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Tadalafil improves short-term memory by suppressing ischemia-induced apoptosis of hippocampal neuronal cells in gerbils

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ABSTRACT

Cerebral ischemia resulting from transient or permanent cerebral artery occlusion leads to neuronal cell death, and eventually causes neurological impairments. Tadalafil (Cialis®) is a long-acting phosphodiesterase type-5 (PDE-5) inhibitor used to treat erectile dysfunction. The therapeutic effects of PDE-5 inhibitors on chronic obstructive pulmonary disease, prostate hyperplasia, hypertension, and coronary heart disease have been reported. The present study investigated the effects of tadalafil on short-term memory, cyclic guanosine monophosphate (cGMP) level, apoptotic neuronal cell death, and cell proliferation in the hippocampus following transient global ischemia in gerbils. For this study, a step-down avoidance task, cGMP assay, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay, and immunohistochemistry for caspase-3 and 5-bromo-2′-deoxyuridine were performed. The results revealed that ischemic injury increased apoptotic neuronal cell death in the hippocampal CA1 region, impaired short-term memory, and decreased cGMP level. Ischemic injury enhanced cell proliferation in the hippocampal dentate gyrus. Tadalafil treatment improved short-term memory by suppressing ischemia-induced apoptotic neuronal cell death in the hippocampal CA1 region, and decreased cGMP level. Also, tadalafil suppressed the ischemia-induced increase in cell proliferation in the hippocampal dentate gyrus. We showed that tadalafil can overcome ischemia-induced apoptotic neuronal cell death, thus facilitates recovery following ischemic cerebral injury.

1. Introduction

Tadalafil (Commercial name: Cialis®) is a long-acting phosphodiesterase type-5 (PDE-5) inhibitor, and it has been widely used to treat erectile dysfunction. Recently, PDE-5 inhibitors have been reported to be effective in the treatment of various disorders such as chronic obstructive pulmonary disease, prostate hyperplasia, hypertension, and coronary heart disease (van Driel, 2006). PDE-5 inhibitors are highly specific for cyclic guanosine monophosphate (cGMP) hydrolysis, and are a key modulator of intracellular cGMP signaling pathways (Rybalkin et al., 2003; Zhang et al., 2006). cGMP plays critical roles in modulating brain functions including neurogenesis, synaptic plasticity, and physiological and pathological apoptosis (Lin et al., 2006; Prickaerts et al., 1997, 2004). Elevated cGMP production also inhibits apoptosis and repairs damage by stimulating neurogenesis (Keynes and Garthwaite, 2004). In addition, PDE-5 inhibitors have been reported to regulate angiogenesis and neurogenesis after stroke (Wang et al., 2005; Zhang et al., 2002, 2003, 2005).

Stroke is a major cause of death and disability in many countries. Cerebral ischemia results from reduced cerebral blood flow due to a transient or permanent cerebral artery occlusion (Dirnagl et al., 1999; Leker and Shohami, 2002). Ischemic injury in the brain leads to neuronal cell death (Benchoua et al., 2001; Lee et al., 2003), and eventually causes neurological impairments. In addition, tissue damage following cerebral ischemia is caused by complex pathophysiological processes such as glutamate excitotoxicity, membrane depolarization, inflammation, and apoptosis (Dirnagl et al., 1999). Pyramidal neurons in the hippocampal CA1 region are particularly vulnerable to ischemic injury (Sugawara et al., 2002).

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Apoptosis is an important mechanism leading to brain damage after cerebral ischemia (Endres et al., 1998). Apoptosis, also known as programmed cell death, is a form of cell death that serves to eliminate dying cells in proliferating or differentiating cell populations (Kerr et al., 1972), thus apoptosis plays a crucial role in normal development and tissue homeostasis (Woodle and Kulkarni, 1998). Nevertheless, inappropriate or excessive apoptosis has been implicated in several neurological disorders (Johnson et al., 1995; Lee et al., 2003; Liu et al., 1998; Smith et al., 1991). Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) detects one characteristic of apoptotic cell death, DNA fragmentation (Gavrieli et al., 1992). In ischemic animal models, TUNEL-positive cells represent apoptotic cell death (Li et al., 1995). Another important characteristic of apoptosis is caspase activation. Caspase-3 is one of the most widely studied caspases, and is a key executor of apoptosis (Cohen, 1997). In ischemic animal models, caspase-3 activation is implicated in neuronal apoptosis (Benchoua et al., 2001).

Neurogenesis encompasses cell proliferation, survival, migration, and neuronal differentiation. Cell proliferation in the hippocampus plays a central role in learning and memory processes (Gage, 2000). Increased cell proliferation in the hippocampal dentate gyrus has also been observed in some pathological states such as seizure, mechanical brain damage, and ischemic injury (Liu et al., 1998; Sim et al., 2004). The up-regulation of cell proliferation during pathological situations is thought to be a compensatory response to lesion-induced cell death (Liu et al., 1998; Lee et al., 2003).

In the present study, we investigated the effects of tadalafil on short-term memory, neuronal apoptosis, and cell proliferation in the hippocampal dentate gyrus following transient global ischemia in gerbils. For this study, a step-down inhibitory avoidance task, cGMP assay, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, and immunohistochemistry for caspase-3 and 5-bromo-2′-deoxyuridine (BrdU) were performed.

2. Materials and methods

2.1. Experimental animals and treatment

Adult male Mongolian gerbils (12–14 weeks old) were used in this experiment. The experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The gerbils were housed under controlled temperature (20±2 °C) and lighting (07:00 to 19:00 h) conditions with food and water available ad libitum. Gerbils were randomly divided into eight groups (n=8 in each group): the sham-operation group, the sham-operation and 0.1 mg/kg tadalafil-treated group, the sham-operation and 1 mg/kg tadalafil-treated group, the sham-operation and 10 mg/kg tadalafil-treated group, the ischemia-induction group, the ischemia-induction and 0.1 mg/kg tadalafil-treated group, the ischemia-induction and 1 mg/kg tadalafil-treated group, the ischemia-induction and 10 mg/kg tadalafil-treated group. All gerbils received 50 mg/kg BrdU (Sigma Chemical Co., St. Louis, MO, USA) intraperitoneally once a day for 7 consecutive days, starting one day after surgery. Gerbils in the tadalafil-treated groups received tadalafil (Eli Lilly Co, Indianapolis, IN, USA) orally once a day for 7 consecutive days, starting one day after surgery. The sham-operation group and ischemic group received an equal amount of distilled water for the same duration.

2.2. Induction of transient global ischemia

Transient global ischemia was induced with a previously described surgical procedure (Sim et al., 2004). In brief, gerbils were anesthetized with Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France). Following bilateral neck incisions, both common carotid arteries were exposed and occluded with aneurysm clips for 7 min. The clips were then removed to restore cerebral blood flow. Body and rectal temperature was maintained at 36±0.5 °C during surgery using Homeothermic Blanket Control Unit (Harvard Apparatus, Massachusetts, MA, USA) that enveloped the body and the head. Regional cerebral blood flow in both sides of the forebrain was measured by a BLF21D laser Doppler flow meter (Transonic Systems Inc, New York, NY, USA) with two probes placed 3.0 mm lateral to the bregma before and after clamping the bilateral common carotid arteries. After recovery, the animals were monitored for an additional 2 h to prevent hypothermia. The animals in the sham-operation group were treated identically, except that the common carotid arteries were not occluded after the neck incisions.

2.3. Step-down avoidance task

The latency of the step-down avoidance task was determined to evaluate short-term memory capability. Gerbils were trained in a step-down avoidance task 6 days after ischemia. One hour after training, the latency (s) of the animals in each group was determined.

Gerbils were placed on a 7×25 cm platform 2.5 cm high. The platform faced a 42×25 cm grid of parallel 0.1 cm-caliber stainless steel bars spaced 1 cm apart. In training sessions, the animals received 0.5 mA, scramble foot shock for 2 s immediately upon stepping down. The interval of gerbils stepping down and placing all four paws on the grid was defined as the latency time. A latency over 180 s was counted as 180 s.

2.4. Tissue preparation

The gerbils were sacrificed immediately after determining the latency of the step-down avoidance task. The animals were anesthetized using Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories), transcardially perfused with 50 mM phosphate-buffered saline (PBS), and fixed with a freshly prepared solution consisting of 4% paraformaldehyde in 100 mM phosphate buffer (PB, pH 7.4). Brains were dissected, post-fixed in the same fixative overnight, and transferred to 30% sucrose for cryoprotection. Forty μm thick coronal sections were made using a freezing microtome (Leica, Nussloch, Germany). Ten slice sections on average in the CA1 region were collected from each gerbil. The sections of 2.5 mm to 2.7 mm posterior from the bregma were used for immunohistochemistry.

2.5. Measurement of cyclic GMP production

In order to determine the effect of tadalafil on cGMP production, cGMP assay was performed using a commercially available cGMP competitive enzyme immunoassay kit (Sapphire Bioscience Pty. Ltd., Redfern, Australia), according to the manufacturer's instructions.

2.6. TUNEL staining

To visualize DNA fragmentation, a marker of apoptosis, TUNEL staining was performed using an In Situ Cell Death Detection Kit® (Roche, Mannheim, Germany) according to the manufacturer's protocol (Sim et al., 2004). Sections were post-fixed in ethanol–acetic acid (2:1) and rinsed. Sections were then incubated with proteinase K (100 μg/ml), rinsed, and incubated in 3% H_2O_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% 3,3′-diaminobenzidine (DAB). Mayer's hematoxylin (DAKO, Glostrup, Denmark) was used as a counterstain, and sections were mounted onto gelatin-coated slides. Slides were air-dried overnight at room temperature, and coverslips were mounted using Permount®.

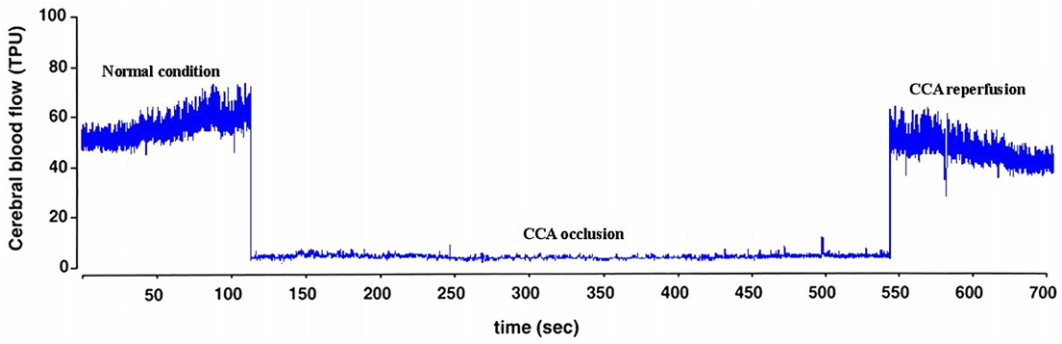

Fig. 1. Changes in cerebral blood flow during occlusion and reperfusion of common carotid arteries. Cerebral blood flow was decreased by occlusion of common carotid arteries and then was increased by reperfusion.

2.7. Caspase-3 immunohistochemistry

To visualize caspase-3 expression, caspase-3 immunohistochemistry was performed as previously described (Sim et al., 2004). Sections were selected from each brain and incubated overnight with mouse anti-caspase-3 antibody (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and then with biotinylated mouse secondary antibody (1:200; Vector Laboratories, Burlingame, CA, USA) for another hour. The secondary antibody was amplified with the Vector Elite ABC kit® (1:100; Vector Laboratories). Antibody–biotin–avidin–peroxidase complexes were visualized using 0.03% DAB, and sections were mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature, and coverslips were mounted using Permount®.

2.8. BrdU immunohistochemistry

To detect newly generated cells in the dentate gyrus, BrdU-specific immunohistochemistry was performed as previously described (Sim et al., 2004). Sections were first permeabilized by incubating in 0.5% Triton X-100 in PBS for 20 min, then pretreated in 50% formamide-2×standard saline citrate (SSC) at 65 °C for 2 h, denatured in 2 N HCl at 37 °C for 30 min, and rinsed twice in 100 mM sodium borate (pH 8.5). Afterwards, sections were incubated overnight at 4 °C with BrdU-specific mouse monoclonal antibody (1:600; Roche, Mannheim, Germany). The sections were then washed three times with PBS and incubated with biotinylated mouse secondary antibody (1:200; Vector Laboratories) for 1 h. The sections were then incubated for another 1 h with an avidin–peroxidase complex (1:100; Vector Laboratories). For visualization, sections were incubated in 50 mM Tris–HCl (pH 7.6) containing 0.03% DAB, 40 mg/ml nickel chloride, and 0.03% hydrogen peroxide for 5 min.

After BrdU staining, BrdU-positive cells differentiation was determined on the same section using a mouse anti-neuronal nucleic (NeuN) antibody (1:1000; Chemicon International, Temecula, CA, USA). The sections were washed three times with PBS, incubated for l h with a biotinylated anti-mouse secondary antibody. For staining, sections were incubated in a reaction mixture consisting of 0.03% DAB and 0.03% hydrogen peroxide for 5 min. The sections were finally mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature, and coverslips were mounted using Permount®.

2.9. Data analysis

The numbers of TUNEL-positive and caspase-3-positive cells in the CA1 region were counted. The area of CA1 region from each slice was measured using Image-Pro® Plus computer-assisted image analysis system (Media Cyberbetics Inc., Silver Spring, MD, USA) attached to a light microscope (Olympus, Tokyo, Japan). The numbers of TUNEL-positive and caspase-3-positive cells in the CA1 region were counted hemilaterally through a light microscope (Olympus, Tokyo, Japan). The numbers of TUNEL-positive and caspase-3-positive cells were expressed as the number of cells per square millimeter of the CA1 region. The number of BrdU-positive cells in the dentate gyrus was counted. The area of the granular layer of the dentate gyrus was measured by Image-Pro® Plus image analysis system (Media Cyberbetics Inc.). The number of BrdU-positive cells in the granular layer of the dentate gyrus was counted hemilaterally through a light microscope (Olympus). The number of BrdU-positive cells was expressed as the number of cells per square millimeter of granular area in the dentate gyrus.

Statistical analysis was performed using one-way ANOVA followed by Duncan's post-hoc test, and the results are expressed as the mean ± standard error of the mean (S.E.M.). Significance was set as $p<0.05$.

3. Results

3.1. Changes in cerebral blood flow

The change of cerebral blood flow during occlusion and reperfusion of common carotid arteries is presented in Fig. 1. This graph showed that cerebral blood flow was decreased by common carotid arteries occlusion and that common carotid arteries reperfusion increased cerebral blood flow.

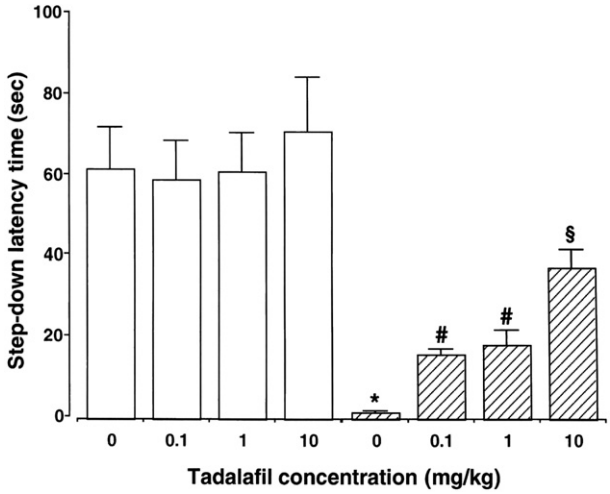

Fig. 2. Effect of tadalafil on latency of a step-down avoidance task. (□) Sham-operation groups, (▨) ischemia-induction groups. * represents $p<0.05$ compared to the sham-operation group. # represents $p<0.05$ compared to the ischemia-induction group. § represents $p<0.05$ compared to the 0.1 mg/kg tadalafil-treated group.

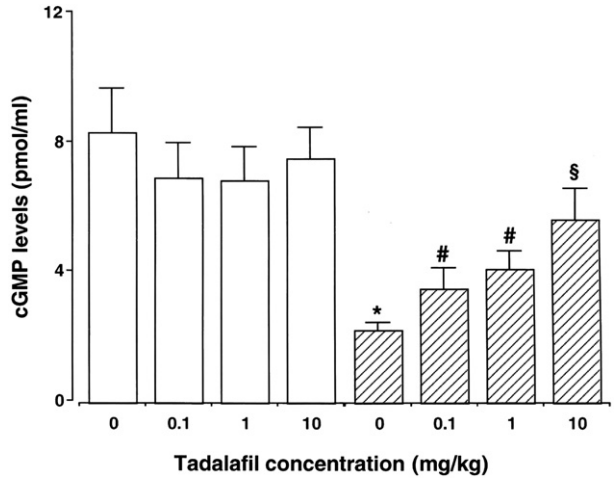

Fig. 3. Effect of tadalafil on cGMP level. (□) Sham-operation groups, (▨) ischemia-induction groups. * represents $p<0.05$ compared to the sham-operation group. # represents $p<0.05$ compared to the ischemia-induction group. § represents $p<0.05$ compared to the 0.1 mg/kg tadalafil-treated group.

3.2. Effect of tadalafil on the step-down avoidance task

The latencies of the step-down avoidance task are presented in Fig. 2. The latency time was 62.00±10.34 s in the sham-operation group, 59.25±9.62 s in the sham-operation and 0.1 mg/kg tadalafil-treated group, 61.25±9.65 s in the sham-operation and 1 mg/kg tadalafil-treated group, 71.25±13.45 s in the sham-operation and 10 mg/kg tadalafil-treated group, 1.75±0.25 s in the ischemia-induction group, 16.00±1.54 s in the ischemia-induction and 0.1 mg/kg tadalafil-treated group, 18.25±3.82 s in the ischemia-induction and 1 mg/kg tadalafil-treated group, and 37.62±4.32 s in the ischemia-induction and 10 mg/kg tadalafil-treated group.

These results showed that short-term memory was disturbed by induction of ischemic injury ($p<0.05$), and tadalafil treatment alleviated ischemia-induced short-term memory impairment as dose-dependently ($p<0.05$). In the normal rats, tadalafil treatment exerted no significant effect on short-term memory.

3.3. Effect of tadalafil on the cGMP level in the hippocampus

The levels of the cGMP are presented in Fig. 3. The cGMP level was 8.35±1.35 pmol/ml in the sham-operation group, 6.95±1.08 pmol/ml in the sham-operation and 0.1 mg/kg tadalafil-treated group, 6.84±1.05 pmol/ml in the sham-operation and 1 mg/kg tadalafil-treated group, 7.55±0.95 pmol/ml in the sham-operation and 10 mg/kg tadalafil-treated group, 2.24±0.25 pmol/ml in the ischemia-induction group, 3.54±0.62 pmol/ml in the ischemia-induction and 0.1 mg/kg tadalafil-treated group, 4.15±0.53 pmol/ml in the ischemia-induction and 1 mg/kg tadalafil-treated group, and 5.65±0.95 pmol/ml in the ischemia-induction and 10 mg/kg tadalafil-treated group.

These results showed that ischemic insult suppressed cGMP level in the hippocampus ($p<0.05$), and tadalafil treatment significantly increased cGMP level as dose-dependently ($p<0.05$). In the normal rats, tadalafil treatment exerted no significant effect on cGMP level.

3.4. Effect of tadalafil on TUNEL-positive cells in the CA1 region

Photomicrographs of TUNEL-positive cells in the hippocampal CA1 region are presented in Fig. 4. The number of TUNEL-positive cells was 4.27±1.29/mm^2 in the sham-operation group, 6.35±2.20/mm^2 in the sham-operation and 0.1 mg/kg tadalafil-treated group, 4.42±1.65/mm^2 in the sham-operation and 1 mg/kg tadalafil-treated group, 4.42±1.58/mm^2 in the sham-operation and 10 mg/kg tadalafil-treated group, 1581.33±232.32/mm^2 in the ischemia-induction group, 1108±159.89/mm^2 in the ischemia-induction and 0.1 mg/kg tadalafil-treated group, 981.35±197.02/mm^2 in the ischemia-induction and 1 mg/kg tadalafil-treated group, and 557.07±64.78/mm^2 in the ischemia-induction and 10 mg/kg tadalafil-treated group.

These results showed that ischemic injury enhanced apoptotic cell death in the CA1 region ($p<0.05$), and tadalafil treatment significantly suppressed the ischemia-induced apoptosis as dose-dependently ($p<0.05$). In the normal rats, tadalafil treatment exerted no significant effect on apoptosis.

3.5. Effect of tadalafil on caspase-3 expression in the CA1 region

Photomicrographs of caspase-3-positive cells in the hippocampal CA1 region are presented in Fig. 5. The number of caspase-3-positive cells was 24.81±6.45/mm^2 in the sham-operation group, 38.67±8.56/mm^2 in the sham-operation and 0.1 mg/kg tadalafil-treated group, 41.91±11.61/mm^2 in the sham-operation and 1 mg/kg tadalafil-treated group, and 32.95±8.72/mm^2 in the sham-operation

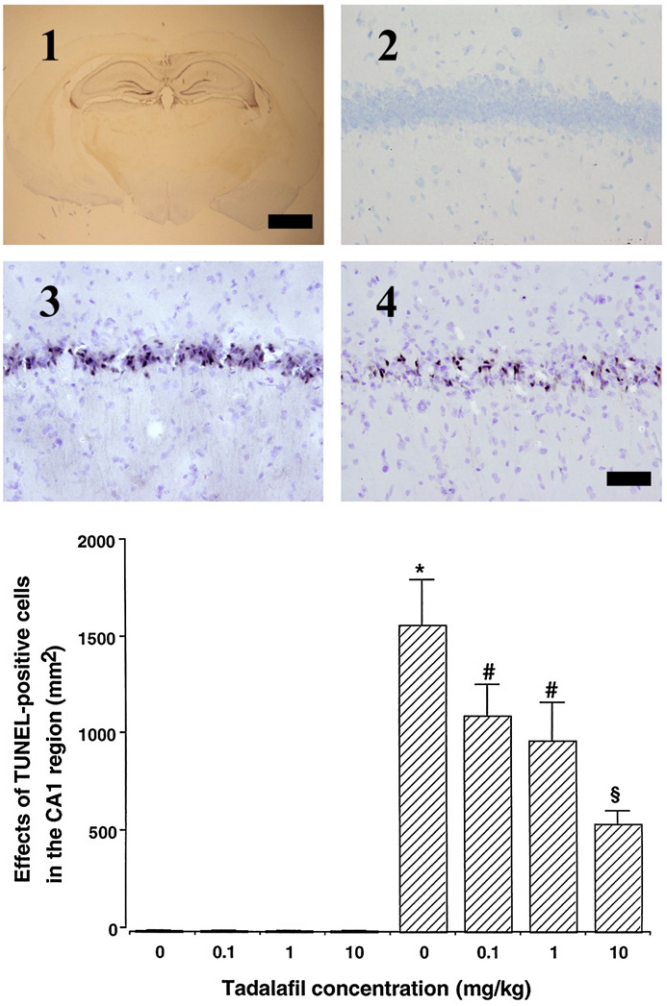

Fig. 4. Effect of tadalafil on DNA fragmentation in the hippocampal CA1 region after transient global ischemia. Upper: Photomicrographs of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-positive cells in the hippocampal CA1 region. (1) Whole brain, (2) sham-operation group, (3) ischemia-induction group, (4) ischemia-induction and 10 mg/kg tadalafil-treated group. The sections were stained for TUNEL. The scale bar represents 1600 μm (1) and 400 μm (2–4). Lower: Number of TUNEL-positive cells in each group. (□) Sham-operation groups, (▨) ischemia-induction groups. * represents $p<0.05$ compared to the sham-operation group. # represents $p<0.05$ compared to the ischemia-induction group. § represents $p<0.05$ compared to the 0.1 mg/kg tadalafil-treated group.

and 10 mg/kg tadalafil-treated group, 1060.00±144.46/mm^2 in the ischemia-induction group, 842.37±64.97/mm^2 in the ischemia-induction and 0.1 mg/kg tadalafil-treated group, 748.60±82.32/mm^2 in the ischemia-induction and 1 mg/kg tadalafil-treated group, 473.51±41.25/mm^2 in the ischemia-induction and 10 mg/kg tadalafil-treated group.

These results showed that ischemic insult enhanced caspase-3 expression in the CA1 region ($p<0.05$), and tadalafil treatment significantly suppressed the ischemia-induced caspase-3 expression as dose-dependently ($p<0.05$). In the normal rats, tadalafil treatment exerted no significant effect on caspase-3 expression.

3.6. Effect of tadalafil on cell proliferation in the dentate gyrus

Photomicrographs of BrdU-positive cells in the hippocampal dentate gyrus are presented in Fig. 6. The number of BrdU-positive cells was 226.05±27.17/mm^2 in the sham-operation group, 251.43±17.95/mm^2 in the sham-operation and 0.1 mg/kg tadalafil-treated group, 305.20±23.80/mm^2 in the sham-operation and 1 mg/kg tadalafil-treated group, 307.36±17.00/mm^2 in the sham-operation and 10 mg/kg tadalafil-treated group, 1369.55±132.01/mm^2 in the ischemia-induction group, 893.27±81.36/mm^2 in the ischemia-induction and 0.1 mg/kg tadalafil-treated group, 802.51±57.57/mm^2 in the ischemia-induction and 1 mg/kg tadalafil-treated group, and 530.86±19.61/mm^2 in the ischemia-induction and 10 mg/kg tadalafil-treated group.

These results showed that ischemic insult enhanced cell proliferation in the dentate gyrus ($p<0.05$), that is the compensatory adaptive response to ischemic brain injury, and tadalafil treatment significantly suppressed the ischemia-induced cell proliferation as dose-dependently ($p<0.05$). In the normal rats, tadalafil treatment exerted no significant effect on cell proliferation.

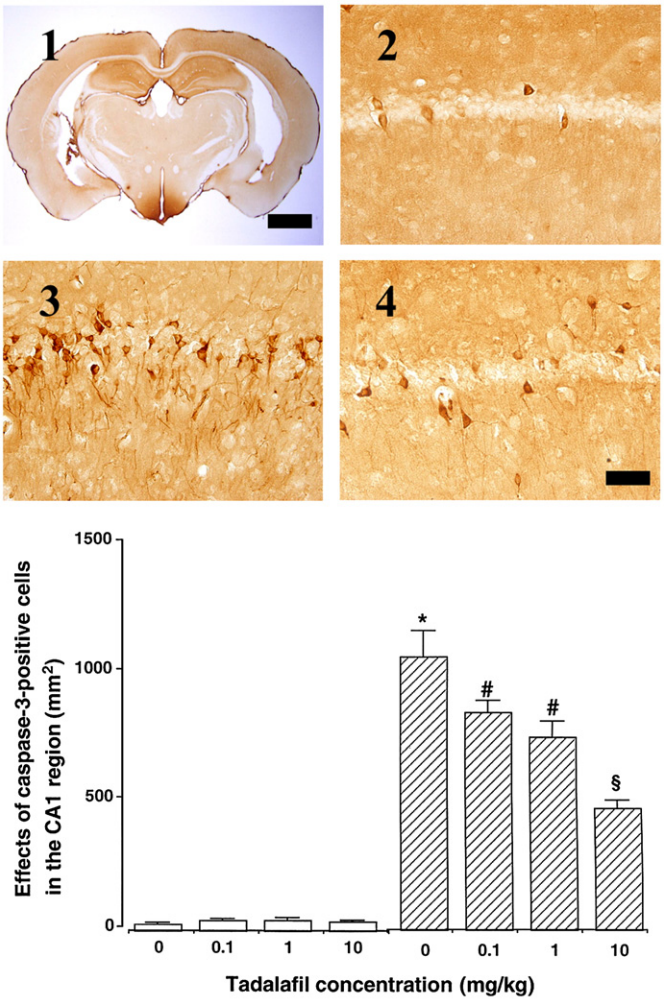

Fig. 5. Effect of tadalafil on caspase-3 expression in the hippocampal CA1 region after transient global ischemia. Upper: Photomicrographs of caspase-3 positive cells in the hippocampal CA1 region. (1) Whole brain, (2) sham-operation group, (3) ischemia-induction group, (4) ischemia-induction and 10 mg/kg tadalafil-treated group. The sections were stained for caspase-3 immunoreactivity (brown). The scale bar represents 1600 μm (1) and 400 μm (2–4). Lower: Number of caspase-3-positive cells in each group. (□) Sham-operation groups, (▨) ischemia-induction groups. * represents $p<0.05$ compared to the sham-operation group. # represents $p<0.05$ compared to the ischemia-induction group. § represents $p<0.05$ compared to the 0.1 mg/kg tadalafil-treated group.

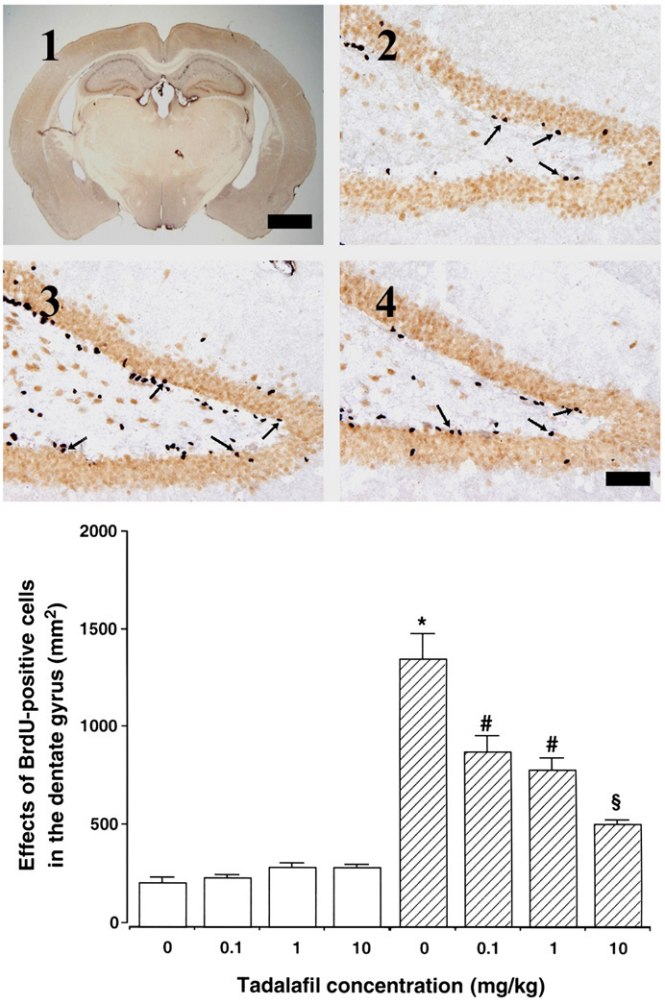

Fig. 6. Effect of tadalafil on cell proliferation in the dentate gyrus after transient global ischemia. Upper: Photomicrographs of 5-bromo-2′-deoxyuridine (BrdU) positive cells in the dentate gyrus. (1) Whole brain, (2) sham-operation group, (3) ischemia-induction group, (4) ischemia-induction and 10 mg/kg tadalafil-treated group. The sections were stained for BrdU (black arrow) and neuronal nuclei (NeuN; brown). The scale bar represents 1600 μm (1) and 400 μm (2–4). Lower: Number of BrdU-positive cells in each group. (□) Sham-operation groups, (▨) ischemia-induction groups. * represents $p<0.05$ compared to the sham-operation group. # represents $p<0.05$ compared to the ischemia-induction group. § represents $p<0.05$ compared to the 0.1 mg/kg tadalafil-treated group.

4. Discussion

Cerebral ischemia deprives oxygen and glucose in the brain, causing tissue infarction and neuronal cell death (Benchoua et al., 2001; Li et al., 1995). Pyramidal neurons in the hippocampal CA1 region are particularly vulnerable to ischemic injury, and neuronal cell

death in the hippocampal CA1 region occurs after ischemia (Schmidt-Kastner and Freund, 1991).

Apoptosis is the major form of cell death following ischemia (Johnson et al., 1995). Apoptosis is also implicated in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (Smith et al., 1991; Thompson, 1995). The morphological characteristics of apoptosis include cell shrinkage, chromatin condensation, membrane blebbing, internucleosomal DNA fragmentation, and the formation of apoptotic bodies (Li et al., 1995). In addition, caspase-3 is up-regulated and activated in the early stages of apoptosis following ischemia (Benchoua et al., 2001; Sim et al., 2004).

In the present study, the numbers of TUNEL-positive cells and caspase-3 positive cells in the hippocampal CA1 region were significantly increased following cerebral ischemia. This indicates that cerebral ischemia induced neuronal apoptosis in the hippocampal CA1 region.

Increased cell proliferation in the hippocampal dentate gyrus and cerebral cortex after ischemia has been previously documented (Liu et al., 1998; Sim et al., 2004). Liu et al. (1998) reported that cell proliferation in the dentate gyrus of gerbils was increased significantly after ischemia, reaching a maximum level at eleven days after ischemia.

The present study showed that the number of BrdU-positive cells in the hippocampal dentate gyrus was significantly increased following cerebral ischemia, indicating that cerebral ischemia enhanced cell proliferation in the hippocampal dentate gyrus. Enhanced cell proliferation in the dentate gyrus is known as a compensatory adaptive response to excessive apoptosis (Liu et al., 1998; Sim et al., 2004).

PDE-5 inhibitors have been suggested to protect against necrosis and apoptosis in various disorders. Ockaili et al. (2002) reported that sildenafil citrate, a potent PDE-5 inhibitor, protected against ischemia/reperfusion cardiac injury in adult rabbits. In addition, sildenafil attenuated cardiomyocyte apoptosis after simulated ischemia/reoxygenation in mice (Fisher et al., 2005; Kukreja et al., 2004; Salloum et al., 2003). Furthermore, PDE-5 inhibitors are known to protect neurons against acute reactive oxygen species (ROS)-induced and chronic glutamate-induced apoptosis (Naula and Seebeck, 2000).

Our present study showed that tadalafil significantly suppressed both the ischemia-induced increase in DNA fragmentation and caspase-3 expression in the hippocampal CA1 region. This inhibiting effect of tadalafil on apoptosis appeared as a dose-dependent manner. In addition, tadalafil also suppressed cerebral ischemia-induced cell proliferation in the dentate gyrus due to the reduced apoptosis.

Ischemic hippocampal injury is known to induce memory impairment (Squire and Zola, 1996). Deficits in learning and memory following cerebral ischemia have a close correlation with neuronal death in the hippocampal CA1 region (Ji et al., 2007). Recent evidences showed that cGMP plays integral roles in memory processes (Edwards and Lindley, 2007; Prickaerts et al., 2004). PDE-5 inhibitors suppress cGMP hydrolysis, increase cGMP level, and thus enhance memory capability (Erceg et al., 2005; Prickaerts et al., 1997, 2004). PDE-5 inhibitors induce vasodilation through cGMP, thus improved memory capability by PDE-5 inhibitors has been suggested to result from increased blood flow and glucose metabolism (Dundore et al., 1992, 1993).

In this study, we evaluated the effect of tadalafil on ischemia-induced short-term memory impairment using a step-down avoidance task. We found that latency was shortened by induction of transient global ischemia, in contrast, latency was significantly improved by tadalafil treatment as a dose-dependent manner. The present study also showed that cGMP level in the hippocampus was decreased by induction of ischemic insult, and cGMP level was significantly increased by tadalafil treatment as a dose-dependent manner.

This study showed that tadalafil alleviated ischemia-induced short-term memory impairment by suppressing ischemia-induced neuronal apoptosis. The anti-apoptotic effect of tadalafil can be ascribed to the enhancing effect of tadalafil on cGMP. Here in this study, we suggest that the PDE-5 inhibitor, tadalafil, can overcome ischemia-induced neuronal apoptosis, thus facilitates recovery following cerebral ischemic injury.

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