Diseases (Stroke Center), College of Oriental Medicine, Kyung Hee University, Seoul;
Department of Physiology, Kyung Hee University School of Medicine, Seoul;
Department of Biochemistry and Molecular Biology, Kyung Hee University School of Medicine, Seoul;
Department of Internal Medicine, Kyung Hee University Hospital at Gangdong, Kyung Hee University School of Medicine, Seoul, Korea

Purpose: Berberine is a type of isoquinoline alkaloid that has been used to treat various diseases. A neuroprotective effect of berberine against cerebral ischemia has been reported; however, the effects of berberine on apoptosis in relation to reactive astrogliosis and microglia activation under ischemic conditions have not yet been fully evaluated. In the present study, we investigated the effects of berberine on global ischemia-induced apoptosis, and focused on the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway in the hippocampus using gerbils.

Methods: Gerbils received berberine orally once a day for 14 consecutive days, starting one day after surgery. In this study, a step-down avoidance task was used to assess short-term memory. Furthermore, we employed the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay to evaluate DNA fragmentation, immunohistochemistry to investigate glial fibrillary acidic protein, CD11b, and caspase-3, and western blot to assess PI3K, Akt, Bax, Bcl-2, and cytochrome c.

Results: Our results revealed that berberine treatment alleviated ischemia-induced short-term memory impairment. Treatment with berberine also attenuated ischemia-induced apoptosis and inhibited reactive astrogliosis and microglia activation. Furthermore, berberine enhanced phospho-PI3K and phospho-Akt expression in the hippocampus of ischemic gerbils.

Conclusions: Berberine exerted a neuroprotective effect against ischemic insult by inhibiting neuronal apoptosis via activation of the PI3K/Akt signaling pathway. The antiapoptotic effect of berberine was achieved through inhibition of reactive astrogliosis and microglia activation. Berberine may therefore serve as a therapeutic agent for stroke-induced neuourological problems.

Keywords: Berberine; Brain ischemia; Short-term memory; Apoptosis; Phosphatidylinositol 3-kinases

INTRODUCTION

Transient cerebral ischemia is induced by temporary deprivation of blood flow to the brain, and results in neuronal degeneration. Pyramidal neurons in the CA1 region are the most vulnerable cells to ischemic-reperfusion injury, and loss of hippocampal CA1 neurons causes long-term learning deficits [1].

Astrocytes are activated (reactive) in response to stroke, trauma, tumor growth, or neurodegenerative diseases. Astrocytic activation results in so-called "reactive astrogliosis," which is an inflammatory response characterized by astrocytic proliferation and hypertrophy following brain injury [2]. Gliial fibrillary acidic protein (GFAP) is the main constituent of intermediate astrocytic filaments. GFAP expression has been shown to increase after transient ischemic stroke in rats [3]. Moreover, alterations in GFAP immunoreactivity are associated with neuronal cell death in the ischemic CA1 region [4], and GFAP expression has been said to represent a hallmark of reactive astrogliosis [5]. Activated microglia readily undergo dramatic changes in morphology and the surface expression of molecules such as major...
In the present study, we investigated the effects of berberine on global ischemia-induced apoptosis focusing on the PI3K/Akt signaling pathway in the gerbil hippocampus. For this, a step-down avoidance task was employed to assess short-term memory, the TUNEL assay was used to evaluate DNA fragmentation, immunohistochemistry for GFAP, CD11b, and caspase-3, and western blot for the identification of PI3K, Akt, Bax, Bcl-2, and cytochrome c levels.

MATERIALS AND METHODS

Experimental Animals

Adult male Mongolian gerbils (13 weeks old) were used in this experiment. All experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health and the Korean Academy of Medical Sciences. Gerbils were housed under controlled temperature (20°C ± 2°C) and lighting (7 AM to 7 PM) conditions with food and water available ad libitum. The gerbils were randomly divided into five groups (n = 10 in each group): sham-operation group, ischemia-induction group, and ischemia-induction and 20 mg/kg berberine-treated group, ischemia-induction and 50 mg/kg berberine group, and ischemia-induction and 80 mg/kg berberine group. Gerbils in berberine-treated groups received berberine (Sigma Chemical Co., St. Louis, MO, USA) orally once a day for 14 consecutive days, starting one day after surgery. Gerbils in sham-operation and ischemia-induction groups received an equal amount of distilled water for the same duration.

Induction of Transient Global Ischemia

Transient global ischemia was induced as previously described [11]. Briefly, gerbils were anesthetized with Zoletil 50 (10 mg/kg, intraperitoneally; Vibac Laboratories, Carros, France), and following bilateral neck incisions, both common carotid arteries were exposed and occluded with aneurysm clips for 7 minutes. Clips were then removed to restore cerebral blood flow. Body and rectal temperature was maintained at 36°C ± 0.5°C during surgery using a Homeothermic Blanket Control Unit (Harvard Apparatus, Massachusetts, MA, USA) that enveloped the body and head. After recovery, animals were monitored for an additional 2 hours to prevent hypothermia. Animals in the sham-operation group were treated identically, except that the common carotid arteries were not occluded after neck incisions.

Step-Down Avoidance Task

In order to evaluate short-term memory, latency in the step-

Histocompatibility complex, which is recognized by the antibody to CD11b. CD11b is located on the plasma membrane of microglia, and CD11b expression is increased during the microglial activation [6]. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been used in animal models of Parkinson disease, and MPTP administration causes enhanced CD11b expression in the striatum and substantia nigra [7].

The phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway plays a central role in intracellular processes related to cell survival and proliferation. PI3K generates phosphatidylinositol triphosphate, which activates the serine/threonine kinase Akt. Akt then modulates cellular activation, the inflammatory response, and apoptosis [8]. Thus, it is not surprising that decreased Akt activity is involved in ischemia-induced cell death [9], while increased Akt activity is associated with neuroprotective effects in ischemic gerbils [10].

Apoptosis maintains homeostasis; however, inappropriate or excessive apoptosis has been implicated in several types of neurodegenerative disorders, including ischemia [7,11,12]. Apoptosis after cerebral ischemia is one of the major pathways that lead to the process of cell death [13]. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining is an assay that detects DNA fragmentation, a characteristic of apoptotic cell death [11]. One important characteristic of apoptosis is the activation of caspases. Caspase-3 is one of the most widely studied members of the caspase family, and is one of the key initiators of apoptosis [14]. In addition, Bax and Bcl-2 are major proteins that have been shown to regulate apoptosis. Specifically, Bcl-2 is an antiapoptotic protein whose expression protects against cell death, while Bax is a proapoptotic protein that it is expressed abundantly during apoptosis. Increasing the ratio of Bax to Bcl-2 has commonly been used to determine the induction of apoptosis in several tissues [12,15].

Alkaloids are found in bacteria, fungi, plants and animals, although their distribution within each kingdom is quite limited. Despite their structural diversity, alkaloids share many physical and chemical properties. Berberine is a type of isoquinoline alkaloid extracted from Berberis vulgaris L, and is used to treat various diseases, including tumors, diabetes mellitus, cardiovascular deregulation, immunology disorders, neurodegeneration, and neuropsychiatric disorders [16,17]. The neuroprotective effect of berberine against cerebral ischemia has been reported [18,19]; however, the effect of berberine on apoptosis under ischemic conditions is not clear.

In the present study, we investigated the effects of berberine...
down avoidance task was determined, as per the previously described method [20]. Fourteen days after surgery, gerbils were trained in a step-down avoidance task. Rats were positioned on a 7 × 25-cm platform with a height of 2.5 cm, and then allowed to rest on the platform for 2 minutes. The platform faced a 42 × 25-cm grid of parallel 0.1-cm caliber stainless steel bars, which were spaced 1 cm apart. In the training sessions, animals received a 0.5-mA scramble foot shock for 3 seconds immediately upon stepping down. Retention time was assessed 15 days after surgery. The interval for rats stepping down and placing all four paws on the grid was defined as the latency. A latency over 300 seconds was counted as 300 seconds.

**Tissue Preparation**

For brain tissue preparation, animals were fully anesthetized with Zoletil 50, transcardially perfused with 50mM phosphate-buffered saline (PBS), and fixed with freshly prepared solution consisting of 4% paraformaldehyde in 100mM phosphate buffer (PB, pH 7.4). Brains were then removed, postfixed in the same fixative overnight, and transferred into a 30% sucrose solution in 0.05M PBS for approximately 5 minutes and was then washed with PBS and mounted onto gelatin-coated slides. Sections were mounted onto gelatin-coated slides. Slides were then air-dried overnight at room temperature, and coverslips were mounted using Permount (Thermo Fisher Scientific Inc., Fair Lawn, NJ, USA).

**Immunohistochemistry**

Immunohistochemistry was performed according to the previously described method [21,22]. Free-floating tissue sections were first incubated in 3% H$_2$O$_2$ for 30 minutes to block endogenous peroxidase activity. Sections were then incubated in blocking solution (1% bovine serum albumin and 10% horse serum in 0.05M PBS) for 2 hours at room temperature, and were incubated overnight with antimouse GFAP antibody (1:1,000; Cell Signaling Technology, Beverly, MA, USA), antirat CD11b antibody (1:500; Serotec, Raleigh, NC, USA), and antimouse caspase-3 (1:500, Santa Cruz Biotechnology, Dallas, TX, USA) at 4°C. The next day, sections were incubated for 1 hour with biotinylated secondary antibody (1:200; Vector Laboratories, Burlingame, CA, USA) at room temperature, and were subsequently incubated with a Vector Elite ABC kit (Vector Laboratories) for 1 hour at room temperature. The antibody–biotin–avidin–peroxidase complex was visualized using 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB) and 0.03% H$_2$O$_2$ in 50mM Tris-buffer (pH 7.6) for approximately 5 minutes and was then washed with PBS and mounted onto gelatin-coated slides. Sections were mounted onto gelatinized glass slides, air dried, and cover slides were mounted using Permount (Thermo Fisher Scientific Inc., Fair Lawn, NJ, USA).

**TUNEL Staining**

To visualize DNA fragmentation, TUNEL staining was performed using an *In Situ* Cell Death Detection Kit (Roche, Mannheim, Germany), according to a previously described method [23]. Sections were postfixed in ethanol-acetic acid (2:1) and rinsed. The sections were then incubated with proteinase K (100 µg/mL), rinsed, and incubated in 3% H$_2$O$_2$, permeabilized with 0.5% Triton X-100, rinsed again, and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using Converter-POD with 0.03% DAB. Mayer’s hematoxylin (DAKO, Glostrup, Denmark) was used as a counterstain, and sections were mounted onto gelatin-coated slides. Slides were then air-dried overnight at room temperature, and coverslips were mounted using Permount (Thermo Fisher Scientific Inc.).

**Western Blot Analysis**

Western blot was performed according to previously described methods [21]. Tissue samples harvested from the hippocampus were lysed in protein lysis buffer containing 50mM Tris-HCl (pH 7.5), 150mM NaCl, 0.5% deoxycholic acid, 1% nonidet-P40 (NP40), 0.1% sodium dodecyl sulfate (SDS), 1mM phenylmethylsulfonyl fluoride, and 100 µm/mL leupeptin. Protein concentration was measured using a colorimetric protein assay kit (Bio-Rad, Hercules, CA, USA). Proteins of 40 µg were separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). The membranes were incubated with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20, and then incubated overnight at 4°C with the following primary antibodies: antimouse β-actin, antimouse Bcl-2, antimouse Bax, antimouse PI3K, antirabbit phospho-PI3K (p-PI3K), antimonouse Akt, and antimouse phospho-Akt (p-Akt) (1:1,000; Santa Cruz Biotechnology). Subsequently, the membranes were incubated for 1 hour with secondary antibodies (1:2,000; Vector Laboratories), and band detection was performed using the enhanced chemiluminescence detection kit (Santa Cruz Biotechnology). The bands were quantified using an Image-Pro Plus computer-assisted image analysis system (Media Cyberbetics Inc., Silver Spring, MD, USA).

**Data Analysis**

To compare the relative expression of bands, densitometric analysis was performed using Molecular Analyst ver. 1.4.1 (Bio-
Rad). Cell counting and optical density measurements were performed using Image-Pro Plus computer-assisted image analysis system attached to a light microscope (Olympus Co., Tokyo, Japan).

All data were analyzed using IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA), and data are expressed as the mean ± standard error of the mean. For comparisons among groups, a one-way analysis of variance and Duncan post hoc test were performed with P < 0.05 as an indication of statistical significance.

RESULTS

Effects of Berberine on Short-Term Memory

The results of step-down avoidance task are presented in Fig. 1. Latency was 149.3 ± 10.37 seconds in the sham-operation group, 11.50 ± 3.16 seconds in the ischemia-induction group, 63.13 ± 20.13 seconds in the ischemia-induction and 20 mg/kg berberine-treated group, 61.63 ± 2.16 seconds in the ischemia-induction and 50 mg/kg berberine-treated group, and 114.00 ± 17.71 seconds in the ischemia-induction and 80 mg/kg berberine-treated group. The present results showed that short-term memory was deteriorated with the induction of ischemia (P < 0.05), but that treatment with berberine alleviated ischemia-induced short-term memory impairment in the ischemic gerbils (P < 0.05).

Effects of Berberine on GFAP and CD11b Expression in the Hippocampal CA1 Region

GFAP and CD11b expression in the hippocampal CA1 region is presented in Fig. 2. When the intensity level of GFAP in the sham-operation group was set at 1.00, GFAP was 1.70 ± 0.05 in the ischemia-induction group, 1.77 ± 0.07 in the ischemia-induction and 20 mg/kg berberine-treated group, 1.27 ± 0.08 in the ischemia-induction and 50 mg/kg berberine-treated group, and 1.11 ± 0.08 in the ischemia-induction and 80 mg/kg berberine-treated group. The present results showed that expression of GFAP in the hippocampal CA1 region was enhanced by the induction of ischemia (P < 0.05), but that treatment with berberine reduced GFAP expression in the ischemic gerbils (P < 0.05).

When the intensity level of CD11b in the sham-operation group was set at 1.00, the level of CD11b was 2.11 ± 0.09 in the ischemia-induction group, 2.09 ± 0.11 in the ischemia-induction and 20 mg/kg berberine-treated group, 1.52 ± 1.35 in the ischemia-induction and 50 mg/kg berberine-treated group, and 1.35 ± 0.08 seconds in the ischemia-induction and 80 mg/kg berberine-treated group. The present results showed that expression of CD11b in the hippocampal CA1 region was enhanced by the induction of ischemia (P < 0.05), but that treatment with berberine reduced CD11b expression in the ischemic gerbils (P < 0.05).

Effects of Berberine on Caspase-3 Expression and DNA Fragmentation in the CA1 Region of the Hippocampus

The numbers of caspase-3-positive and TUNEL positive cells in the hippocampal CA1 region are presented in Fig. 3. The number of caspase-3-positive cells was 7.01 ± 1.85/mm² in the sham-operation group, 153.15 ± 28.36/mm² in the ischemia-induction group, 152.06 ± 20.67/mm² in the ischemia-induction and 20 mg/kg berberine-treated group, 82.50 ± 4.45/mm² in the ischemia-induction and 50 mg/kg berberine-treated group, and 64.50 ± 10.84/mm² in the ischemia-induction and 80 mg/kg berberine-treated group. The present results showed that caspase-3 expression in the hippocampal CA1 region was enhanced by the induction of ischemia (P < 0.05), but that treatment with berberine reduced caspase-3 expression in the ischemic gerbils (P < 0.05).

The number of the TUNEL-positive cells in the CA1 region was 3.01 ± 1.06/mm² in the sham-operation group, 64.74 ± 8.99/mm² in the ischemia-induction group, 64.50 ± 10.84/mm² in the ischemia-induction and 20 mg/kg berberine-treated group, 45.50 ± 4.45/mm² in the ischemia-induction and 50 mg/kg berberine-treated group, and 34.79 ± 3.63/mm² in the ischemia-induction and 80 mg/kg berberine-treated group. The present re-
Results showed that DNA fragmentation in the hippocampal CA1 region was enhanced by the induction of ischemia (P < 0.05), but that treatment with berberine reduced DNA fragmentation in the ischemic gerbils (P < 0.05).

Effects of Berberine on Akt and PI3K Expression in the Hippocampus

We determined the relative expression of PI3K, p-PI3K, Akt, and p-Akt in the hippocampus (Fig. 4). When the ratio of p-PI3K/PI3K (73–82 kDa) in the sham-operation group was set at 1.00, the ratio of p-PI3K/PI3K was 0.34 ± 0.05 in the ischemia-induction group, 0.28 ± 0.10 in the ischemia-induction and 20 mg/kg berberine-treated group, 0.58 ± 0.05 in the ischemia-induction and 50 mg/kg berberine-treated group, and 1.01 ± 0.08 in the ischemia-induction and 80 mg/kg berberine-treated group (Fig. 4). The present results showed that the ratio of p-PI3K/PI3K in the hippocampus was decreased by the induction of ischemia (P < 0.05), but that treatment with berberine increased the ratio of p-PI3K/PI3K in the ischemic gerbils (P < 0.05).

When the ratio of p-Akt/Akt (60 kDa) in the sham-operation group was set at 1.00, the ratio of p-Akt/Akt was 0.47 ± 0.02 in the ischemia-induction group, 0.59 ± 0.07 in the ischemia-induction and 20 mg/kg berberine-treated group, 0.85 ± 0.10 in the ischemia-induction and 50 mg/kg berberine-treated group, and 0.88 ± 0.03 in the ischemia-induction and 80 mg/kg berberine-treated group (Fig. 4). The present results showed that the ratio of p-Akt/Akt in the hippocampus was decreased by the induction of ischemia (P < 0.05), but that treatment with berberine increased the ratio of p-Akt/Akt in the ischemic gerbils (P < 0.05).

Effects of Berberine on the Expression of Bax, Bcl-2, and Cytochrome c in the Hippocampus

We determined the relative expression of Bax, Bcl-2, and cytochrome c in the hippocampus (Fig. 5). When the level of the Bax
(23 kDa) in the sham-operation group was set at 1.00, the level of Bax was 1.65 ± 0.07 in the ischemia-induction group, 1.38 ± 0.15 in the ischemia-induction and 20 mg/kg berberine-treated group, 0.75 ± 0.07 in the ischemia-induction and 50 mg/kg berberine-treated group, and 0.61 ± 0.08 in the ischemia-induction and 80 mg/kg berberine-treated group. The present results showed that the level of Bax expression in the hippocampus was increased by the induction of ischemia (P < 0.05), but that treatment with berberine decreased the level of Bax expression in the ischemic gerbils (P < 0.05).

When the level of Bcl-2 (26 kDa) in the sham-operation group was set at 1.00, the level of Bcl-2 was 0.28 ± 0.02 in the ischemia-induction group, 0.70 ± 0.02 in the ischemia-induction and 20 mg/kg berberine-treated group, 0.93 ± 0.05 in the ischemia-induction and 50 mg/kg berberine-treated group, and 1.09 ± 0.09 in the ischemia-induction and 80 mg/kg berberine-treated group. The present results showed that the level of Bcl-2 expression in the hippocampus was decreased by the induction of ischemia (P < 0.05), but that treatment with berberine recovered the level of Bcl-2 expression in the ischemic gerbils near to the control level (P < 0.05).

The ratio of Bax to Bcl-2 was calculated. When the ratio of Bax to Bcl-2 in the sham-operation group was set at 1.00, the ratio of Bax to Bcl-2 was 6.38 ± 0.74 in the ischemia-induction group, 1.74 ± 0.28 in the ischemia-induction and 20 mg/kg berberine-treated group, 0.66 ± 0.11 in the ischemia-induction and 50 mg/kg berberine-treated group, and 0.55 ± 0.13 in the ischemia-induction and 80 mg/kg berberine-treated group. The present results showed that the ratio of Bax to Bcl-2 in the hippocampus was increased by the induction of ischemia (P < 0.05), but that treatment with berberine decreased the ratio of Bax to Bcl-2 in the ischemic gerbils (P < 0.05).

When the level of cytochrome c (15 kDa) in the sham-operation group was set at 1.00, the level of cytochrome c was 3.07 ±
0.46 in the ischemia-induction group, 3.16 ± 0.57 in the ischemia-induction and 20 mg/kg berberine-treated group, 2.70 ± 0.44 in the ischemia-induction and 50 mg/kg berberine-treated group, and 1.65 ± 0.24 in the ischemia-induction and 80 mg/kg berberine-treated group. The present results showed that the level of cytochrome c expression in the hippocampus was increased by the induction of ischemia (P < 0.05), but that treatment with berberine suppressed the level of cytochrome c expression in the ischemic gerbils (P < 0.05).

**DISCUSSION**

Global cerebral ischemia induces neuronal damage in the hippocampus, and causes deficits in learning ability and memory function [1,11]. Berberine has been shown to protect against ischemic brain injury by decreasing intracellular reactive oxygen species levels and subsequently inhibiting the mitochondrial apoptotic pathway [24]. Berberine also prevents changes in oxidative stress and choline esterase activity, and consequently can improve the memory impairment seen in streptozotocin-induced diabetic rats [16]. In the present study, ischemic insult decreased latency in the step-down avoidance task, indicating that ischemia deteriorated short-term memory. In contrast, berberine enhanced latency in the step-down avoidance task. These results indicate that berberine alleviated the ischemia-induced short-term memory impairment.

Reactive astrogliosis inhibits axonal regeneration and exacerbates apoptotic neuronal cell death following ischemic damage.

**Fig. 4.** Effects of berberine on the phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) expressions in the hippocampus. (A) Sham-operation group, (B) ischemia-induction group, (C) ischemia-induction and 20 mg/kg berberine-treated group, (D) ischemia-induction and 50 mg/kg berberine-treated group, and (E) ischemia-induction and 80 mg/kg berberine-treated group. The data are presented as the mean ± standard error of the mean. *P < 0.05 compared to the sham-operation group. #P < 0.05 compared to the ischemia-induction group. p-PI3K, phospho-PI3K; p-Akt, phospho-Akt.
Fig. 5. Effects of berberine on Bax, Bcl-2, and cytochrome c expressions in the hippocampus. (A) Sham-operation group, (B) ischemia-induction group, (C) ischemia-induction and 20 mg/kg berberine-treated group, (D) ischemia-induction and 50 mg/kg berberine-treated group, and (E) ischemia-induction and 80 mg/kg berberine-treated group. The data are presented as the mean ± standard error of the mean. *P < 0.05 compared to the sham-operation group. #P < 0.05 compared to the ischemia-induction group.

[2]. Additionally, microglia activation is associated with neuronal death in ischemic gerbils [25]. Therefore, it is not surprising that inhibiting microglia activation attenuates injury-induced neuronal death [7,26]. Reactive astrogliosis and microglia acti-
viation have been implicated in the neuronal dysfunction and cell death [4,7]. In the present study, ischemia insult increased GFAP and CD11b expression in the hippocampal CA1 region. In contrast, berberine treatment suppressed ischemia-induced increments of GFAP and CD11b expression, showing that berberine can attenuate ischemic injury by inhibiting reactive astrogliosis and microglia activation.

Apoptosis has been implicated in the pathogenesis of cerebrovascular disease; specifically TUNEL-positive and caspase-3-positive cells appear to increase in ischemic gerbils and in intracerebral hemorrhagic rats [11,27]. In contrast, decrements of these numbers indicate the suppression of apoptosis [11,27]. In the present study, caspase-3-positive and TUNEL-positive cells in the hippocampal CA1 region were observed to increase following ischemic insult, while berberine suppressed these numbers.

Bcl-2 is also involved in the inhibition of apoptosis, while Bax is associated with apoptosis promotion [28]. Decrements of Bcl-2 expression have been observed in alcohol-induced apoptotic neuronal death in the hippocampus of gerbils [21]. Moreover, enhanced Bax and suppressed Bcl-2 expression represents apoptotic neuronal death in cerebrovascular disease [27]. In the present study, ischemic insult was found to increase Bax expression and decrease Bcl-2 expression, resulting in an increased Bax to Bcl-2 ratio. We also found that treatment with berberine suppressed Bax and enhanced Bcl-2 expression in ischemic gerbils.

The pro- and antiapoptotic members of the Bcl-2 family control the release of cytochrome c. A decreased Bax to Bcl-2 ratio can inhibit cytochrome c release, and consequently suppresses apoptosis [29], since the release of cytochrome c from the mitochondria is known to activate apoptosis. In the present study, while cytochrome c expression was increased in the hippocampus of ischemic gerbils, berberine was observed to decrease this expression.

Based on these results, we found that berberine exerted inhibitory effects against ischemia-induced apoptosis in gerbils through altering apoptosis-related proteins. Wang et al. [18] reported that berberine blocks K+ channels of hippocampal CA1 neurons, which subsequently restores cation balance in neurons following anoxic/ischemic injury. This situation then leads to the suppression of apoptosis and a substantial increase in cell survival. Thus, berberine protects against ischemic brain injury by decreasing intracellular reactive oxygen species and subsequently inhibiting the mitochondrial apoptotic pathway [24].

Akt is a serine/threonine kinase and a major downstream target of PI3K. Activation of the PI3K/Akt pathway can promote angiogenesis and diminish microglial/astrotic proliferation, resulting in the reduction of infarct volumes and behavioral recovery in ischemic rats [30]. Koh et al. [31] reported that global ischemia decreased Akt phosphorylation in the hippocampal CA1 region, while in contrast, Akt activation exerted a protective effect on global ischemic injury. Neuroprotective and post-conditioning effects of transient middle cerebral artery occlusion in rats have also been reported to be maintained through activation of the PI3K/Akt pathway [32]. Furthermore, PI3K/Akt pathway can prompt survival signaling in neonatal rats subjected to hypoxic/ischemic conditions [33]. In the present study, ischemic insult suppressed p-Akt and p-PI3K expressions in the hippocampus. Treatment with berberine enhanced p-Akt and p-PI3K expression in ischemic gerbils, showing that berberine activated the PI3K/Akt pathway. These data suggest that phosphorylation of PI3K and Akt mediate a berberine-induced neuroprotective effect through suppressing apoptosis, and that this contributes to hippocampal cell survival.

In the urological aspect, berberine has been suggested as a novel chemotherapy drug to treat bladder cancer via suppressing tumor growth [34]. Yu et al. [35] demonstrated that berberine pretreatment serves a protective role against hypoxia/reoxygenation induced apoptosis in human renal proximal tubular cells by suppression of mitochondrial and endoplasmic reticulum stress pathways. Taken together, berberine exerts its neuroprotective effect against ischemic insult by inhibiting neuronal apoptosis via activation of the PI3K/Akt signaling pathway. This antiapoptotic effect is achieved through inhibition of reactive astrogliosis and microglia activation. Based on the present results, we suggest that berberine may serve as a therapeutic agent for stroke-induced neurourological problems.

CONFLICT OF INTEREST

The authors report no potential conflict of interest relevant to this article.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2010-0022895).

REFERENCES

1. Hartman RE, Lee JM, Zipfel GJ, Wozniak DF. Characterizing learn-
ing deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. Brain Res 2005;1043:48-56.

2. Walker EL, Rosenberg GA. TIMP-3 and MMP-3 contribute to delayed inflammation and hippocampal neuronal death following global ischemia. Exp Neurol 2009;216:122-31.

3. Lively S, Moxon-Emre I, Schlichter LC. SC1/hevin and reactive gliosis after transient ischemic stroke in young and aged rats. J Neuroophathol Exp Neurol 2011;70:913-29.

4. McPherson CA, Kraft AD, Harry GJ. Injury-induced neurogenesis: consideration of resident microglia as supportive of neural progenitor cells. Neurotox Res 2011;19:341-52.

5. Kim K, Shin MS, Cho HS, Kim YP. Effects of endurance exercise on expressions of glial fibrillary acidic protein and myelin basic protein in developing rats with maternal infection-induced cerebral palsy. J Exerc Rehabil 2014;10:9-14.

6. Singh AK, Tiwari MN, Dixa A, Upadhyay G, Patel DK, Singh D, et al. Nigrostriatal proteomics of cypermethrin-induced dopaminergic neurodegeneration: microglial activation-dependent and independent regulations. Toxicol Sci 2011;122:526-38.

7. Sung YH, Kim SC, Hong HP, Park CY, Shin MS, Kim CJ, et al. Treadmill exercise ameliorates dopaminergic neuronal loss through suppressing microglial activation in Parkinson’s disease mice. Life Sci 2012;91:1309-16.

8. Cantley LC. The phosphoinositide 3-kinase pathway. Science 2002;296:1655-7.

9. Kawano T, Fukunaga K, Takeuchi Y, Morioka M, Yano S, Hamada J, et al. Neuroprotective effect of sodium orthovanadate on delayed neuronal death after transient forebrain ischemia in gerbil hippocampus. J Cereb Blood Flow Metab 2001;21:1268-80.

10. Yano S, Morioka M, Fukunaga K, Kawano T, Hara T, Kai Y, et al. Activation of Akt/protein kinase B contributes to induction of ischemic tolerance in the CA1 subfield of gerbil hippocampus. J Cereb Blood Flow Metab 2001;21:351-60.

11. Ko IG, Shin MS, Kim BK, Kim SE, Sung YH, Kim TS, et al. Tadalafil improves short-term memory by suppressing ischemia-induced apoptosis of hippocampal neuronal cells in gerbils. Pharmacol Biochem Behav 2009;91:629-35.

12. Kim M, Cho KH, Shin MS, Lee JM, Cho HS, Kim CJ, et al. Berberine prevents nigrostriatal dopaminergic neuronal loss and suppresses hippocampal apoptosis in mice with Parkinson’s disease. Int J Mol Med 2014;33:870-8.

13. Broughton BR, Reutens DC, Sobey CG. Apoptotic mechanisms after cerebral ischemia. Stroke 2009;40:e331-9.

14. Fan TJ, Han LH, Cong RS, Liang J. Caspase family proteases and apoptosis. Acta Biochim Biophys Sin (Shanghai) 2005;37:719-27.

15. Ghobrial IM, Witzig TE, Adjei AA. Targeting apoptosis pathways in cancer therapy. CA Cancer J Clin 2005;55:178-94.

16. Bhutada P, Mundhada Y, Bansod K, Tawari S, Patil S, Dixit P, et al. Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav Brain Res 2011;220:30-41.

17. Imanshahidi M, Hosseinzadeh H. Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. Phytother Res 2008;22:999-1012.

18. Wang F, Zhao G, Cheng L, Zhou HY, Fu LY, Yao WX. Effects of berberine on potassium currents in acutely isolated CA1 pyramidal neurons of rat hippocampus. Brain Res 2004;999:91-7.

19. Yoo KY, Hwang IK, Lim BO, Kang TC, Kim DW, Kim SM, et al. Berberine extract reduces neuronal damage and N-Methyl-D-aspartate receptor 1 immunoreactivity in the gerbil hippocampus after transient forebrain ischemia. Biol Pharm Bull 2006;29:623-8.

20. Kim YM, Ji ES, Yoon SJ, Yoon JH. Sudden detraining deteriorates swimming training-induced enhancement of short-term and spatial learning memories in mice. J Exerc Rehabil 2013;9:243-9.

21. Kim JE, Ji ES, Seo JH, Lee MH, Cho S, Pak YK, et al. Alcohol exposure induces depression-like behavior by decreasing hippocampal neuronal proliferation through inhibition of the BDNF-ERK pathway in gerbils. Anim Cell Syst 2012;16:190-7.

22. Seo TB, Cho HS, Shin MS, Kim CJ, Ji ES, Baek SS. Treadmill exercise improves behavioral outcomes and spatial learning memory through up-regulation of reelin signaling pathway in autistic rats. J Exerc Rehabil 2013;9:220-9.

23. Yoon JH, Lee HH, Yi ES, Baek SG. Age-dependent effect of treadmill exercise on hemorrhage-induced neuronal cell death in rats. J Exerc Rehabil 2013;9:506-10.

24. Zhou XQ, Zeng XN, Kong H, Sun XL. Neuroprotective effects of berberine on stroke models in vitro and in vivo. Neurosci Lett 2008;447:31-6.

25. Hwang IK, Yoo KY, Kim DW, Choi SY, Kang TC, Kim YS, et al. Ionized calcium-binding adapter molecule 1 immunoreactivity in the gerbil hippocampus after transient forebrain ischemia. Neurochem Res 2006;31:957-65.

26. Hamby ME, Sofroniew MV. Reactive astrocytes as therapeutic targets for CNS disorders. Neurotherapeutics 2010;7:494-506.

27. Hwang L, Choi IY, Kim SE, Ko IG, Shin MS, Kim CJ, et al. Dexamethomidine ameliorates intracerebral hemorrhage-induced memory impairment by inhibiting apoptosis and enhancing brain-derived neurotrophic factor expression in the rat hippocampus. Int J Mol Med 2013;31:1047-56.

28. Elmore S. Apoptosis: a review of programmed cell death. Toxicol
29. Pollack M, Phaneuf S, Dirks A, Leeuwenburgh C. The role of apoptosis in the normal aging brain, skeletal muscle, and heart. Ann N Y Acad Sci 2002;959:93-107.
30. Baba T, Kameda M, Yasuhara T, Morimoto T, Kondo A, Shingo T, et al. Electrical stimulation of the cerebral cortex exerts antiapoptotic, angiogenic, and anti-inflammatory effects in ischemic stroke rats through phosphoinositide 3-kinase/Akt signaling pathway. Stroke 2009;40:e598-605.
31. Koh PO, Cho GJ, Choi WS. 17beta-estradiol pretreatment prevents the global ischemic injury-induced decrease of Akt activation and bad phosphorylation in gerbils. J Vet Med Sci 2006;68:1019-22.
32. Wang HY, Wang GL, Yu YH, Wang Y. The role of phosphoinositide-3-kinase/Akt pathway in propofol-induced postconditioning against focal cerebral ischemia-reperfusion injury in rats. Brain Res 2009;1297:177-84.
33. Carloni S, Girelli S, Scopa C, Buonocore G, Longini M, Balduini W. Activation of autophagy and Akt/CREB signaling play an equivalent role in the neuroprotective effect of rapamycin in neonatal hypoxia-ischemia. Autophagy 2010;6:366-77.
34. Yan K, Zhang C, Feng J, Hou L, Yan L, Zhou Z, et al. Induction of G1 cell cycle arrest and apoptosis by berberine in bladder cancer cells. Eur J Pharmacol 2011;661:1-7.
35. Yu W, Sheng M, Xu R, Yu J, Cui K, Tong J, et al. Berberine protects human renal proximal tubular cells from hypoxia/reoxygenation injury via inhibiting endoplasmic reticulum and mitochondrial stress pathways. J Transl Med 2013;11:24.