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Panax ginseng ginsenoside-Rg_2 protects memory impairment via anti-apoptosis in a rat model with vascular dementia

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Abstract

Ethnopharmacological relevance: Ginsenosides, the major active ingredients of Panax ginseng, produce a variety of pharmacological or physiological responses with effects on the central and peripheral nervous systems.
Aim of the Study: In this report, we investigated the effects of ginsenoside Rg_2 on cerebral ischemia-reperfusion induced impairment of neurological responses, memory and caudate-putamen neuronal apoptosis in a vascular dementia (VD) rat model.
Materials and Methods: Neurological evaluation was performed 24 h after reperfusion and Y-maze memory performance was assessed at 48 h after reperfusion. Immunocytochemical techniques were employed to check the protein expression of BCL-2, BAX, heat shock protein 70 and P53, which are related with cell apoptosis.
Results: Neurological responses and memory ability of the ginsenoside Rg_2 or nimodipine groups improved significantly compared with the VD group. The expression of BCL-2 and HSP70 were decreased, while BAX and P53 were increased in the VD model. The expression of BCL-2 and HSP70 proteins were increased, while BAX and P53 decreased after ginsenoside Rg_2 (2.5, 5 and 10 mg/kg) and nimodipine (50 μg/kg) treatment compared with the VD group. The study suggests that ginsenoside Rg_2 improved neurological performance and memory ability of VD rats through mechanisms related to anti-apoptosis.
Conclusions: The capacity for ginsenoside Rg_2 to modulate the expression of apoptotic related proteins suggests that ginsenoside Rg_2 may represent a potential treatment strategy for vascular dementia or other ischemic insults.

Keywords: Vascular dementia; Apoptosis; *Panax ginseng*; Neuroprotection

1. Introduction

Dementia represents an increasingly common diagnosis in our aging population in the last decades. Global prevalence data have documented that the number will double between 1990 and 2020 (Fratiglioni et al., 1999; Sloane et al., 2002). VD is the second most common cause of dementia, accounting for about 20% of all cases (Stewart, 2007). A major consequence of VD is an impairment of memory. Patients with VD lose executive function, drive, disinhibited behaviors and commonly develop treatable mood disorders like depression. Once the symptoms are recognized, the clinician is in an ideal position to apply treatments.

Apoptosis is an active process of cell death that occurs in many important physiological conditions, such as embryonic development and tissue remodeling (Vaux and Korsmeyer, 1999; Walsh et al., 2000). Many diseases are associated with either too much or too little apoptosis, including cancer, autoimmunity, sepsis, and neurodegenerative diseases (Kam and Ferch, 2000). Many studies show that cell apoptosis is one of the mechanisms in cerebral ischemia and reperfusion injury (Tatton et al., 1997; Waldmeier, 2003).

Ginseng, one of the seven top-selling herbal medicinal products, has a wide range of actions in the CNS (Van Kampen et al., 2003). For example, it was reported that ginseng improved learning and memory deficits in different disease models (Hsieh et al., 2000; Wesnes et al., 2000), and senile dementia (Gold et al., 2001). The main active components of Panax ginseng are ginsenosides, which have been shown to have a variety of beneficial consequences, including antiinflammatory,

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antioxidant, anticancer effects, antimutagenic, antiaging activities and tonic, immunomodulatory, and adaptogenic actions (Kiefer and Pantuso, 2003; Lee et al., 2005). Ginsenoside Rg_2 is protopanaxatriol-type compound that is one of the major active components in the root and stem leaves of ginseng. It has been suggested that ginsenoside Rg_2 acts on a wide range of mechanisms. Little research has been conducted to determine whether the effects of ginsenoside Rg_2 on cerebral ischemia-reperfusion induced memory impairment and whether the effects afforded by ginsenoside Rg_2 relate to reduction in apoptosis during ischemia/reperfusion. Therefore, the primary objective of this study was to determine whether ginsenoside Rg_2 could protect memory functions and caudate-putamen neuronal cells against ischemia/reperfusion-induced apoptosis in the rat. Since the BCL-2 family of proteins plays a major role in determining the ultimate sensitivity or resistance of cells to the myriad of stimuli and insults that induce apoptosis (Chao and Korsmeyer, 1998; Rosse et al., 1998; Cory et al., 2003), we examined the effects of ginsenoside Rg_2 on gene expression of the BCL-2 family as an approach to understand the underlying protective mechanisms of ginsenoside Rg_2 on cerebral ischemia injury. Furthermore, HSP70 and P53 were also examined as other factors that show a relationship with cell apoptosis. The specific goals of this report consisted of investigating: (1) the relationship of memory impairment and neuron apoptosis, (2) whether ginsenoside Rg_2 could affect of expression of BCL-2, HSP70, BAX, and P53, which were related with cell apoptosis, and (3) the interaction among BCL-2, HSP70, BAX, P53 and apoptosis following induction of VD in a rat model.

2. Materials and methods

2.1. Animals

Sixty-six male Sprague–Dawley rats weighing 250–300 g were purchased from Henan medical college (Zhengzhou, China). The rats were housed 2–3 per cage and provided with food and water *ad libitum*. They were maintained at ambient room temperature under an artificial light of 12 h each day. All animal experiments were carried out in accordance with the ethic guidelines of Qingdao University for animal care.

2.2. Materials

Ginsenoside Rg_2 was provided by Jilin Academy of Traditional Chinese Medicine and Materia Medica (Changchun, China), with the chemical structure illustrated previously (Li et al., 2007). Rabbit-anti-BCL-2, Rabbit-anti-BAX, Rabbit-anti-HSP70, Rabbit-anti-P53 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). SABC, DAB kits were purchased from Boster Biological Technology Ltd. (Wuhan, China). 2,3,5-Triphenyl tetrazolium chloride (TTC) was purchased from the Third Shanghai Chemical Company (Shanghai, China).

2.3. Animal model and treatments

The rats were randomly divided into seven groups with eight rats in each group: including control, sham-operated, VD model, ginsenoside Rg_2 (2.5, 5 and 10 mg/kg)-treated VD model and nimodipine (50 μg/kg)-treated VD model groups. Animals who were sensitive to the electric stimulus and achieved 9/10 consecutive avoidances were selected for the experiment. Rat models of VD were induced by the middle cerebral artery occlusion (MCAO)-reperfusion method as described previously (Longa et al., 1989). Ischemia was induced by occluding the middle cerebral artery with intraluminal filament (18.5 ± 0.5 mm) for 1 h, the circulation was then restored for 48 h (VD group). Rats in the sham-operated group were treated with short intraluminal filament (10 mm), which cannot contact the MCA. Fifteen minutes before reperfusion and 24 h after reperfusion, test agents (saline, ginsenoside Rg_2, or nimodipine) were administered through the tail vein in 2.5 ml/kg. Rats in the control and the sham-operated groups were injected with saline. Rats in the VD model groups were treated with menstruum, those in the ginsenoside Rg_2-treated and nimodipine-treated groups were injected with ginsenoside Rg_2 (2.5, 5 and 10 mg/kg) or nimodipine (50 μg/kg). Sixty-six male rats were initially planned for use in this study; however, 10 rats were selected out or died during the course of the experiment.

2.4. Neurological evaluation

A neurological evaluation was performed 24 h after the reperfusion and scored on a 6-point scale (Schabitz et al., 1999), as modified from a scale proposed by Longa et al. (1989): 0 = no neurological deficit, 1 = failure to extend left forepaw fully, 2 = circling to the left, 3 = falling to the left, 4 = no spontaneous walking with depressed level of consciousness and 5 = death.

2.5. TTC staining

The rats were anesthetized with 8% chloral hydrate and the brains were quickly removed 48 h after the induction of reperfusion as described above. The brain was sectioned coronally into 2 mm thick slices. The sections were immediately immersed into a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) at 37 °C for 30 min. Tissues from controls were stained red and the infarct tissues white, thereby demonstrating the occurrence of brain tissue necrosis that results from the cerebral ischemia-reperfusion.

2.6. Y-maze task

The Y-maze had three equal-sized arms with a 120° angle. On the floor of each arm of the Y-maze was an array of copper wires. Each arm was 45 cm long and contained a 15 W lamp suspended above the arm, which could be turned on whenever electric current was present in the arm. The rats were trained individually in the Y-maze at 24 h before operation (learning task) and 48 h after reperfusion (memory task). At the start of testing, the rat was placed into the Y-maze and allowed 3–5 min

to adapt to the apparatus, followed by a series of tests (<30) with an inter-trial interval of 45 s. A voltage of 40 V was used with each test, while the lamp of the arm was illuminated to indicate the non-stimulation area. If the rat went directly into the lighted arm (non-stimulated), one correct response was recorded. If the rat went to the dark arm (stimulated), one wrong response was recorded. A criterion of 9/10 consecutive avoidances was used for checking their memory and learning ability (Fletcher, 1989).

2.7. Tissue preparation and HE and Nissl staining

The rats were anesthetized with 8% chloral hydrate (400 mg/kg) 48 h after reperfusion and perfused transcardially with normal saline 400–500 ml, followed by brain tissue pre-fixed by 4% paraformaldehyde. Brains were then removed and post-fixed in 4% paraformaldehyde for 10–12 h. Thereafter the tissues were dehydrated with different concentrations of ethanol, embedded with paraffin wax and serially sectioned in 7 μm thick coronal sections before 1.2 mm anterior fontanel. Sections were then subjected to HE and Nissl staining for histological viewing.

2.8. Immunohistochemistry for BCL-2, HSP70, BAX, P53

Paraffin wax sections of the sham-operated, VD model, ginsenoside Rg_2 or nimodipine-treated VD model groups were simultaneously processed for immunohistochemistry. The sections were treated with 10% normal goat serum while incubating at 37 °C for 20 min. Before normal goat serum pretreatment, 3% H_2O_2 was used to incubate sections for 10 min which were then washed with PBS (0.01 M, pH 7.4) for 3 times 5 min. After 20 min, the sections were wiped to dry, and processed for immunohistochemistry. The sections were incubated with primary antiserums, rat-anti-BCL-2, rat-anti-BAX, rat-anti-HSP70, rabbit-anti-P53 (1:100, Santa Cruz, USA) and incubated overnight at 4 °C. After washing 3 times 5 min with PBS, the sections were further incubated for 30 min with a biotinylated secondary antibody. Between incubations, the tissues were washed with PBS 3 times 5 min. The sections were visualized using 0.05% 3,3-diaminobenzidine tetrachloride (DAB), and the whole reaction was developed for 5 min in 3% H_2O_2 until the desired stain intensity was obtained. A negative control group was included and consisted of substituting normal rat serum for primary antibody. Control groups were included to determine the specificity of the immunostaining. The staining of BCL-2, HSP70, BAX, and P53 was observed and imaged with an Olympus BX50-camera (Japan).

2.9. Statistical analysis

Data were expressed as the mean ± S.D. Statistical differences were evaluated by using one-way ANOVA. The level for a statistically significant difference was set at $p < 0.05$.

3. Results

3.1. Neurological and Y-maze task results

All animals were fully awake about 40 min after MCAO. Rats in the sham-operated group had no neurological deficit. The VD model rats failed to extend their right forepaw fully or circled to the right or failed to the right. However, ginsenoside Rg_2 administration at the doses of 2.5, 5 and 10 mg/kg improved the behavior. Neurological responses of the ginsenoside Rg_2 (2.5, 5 and 10 mg/kg) or nimodipine (50 μg/kg) groups improved compared with the VD group ($p < 0.01$ or 0.05). Ginsenoside Rg_2 at 10 mg/kg was more effective than at 2.5 mg/kg ($p < 0.01$). Rats were tested for memory performance in the Y-maze at 48 h after reperfusion. The memory ability of the VD rats was significantly lower than that of the sham, ginsenoside Rg_2 (2.5, 5 and 10 mg/kg)-treated and nimodipine (50 μg/kg)-treated groups ($p < 0.01$) (shock times needed to achieve a 9/10 consecutive avoidances were significantly decreased in these latter groups). In addition, no significant differences were observed among the ginsenoside Rg_2-treated at the doses of 2.5, 5 or 10 mg/kg (Table 1, $F_{\text{neurological score}} = 74.092$; $F_{\text{9/10 consecutive avoidances}} = 40.998$).

3.2. Histopathological observation

TTC staining (Fig. 1a) showed that the normal tissues were stained red and the infarct tissues white, which indicated brain tissue ischemia in rats with MCAO. HE staining (Fig. 1b) indicated that for rats in the VD group many neurons became pyknotic and deep-stained around the infarcts, but rats in the sham-operated group showed no neuronal defects. Nissl staining (Fig. 1c) indicated that the infarct areas were located

Table 1
Effects of ginsenosides Rg_2 on neurological responses and memory performance in rats subjected to MCAO-reperfusion

Group	n	Neurological score 24 h after MCAO-reperfusion	9/10 consecutive avoidances
Control	8	0.00 ± 0.00[a]	1.0 ± 0.00[a]
Sham-operation	8	0.00 ± 0.00[a]	1.0 ± 0.00[a]
Model	8	3.13 ± 0.35	25.4 ± 6.44
Nimodipine (50 μg/kg)	8	2.38 ± 0.52[a]	3.5 ± 2.93[a]
Ginsenoside Rg_2 (2.5 mg/kg)	8	2.63 ± 0.52[a,b]	7.6 ± 5.10[a]
Ginsenoside Rg_2 (5 mg/kg)	8	2.00 ± 0.53[a]	3.5 ± 3.21[a]
Ginsenoside Rg_2 (10 mg/kg)	8	1.75 ± 0.46[a]	3.5 ± 2.93[a]

$N = 8$. Data represent mean ± S.D.
[a] $p < 0.01$.
[b] $p < 0.05$ vs. model.

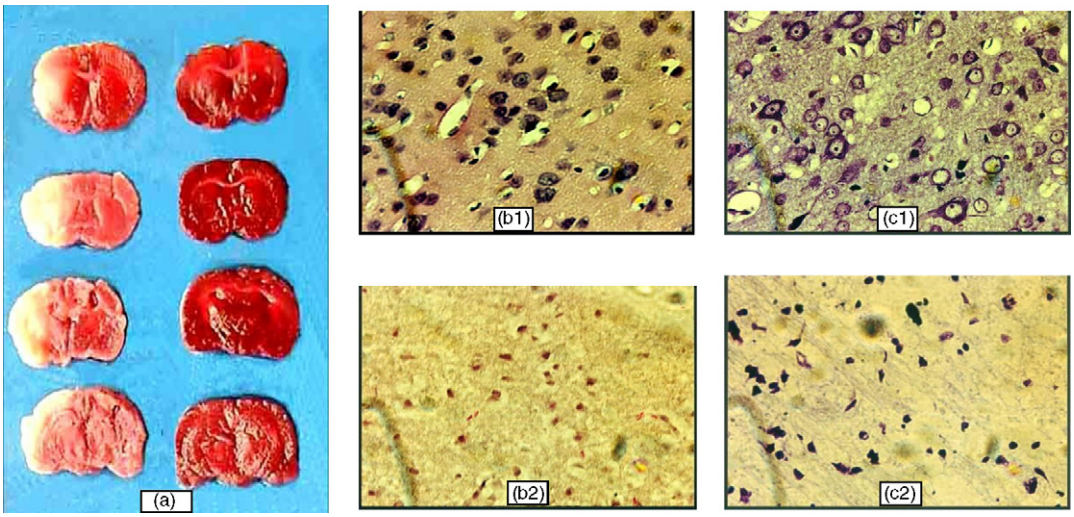

Fig. 1. (a) Two-millimeter thick, TTC stained coronal sections of brains from rats subjected to transient occlusion of the middle cerebral artery for 1 h followed by 48 h of reperfusion. The highlighted non-stained core area is white, which is surrounded by a pink penumbra in the cortex, and a smaller penumbra area is also identified within the striatal region. The viable brain tissue is red in the figure. (b) Representative photographs representing HE staining of the cerebral cortex and the striatal region. *Note*: many neurons become pyknotic and deep-stained around the infarcts (b2) and no evident cell damage is present in the cerebral cortex and striatal region (b1). (c) Representative photographs representing Nissl staining of the cerebral cortex and the striatal region. There are a reduced number of neurons in ischemia-reperfusion side (c2) as compared with the no ischemia-reperfusion side (c1). Neurons were counted in the caudate-putamen area under microscope (40 × 10). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

mainly at the corpus striatum and the parietal lobe. The number of neuronal cells was reduced greatly after MCAO in caudate-putamen and the number of neuronal cells in the sham-operated group was normal.

3.3. Immunocytochemistry result

The positive cell number of BCL-2 (Fig. 2) and HSP70 (Fig. 3) was significantly decreased in the caudate-putamen of VD model rats compared to that of ginsenoside Rg_2 (2.5, 5 and 10 mg/kg)-treated and nimodipine (50 μg/kg)-treated rats ($p < 0.01$). Moreover, the positive cell number of BCL-2 and HSP70 was lower in the ginsenoside Rg_2 (2.5 mg/kg)-treated ($p < 0.01$), and higher in ginsenoside Rg_2 (5 and 10 mg/kg)-treated group compared with that of nimodipine-treated group ($p < 0.01$ or 0.05, Table 2, $F_{bcl\text{-}2} = 457.252$; $F_{hsp70} = 2081.223$).

In contrast, the positive cell number of BAX (Fig. 4) and P53 (Fig. 5) was increased in the VD group when compared with the sham group ($p < 0.01$). Ginsenoside Rg_2 (2.5, 5 and 10 mg/kg) and nimodipine (50 μg/kg) treatment decreased the positive cell number compared to the VD group ($p < 0.01$). No significant change in the number of BAX and P53-positive cells was seen in the ginsenoside Rg_2 treatment at the dose of 5 mg/kg group

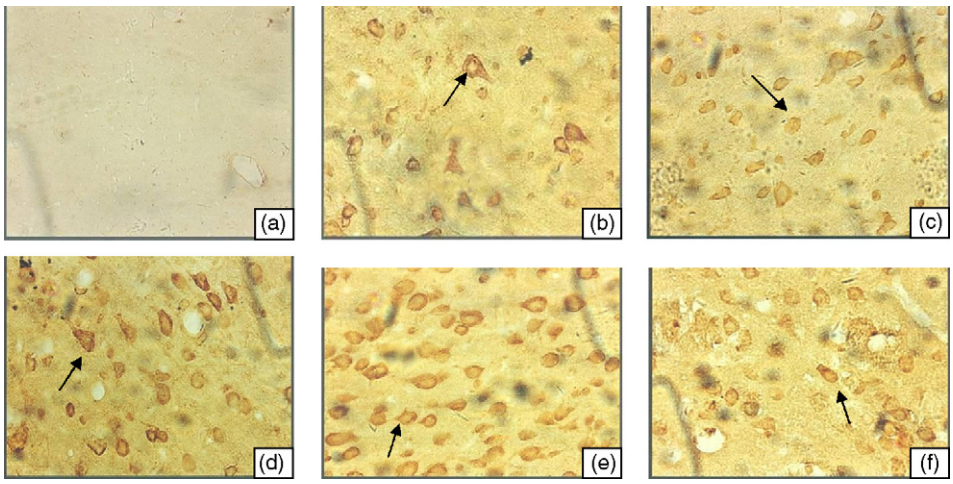

Fig. 2. Changes of immunohistochemical staining against BCL-2 in the neuronal cells of the caudate-putamen area after 1 h followed by 48 h of reperfusion. Panels show sham group (a), VD group (b), ginsenoside Rg_2 (2.5 mg/kg) group (c), ginsenoside Rg_2 (5 mg/kg) group (d), ginsenoside Rg_2 (10 mg/kg) group (e) and nimodipine group (50 ug/kg) (f). The positive number of BCL-2 cells in the VD group was decreased compared with sham group. The positive number of BCL-2 cells was significantly increased in the ginsenosides Rg_2 and nimodipine treatment groups. Moreover, cells of the ginsenoside Rg_2 (2.5 mg/kg) group (c) was lower than that of nimodipine group (f) and the ginsenosides Rg_2 (5 mg/kg) (d) and (10 mg/kg) groups (e) were higher than that of nimodipine group (f). Neurons were counted under microscope (40 × 10) and five different visual field was counted.

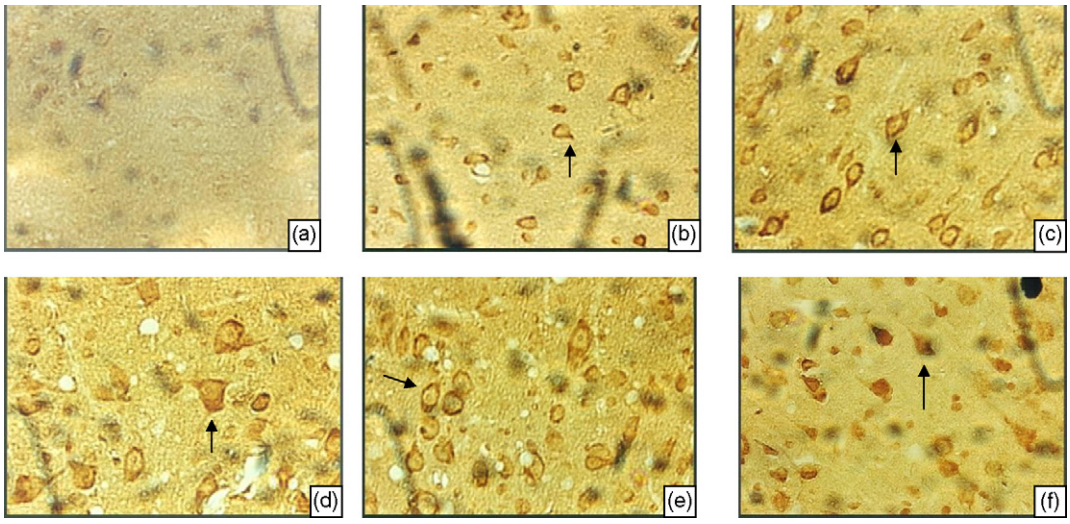

Fig. 3. Changes of immunohistochemical staining against HSP70 in the neuronal cells of the caudate-putamen area after 1 h followed by 48 h of reperfusion. Panels show sham group brain (a), VD group (b), ginsenoside Rg_2 (2.5 mg/kg) group brain (c), ginsenoside Rg_2 (5 mg/kg) group brain (d), ginsenoside Rg_2 (10 mg/kg) group brain (e) and nimodipine group (50 μg/kg) brain (f). The positive number of HSP70 cells in the VD group was decreased compared with sham group. The positive number of HSP70 cells was significantly increased in the ginsenosides Rg_2 and nimodipine treatment group. Moreover, cells of the ginsenosides Rg_2 (2.5 mg/kg) group (c) was lower than that of nimodipine group (f) and ginsenosides Rg_2 (5 mg/kg) (d) and (10 mg/kg) group (e) were higher than that of nimodipine group (f). Neurons were counted under microscope (40 × 10) and five different visual field was counted.

Table 2
Effects of ginsenoside Rg_2 on BCL-2, HSP70 positive neuronal numbers of the caudate-putamen in rats subjected to MCAO-reperfusion

Group	*n*	BCL-2 positive neuronal numbers	HSP70 positive neuronal numbers
Control	8	0.0 ± 0.00[a]	0.0 ± 0.00[a]
Sham-operation	8	1.8 ± 0.53[a]	1.8 ± 1.07[a]
Model	8	18.3 ± 1.35	42.9 ± 1.79
Nimodipine (50 μg/kg)	8	54.8 ± 6.93[a]	94.9 ± 4.93[a]
Ginsenoside Rg_2 (2.5 mg/kg)	8	28.6 ± 4.18[a,b]	72.9 ± 1.86[a,b]
Ginsenoside Rg_2 (5 mg/kg)	8	62.7 ± 1.90[a,c]	98.7 ± 2.07[a,c]
Ginsenoside Rg_2 (10 mg/kg)	8	120.5 ± 3.21[a,b]	134.8 ± 3.46[a,b]

$N = 8$. Data represent mean ± S.D.
[a] $p < 0.01$ vs. model.
[b] $p < 0.01$.
[c] $p < 0.05$ vs. nimodipine.

versus the nimodipine-treated group ($p > 0.05$). However, ginsenoside Rg_2 at the doses of 2.5 and 10 mg/kg group significantly changed the cell numbers when compared with the nimodipine (50 μg/kg)-treated group ($p < 0.01$, Table 3, $F_{bax} = 388.248$; $F_{p53} = 1016.711$).

4. Discussion

In this work we have studied the protective effect of ginsenoside Rg_2 (2, 5, or 10 mg/kg) on memory impairment induced by cerebral ischemia-reperfusion, and the expression of neu-

Table 3
Effects of ginsenoside Rg_2 on BAX, P53 positive neuronal numbers of the caudate-putamen in rats subjected to MCAO-reperfusion

Group	*n*	BAX positive numbers	P53 positive neuronal numbers neurons
Control	8	0.0 ± 0.00[a]	0.0 ± 0.00[a]
Sham-operation	8	1.4 ± 0.51[a]	1.1 ± 0.68[a]
Model	8	165 ± 12.40	137.5 ± 3.15
Nimodipine (50 μg/kg)	8	90.1 ± 2.82[a]	77.2 ± 5.04[a]
Ginsenoside Rg_2 (2.5 mg/kg)	8	120 ± 6.13[a,b]	98.4 ± 2.41[a,b]
Ginsenoside Rg_2 (5 mg/kg)	8	91.9 ± 1.27[a,c]	75.9 ± 3.69[a,c]
Ginsenoside Rg_2 (10 mg/kg)	8	55.4 ± 4.61[a,b]	45.3 ± 1.67[a,b]

$N = 8$. Data represent mean ± S.D.
[a] $p < 0.01$ vs. model.
[b] $p < 0.01$.
[c] $p < 0.05$ vs. nimodipine.

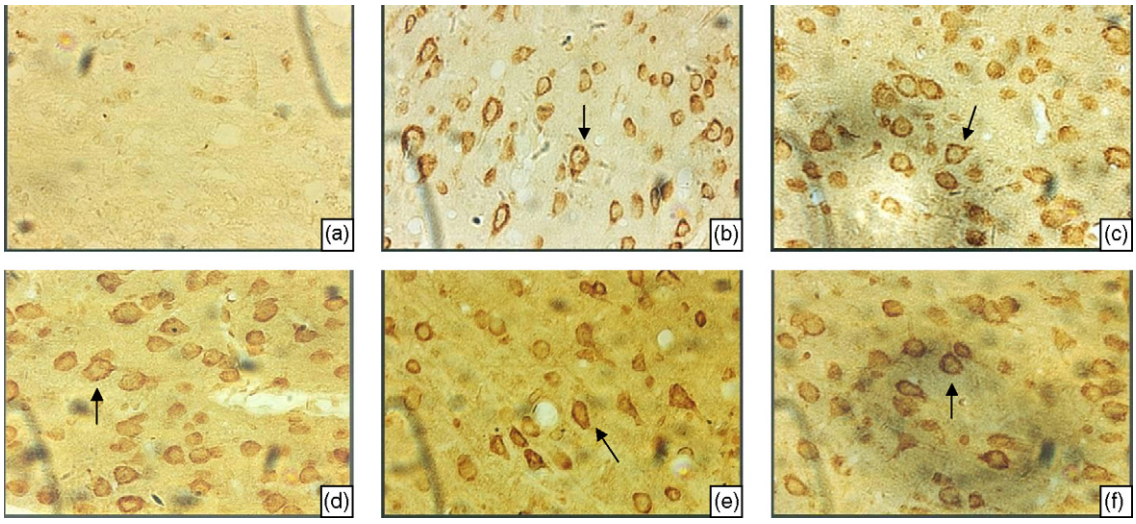

Fig. 4. Changes of immunohistochemical staining against BAX in the neuronal cells of the caudate-putamen area after 1 h followed by 48 h of reperfusion. Panels show sham group brain (a), VD group (b), ginsenoside Rg_2 (2.5 mg/kg) group brain (c), ginsenoside Rg_2 (5 mg/kg) group brain (d), ginsenoside Rg_2 (10 mg/kg) group brain (e) and nimodipine (50 μg/kg) group brain (f). The positive number of BAX cells in the VD group was increased compared with sham group. The positive number of BAX cells was significantly decreased in the ginsenosides Rg_2 and nimodipine treatment group. No significant change of the number of BAX positive cells was seen when ginsenosides Rg_2 (5 mg/kg) group (d) compared with the nimodipine group (f). The positive number of BAX cells in ginsenosides Rg_2 (2.5 mg/kg) and ginsenosides Rg_2 (10 mg/kg) group changed significantly when compared with nimodipine group (f). Neurons were counted under microscope (40 × 10) and five different visual field was counted.

ronal apoptosis as a possible mechanism related to this memory impairment.

The doses of ginsenoside Rg_2 (2.5, 5, 10 mg/kg) selected were based on a previous study, which demonstrated that ginsenoside Rg_2 (2.5, 5, 10 mg/kg) can improve chemical myocardial ischemia in rats (Tian et al., 2003). Ginseng and Ginkgo are the commonly used herbal remedies. They have been shown to produce differential cognitive improvements following a single dose of each and a combination of standardized extracts of Ginkgo and Panax ginseng may improve aspects of cognitive performance present both in pathological and healthy middle aged populations (Kennedy et al., 2001). Ginkgo and ginseng also have some effects on dementia and intermittent claudication (Gold et al., 2001; Ernst, 2002).

Research examining the neurobiological bases of memory in mammals suggests that memory is organized in multiple brain systems (McDonald and White, 1993; White and McDonald, 2002). In rats, double dissociations between the mnemonic func-

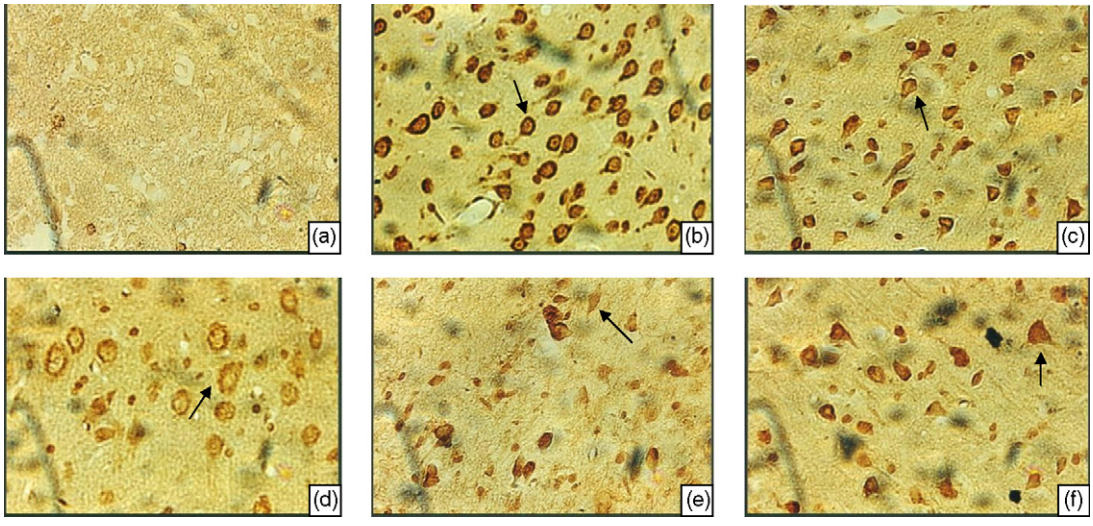

Fig. 5. Changes of immunohistochemical staining against P53 in the neuronal cells of the caudate-putamen area after 1 h followed by 48 h of reperfusion. Panels show sham group brain (a), VD group (b), ginsenoside Rg_2 (2.5 mg/kg) group brain (c), ginsenoside Rg_2 (5 mg/kg) group brain (d), ginsenoside Rg_2 (10 mg/kg) group brain (e) and nimodipine (50 μg/kg) group brain (f). The positive number of P53 cells in the VD group was increased compared with sham group. The positive number of P53 cells was significantly decreased in the ginsenosides Rg_2 and nimodipine treatment group. No significant change of the number of P53 positive cells was seen when ginsenosides Rg_2 (5 mg/kg) group (d) compared with the nimodipine group (f). The positive number of P53 cells in ginsenosides Rg_2 (2.5 mg/kg) and ginsenosides Rg_2 (10 mg/kg) group changed significantly when compared with nimodipine group (f). Neurons were counted under microscope (40 × 10) and five different visual field was counted.

tions of the hippocampal system and caudate-putamen have been observed following irreversible (Kesner et al., 1993) and reversible (Packard and McGaugh, 1996) memory perturbations. Animal studies have suggested that caudate nuclei are essential for cognitive functions. It was reported that electrical stimulation of the caudate nucleus produced a declarative memory disturbance (Rosvold and Delgado, 1956). More recently, it has been demonstrated that damage to the medial caudate nucleus results in short-term memory deficits for directional information while the left caudate nucleus may be related with both declarative memory and procedural memory (Mizuta and Motomura, 2006). Accordingly, we choose the caudate-putamen as our region to study the caudate-putamen dependent memory.

Our data indicated that ischemia 1 h and reperfusion 48 h can induce memory impairment in rats. The ethological results showed ginsenoside Rg_2 can improve the cerebral ischemia-reperfusion-induced memory impairment. These results are consistent with earlier reports on the cyproheptadine (CYP)-induced recognitional deficits (Ma and Yu, 1993), which reduces the acetylcholine-evoked catecholamine release from bovine adrenal chromaffin cells (Tachikawa et al., 1995), and ginsenoside Rg_2 blocks human neuronal nicotinic acetylcholine receptors by a noncompetitive mechanism in Xenopus oocytes (Sala et al., 2002). The previous studies suggested that cerebral ischemia-reperfusion might be related to the cell apoptosis (Ferrer et al., 2003; Greco et al., 2007). Although a considerable amount of research on other functions of ginsenosides had been performed in previous studies, the potential for ginsenoside Rg_2 to affect memory impairment via anti-apoptosis has not been reported. To clarify the relationship between cell apoptosis and the memory impairment induced by cerebral ischemia-reperfusion, and the effects of ginsenoside Rg_2 on this process, we examined the effects of ginsenoside Rg_2 on a vascular dementia rat model.

Cell apoptosis is an essential component for the normal development of any multicellular organism (Ditzel and Meier, 2002). Early cell changes that occur during apoptosis are associated with mitochondrial changes mediated by the BCL-2 family of proteins, including the anti-apoptotic BCL-2 and pro-apoptotic BAX proteins (Stewart and Pietenpol, 2001). Some reports indicate that HSP70 enhances neuronal survival during transient focal cerebral ischemia or excitotoxin-induced seizures (Yenari et al., 1998; Lee et al., 2004). Numerous studies have indicated that P53 plays a key role in apoptotic cell death following certain types of toxic stress (Morrison et al., 1996; Uberti et al., 1998; Katayama et al., 2001; Ueno et al., 2002). P53-induced apoptosis is proposed to be mediated by the transactivation of BAX (Miyashita and Reed, 1995) and P53-inducible genes (Polyak et al., 1997). BAX expression is up-regulated during P53-dependent apoptosis (Miyashita and Reed, 1995) and Bax activates apoptosis (Nataraj et al., 1995). We observed that the protein expression of BAX and P53 increased and BCL-2 and HSP70 decreased after cerebral ischemia and reperfusion injury. BCL-2 and HSP70 were increased and BAX and P53 were inhibited by ginsenosideRg_2. These results are consistent with earlier report on the ultraviolet B-induced DNA repair synthesis and apoptosis in NIH3T3 cells (Jeong et al., 2007). Moreover, we found an effect of ginsenoside Rg_2 (5 mg/kg) on regular protein expression of BAX, P53 and BCL-2, HSP70 as well as with nimodipine (50 μg/kg). BCL-2 and HSP70 are the most important anti-apoptotic proteins and can antagonize apoptosis by depressing calcium overload and free radical production, preventing signal transmission of pro-apoptotic genes and expression of gene products (MacManus and Linnik, 1997); however, BAX and P53 play a passive role in cell apoptosis (Kaneda et al., 1999). It has been suggested that ginsenoside Rg_2 can block calcium channels and display anti-free-radical activity (Jiang et al., 1996). Within our own laboratory we have shown that ginsenoside Rg_2 can increase cell viability, decrease [Ca2+]i, lipid peroxidation (the excessive production of MDA, NO) and the protein expression levels of calpain II, caspase-3 and Aβ1–40 in PC12 cells introduced by glutamate (Li et al., 2007). Moreover, it has been suggested that ginsenoside Rg_2 acts on nicotinic acetylcholine receptor-operated cation channels, inhibiting Na^+ influx through the channels and consequently reducing both Ca^{2+} influx and catecholamine secretion in chromaffin cells (Tachikawa et al., 1995).

In conclusion, the present study demonstrates a beneficial effect of ginsenoside Rg_2 on the memory impairment induced by ischemia-reperfusion, possibly through prevention of the development of apoptosis. Ginsenoside Rg_2 may mediate this effect by down-regulating the expression of pro-apoptotic factors BAX and P53 and up-regulating BCL-2 and HSP70. According to previous studies and our current results study, the data indicate that ginsenoside Rg_2 (at a range of some dosage) acts as a neuroprotectant by various pathways. These results demonstrate that ginsenoside Rg_2 has a neuroprotective effect and that this drug may be useful for treating cerebro-vascular dementia that develops consequent to stroke or other ischemic insults.

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