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Original Article

Sevoflurane Postconditioning Increases the Activities of Antioxidant Enzymes and Improves Spatial Learning and Memory after Cardiac Arrest in Rats

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ABSTRACT

Background: Sevoflurane, as a novel volatile anesthetic with minimal pungency, low solubility, and less toxicity, is widely used in anesthetic practice. The purpose of this study was to investigate the effect of sevoflurane postconditioning on endogenous antioxidant status and spatial learning and memory ability after cardiac arrest (CA) and the potential underlying mechanisms.

Methods: A rat model of CA was established by delivering an alternating current between the esophagus and chest wall to induce ventricular fibrillation. Animals were randomly divided into three groups: sham group (no CA), CA group, and CA + sevoflurane postconditioning (CA + SE) group. Sevoflurane postconditioning was achieved by administration of 2.5% sevoflurane for 60 min after resuscitation. The spatial learning and memory ability of rats was measured by the Morris water maze. The antioxidant enzymes activities were assessed at 4 hours, 1 day and 8 days after resuscitation. Moreover, the expressions of hippocampal proteins which could serve as memory enhancement biomarkers including growth-associated protein-43 (GAP-43), postsynaptic density-95 (PSD-95) and the transcription factor c-jun/activator protein-1 (AP-1), were detected at 8 days after resuscitation.

Results: We found that CA significantly decreased the ability of spatial learning and memory in contrast to the sham controls. However, sevoflurane postconditioning significantly increased survival rate and antioxidant enzyme activities as well as ameliorated the spatial learning and memory deficits induced by CA. Furthermore, sevoflurane postconditioning regulated hippocampal memory enhancement proteins.

Conclusions: Postconditioning with sevoflurane improved learning and memory deficits in a CA model possibly due to the reduction of oxidative stress and up-regulation of the memory enhancement biomarkers in hippocampus.

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Cardiac arrest (CA), a serious threat to human life, is the cessation of normal circulation of the blood due to failure of the heart to contract effectively. CA causes a temporary or permanent reduction of cerebral blood flow that leads to various brain, blood and oxygen supply disorders in conjunction with global hypoxic-ischemia, and even causes the death of neurons.

Although different mechanisms are involved in the pathogenesis of CA, increasing evidences have indicated that oxidative stress accounts for its pathogenic progression. A substantial body of evidence has suggested that it links the production of reactive oxygen species (ROS) generated after cerebral ischemic reperfusion and subsequent oxidative damage to the pathogenesis of ischemia-

reperfusion (1-3). ROS are also threatened to neuronal survival by their ability to propagate the initial attack on lipid rich membranes of the brain to cause lipid peroxidation (LPO). Superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) are involved in the intracellular defense against ROS, which are usually scavenged by these antioxidant enzymes (4-6).

Sevoflurane, a novel volatile anesthetic with minimal pungency, low solubility and less toxicity, is widely used in anesthetic practice. Recently, many studies have demonstrated that sevoflurane preconditioning could alleviate hypoxic and ischemia-reperfusion injury in the brain (7-9). However, the clinical use of sevoflurane is limited as ischemic episodes are mostly unpredictable. On the contrast, the onset of reperfusion is more often predictable. Therefore, the concept of postconditioning through modulation of reperfusion rather than ischemia has been proposed. Previous studies have suggested that the effect of sevoflurane to protect organs from ischemia-reperfusion injury and neuron death by administration immediately at the onset of reperfusion may be attributed to sevoflurane's antioxidant and free radical scavenging actions (10, 11).

The hippocampus and cortex of rats are the most vulnerable brain regions to global ischemia/reperfusion injury in terms of cognitive dysfunction, particularly memory decline (12, 13), which are often accompanied with cardiopulmonary resuscitation after CA. Moreover, spatial memory in rats and humans is largely dependent on the hippocampus, and hippocampal neuronal damage induced by ischemia is associated with spatial memory impairment (12-14). Brain functions, such as memory formation and recovery of function after injury, depend on subtle regulation of hippocampal proteins, including growth-associated protein-43 (GAP-43) (15), postsynaptic density-95 (PSD-95) (16) and the transcription factor c-jun/activator protein-1 (AP-1) (17). The functions of these hippocampal proteins range from synaptic vesicle recycling and exocytosis to neurotransmission and signal transduction. Reducing the expression of these hippocampal proteins leads to the impairment of memory and synaptic function. As shown in previous studies, preconditioning with sevoflurane could ameliorate the ability of spatial learning and memory after focal cerebral ischemia-reperfusion in rats (18). However, there have been few studies reporting the mechanisms underlying the effect of sevoflurane postconditioning on memory in cerebral ischemia animal models.

Therefore, the purpose of the present study was to investigate the effect of sevoflurane postconditioning on the up-regulation of antioxidant enzymes activities, spatial learning and memory ability and the expression of hippocampus proteins that serve as memory enhancement biomarkers after global cerebral ischemia-reperfusion injury induced by CA.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats weighing 350-450 g from Central South University were used. They were housed in a temperature-controlled (22-24℃) room with a 12-h dark-light cycle with free access to food and water ad libitum. All procedures used in this study were approved by the Ethics Committee for the use of experimental animals at Xiangya Hospital affiliated to Central South University.

Establishment of a CA Animal Model

A rat model of CA was established by delivering an alternating current between the esophagus and chest wall to induce ventricular fibrillation as previously reported (19). All animals were fasted but given free access to water overnight prior to the experiment. Animals were anaesthetized by a single dose intraperitoneal injection of chloral hydrate (350 mg/kg). Animals were intubated and mechanically ventilated (Harvard Model 683 Small Animal Ventilator, Harvard Apparatus, Massachusetts) with a mixture of 70% N_2O and 30% O_2 at a tidal volume of 8 ml/kg. The breathing rate was approximately 60 breaths per min and adapted to maintain normocapnia (assessed by blood gas analyses) and no positive end-expiratory pressure. Polyethylene catheters were inserted into the left femoral artery and vein for arterial blood pressure monitoring or drug injection. An electrocardiogram was obtained with three subcutaneous needle electrodes, and the tympanic temperature was recorded and kept with-

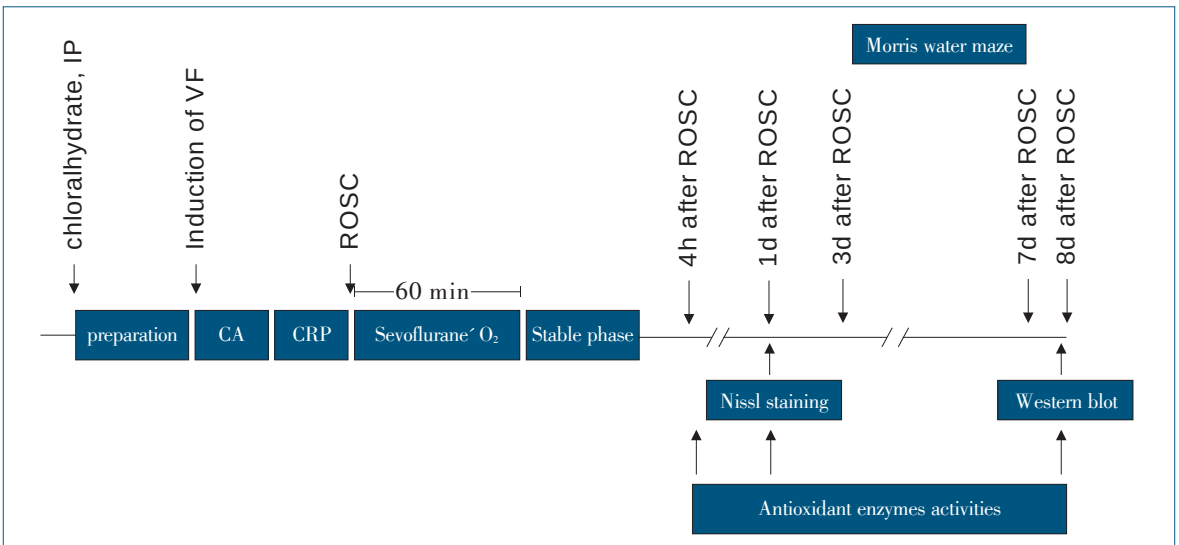

Figure 1. Experimental Procedures.

in a range of 36.5-37.5℃ by a heating plate (Hot Plates Typ 062, Labotect, Germany) on which the animals were placed. A BL-420s multichannel physiological signal recording system (Chengdu Taimeng Science and Technology Co., Ltd., Chengdu, China) was used to record the electrocardiogram, rectal and tympanic temperature and arterial blood pressure. Ventricular fibrillation was initiated using an alternating current (12 V, 50 Hz) via an oesophageal electrode and ventilation was stopped. CA was identified using the following criteria: 1) the systolic arterial pressure after electrical stimulation gradually fell to below 25 mm Hg; 2) pulsations in the arterial pressure waveform disappeared. After reaching the criteria for CA, electrical stimulation was performed for 2 minutes followed by 5 minutes of observation without treatment. After 7 minutes of CA, cardiopulmonary resuscitation (CPR) was started as follows: 60 breaths/min (100% oxygen), external manual chest compressions at a rate of 200 min^{-1}, duty cycle 50%, and a compression depth of 25% of the anterior-posterior diameter of the chest wall. After 2 minutes of CPR without return of spontaneous circulation (ROSC), a defibrillation attempt was performed with one biphasic shock of 1 Joule (M-Series, Zoll Corporation, Germany). CPR was continued and adrenaline was administered (epinephrine, 20 μg/kg) if ROSC could not be achieved within 30 seconds after the first defibrillation attempt. Defibrillation procedures were repeated every 30 seconds for up to 6 minutes. In case of unsuccessful CPR, resuscitation was stopped and the animal was declared dead. ROSC was defined as maintenance of an unassisted mean arterial pressure (MAP) beyond values of 50 mm Hg for at least 10 consecutive minutes. No further defibrillation or cardioversion, vasopressors or antiarrhythmic drugs were allowed once ROSC was achieved. Breathing rate was adjusted to reach normocapnia 20 minutes after ROSC and maintained until adequate spontaneous ventilation and extubation. Application of sodium bicarbonate (8.4%) was titrated according to the blood gas analyses aiming at a base excess of 0 to -5 mM for 20 minutes after ROSC. Animals without ROSC following standard CPR for 15 minutes were defined as resuscitation failures.

Experimental Design

Rats were randomly assigned to one of the 3 treatment groups: sham group (no CA), CA group, and CA + sevoflurane postcondtioning (CA + SE) group. The core temperature of the rats was continuously measured with a rectal temperature probe and maintained at 37 ± 0.5℃ using an infrared thermolamp until the animals awoke or 4 hours after ROSC. Arterial and venous catheterization, anesthesia and endotracheal intubation were similarly performed in the sham group. An esophageal electrode was implanted in the sham group with a length of 10

cm from the incisor, and then electrical stimulation using the same parameters was performed for 90 seconds to induce generalized twitching but not CA. In the CA group, ventricular fibrillation was induced for 7 minutes and then standard CPR was performed. Rats inhaled 2.5% Sevoflurane for 30 minutes after ROSC in the CA + SE group. The experiment procedure of our study was shown in figure 1.

Morris Water Maze Test

The Morris water maze test was performed as described previously (20). The test was started on day 3 after operation and conducted at 9:00 to 12:00 AM. The water maze apparatus consisted of a circular pool 150 cm in diameter and 60 cm in height, and was filled to a depth of 30 cm with water at 23±1℃ to cover a black platform (diameter 10 cm). The platform was submerged approximately 1.5 cm below the surface of the water, which was darkened with 20 ml of Chinese ink. The pool was divided into four quadrants with four starting locations called north (N), east (E), south (S) and west (W) at equal distances on the rim. The platform was placed in the center of the northeast quadrant equidistant from the center and the edge of the pool. Spatial navigation the location of the hidden platform was kept constant during all trials and the rats were given four trials per day to find the hidden platform for 5 consecutive days (maximum trial duration 60 seconds, 15 seconds reinforcement on the platform, 30 seconds recovery period between trials). Each rat was placed in the water facing the wall at one of the three starting locations (S, W or E). The rats that failed to find the platform within 60 seconds were then physically placed on the platform for 15 seconds. The parameters including escape latency (i.e. time to reach the platform, seconds), path length (i.e. distance to reach the platform, cm), swim speed (cm/s) and thigmotaxis (i.e. the proportion of the total distance that the rats swam within 10 cm of the maze wall, %) were analyzed for all 5 days. Following a probe test conducted 24 hours after the last training session, the platform was removed and rats were allowed to swim for 60 seconds in search of it. The proportion of time spent in the target quadrant, the number of platform crossings, the path in target quadrant and the number of target quadrant entries were monitored and recorded by a video camera linked to a computer-based image analyzer. To prevent hypothermia, the animals were gently dried with a cotton towel between and after the trial. All trial videos were recorded and analyzed using Smart TM video tracking software (San Diego, CA). Rats were tested in the Morris water maze and data were recorded and analyzed by investigators who were blinded with respect to the groups.

Tissue Harvest

Tissues were prepared for antioxidant enzyme and glutathione assays as previously described (21). At 4 hours, 1 day and 8 days after resuscitation, the animals were sacrificed and their brains were harvested. Hippocampal tissue was digested to give 5% (w/v) homogenate (10 mM PB, pH 7.0, containing 10 μl/ml protease inhibitors, 5 mM leupeptin, 1.5 mM aprotinin, 2 mM phenylethylsulfonylfluoride [PMSF], 3 mM peptastatin A, 0.1 mM EGTA, 1 mM benzamidine and 0.04% butylated hydroxytoluene) and centrifuged at 800 g for 5 minutes at 4 ℃. The supernatant (S1) was used for the assay of thiobarbituric acid reactive substances (TBARS). The remaining S1 was recentrifuged at 10,500 g for 15 minutes at 4℃ (S2) to separate post mitochondrial supernatant (PMS), which was used for the estimation of antioxidant enzymes and GSH.

Assay for Lipid Peroxidation

Lipid peroxidation (LPO) was estimated by measuring the levels of TBARS. Briefly, 0.2 ml S1 was pipetted in 2.0 ml flat bottom eppendorf tube and incubated at 37℃ in a water bath shaker at 120 strokes up and down. 0.2 ml of the same S1 was pipetted in an eppendorf tube and incubated at 0℃. After 1 h of incubation, 0.4 ml of 5% trichloroacetic acid (TCA) and 0.4 ml of 0.67% thiobarbituric acid (TBA) was added in both samples (i.e. 37℃ and 0℃). The reaction mixture was centrifuged at 3000 g for 15 minutes. The supernatant was transferred to another test tube and placed in a boiling water bath for 10 minutes. Thereafter, the test tubes were cooled and the absorbance was read at 535 nm.

The rate of LPO was expressed as nmol of TBARS.

Assay for GSH

0.1 ml PMS was precipitated with 0.1 ml of sulfosalicylic acid (4%). The samples were kept at 4℃ for 1 hours and then centrifugated at 1200 g for 15 minutes at 4℃. The assay mixture contained 0.1 ml of a filtered aliquot, 1.7 ml PB (0.1 M, pH 7.4) and 0.2 ml DTNB (4 mg/ ml, 0.1 M PB, pH 7.4) in a total volume of 2.0 ml and the absorbance was read immediately at 412 nm. The GSH content was calculated as nmol GSH mg^{-1} protein, using molar extinction coefficient of 13.6×10^{3} $M^{-1}cm^{-1}$.

Determination of Superoxide Dismutase (SOD) Activity

SOD activity was measured spectrophotometrically by monitoring the auto-oxidation of (-)-epinephrine at pH 10.4 for 3 minutes at 480 nm. The reaction mixture contained glycine buffer (50 mM, pH 10.4) and 0.2 ml of PMS. The reaction was initiated by the addition of (-)-epinephrine. The enzyme activity was calculated as nmol (-)-epinephrine protected from oxidation/ min/mg of protein using molar extinction coefficient of 4.02×10^{3} $M^{-1}cm^{-1}$.

Determination of Catalase Activity

Briefly, the assay mixture consisted of 0.05 M PB (pH 7.0), 0.019 M hydrogen peroxide, and 0.05 ml PMS in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated as nmol H_2O_2 consumed/min/mg of protein.

Western Blotting

For Western blotting analysis, an equal amount of hippocampus protein (50-100 μg) was loaded on the gel and subjected to 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were then transferred to polyvinylidinene fluoride membranes (Millipore, Bedford, MA, USA), which were blocked in 5% non-fat dry milk prepared in 1 × TBST and incubated with primary antibodies overnight at 4℃. The following primary antibodies were used: GAP-43, PSD-95 and AP-1 antibodies (1:500, Cell Signaling Technology, Beverly, MA, USA), β-actin antibody (1:2000, Cell Signaling Technology, Beverly, MA, USA). The membranes were washed with 1 TBST solution and then incubated with secondary antibodies for 2 hours at room temperature. The blots were developed using ECL plus detection system (Beyotime Institute of Biotechnology) and the relative band density was measured using FluorChem FC2 System (NatureGene Corp., USA).

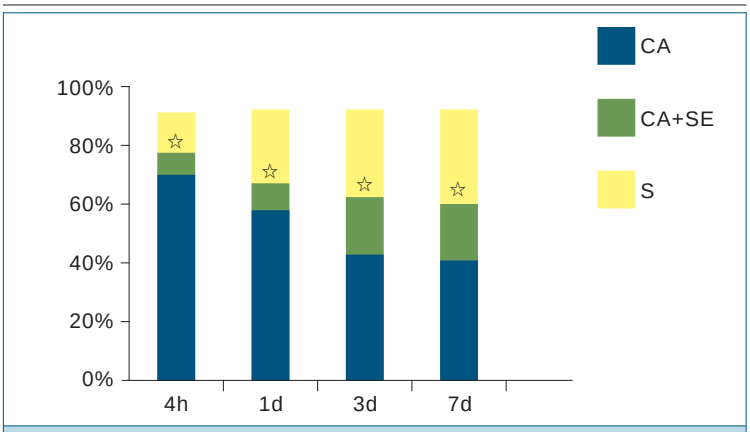

Figure 2. Survival Rates.
*P<0.05 vs. CA group.

Statistical Analysis

All measurement data were expressed as means ± standard deviation (SD). Group differences in the spatial acquisition trials in the Morris water maze task were analyzed using two-way repeated measures ANOVA . Group differences in the probe trials were evaluated with one-way ANOVA followed by Duncan′ s multiple range testing. Statistical Package for the social sciences (SPSS version 13.0) was used for the analysis. Differences were deemed statistically significant at $P<0.05$.

RESULTS

Survival Rate of Rats

Ventricular fibrillation was successfully induced in all rats in the CA and CA + SE groups. There were no significant differences in the resuscitation parameters among these groups ($P>0.05$, data not shown). The survival rate of rats was measured at 4 hour, 1 day, 3 days and 7 days after resuscitation. The survival rate of rats in the sham group was significantly different from those in the other 2 groups with CA. In addition, sevoflurane postconditioning significantly

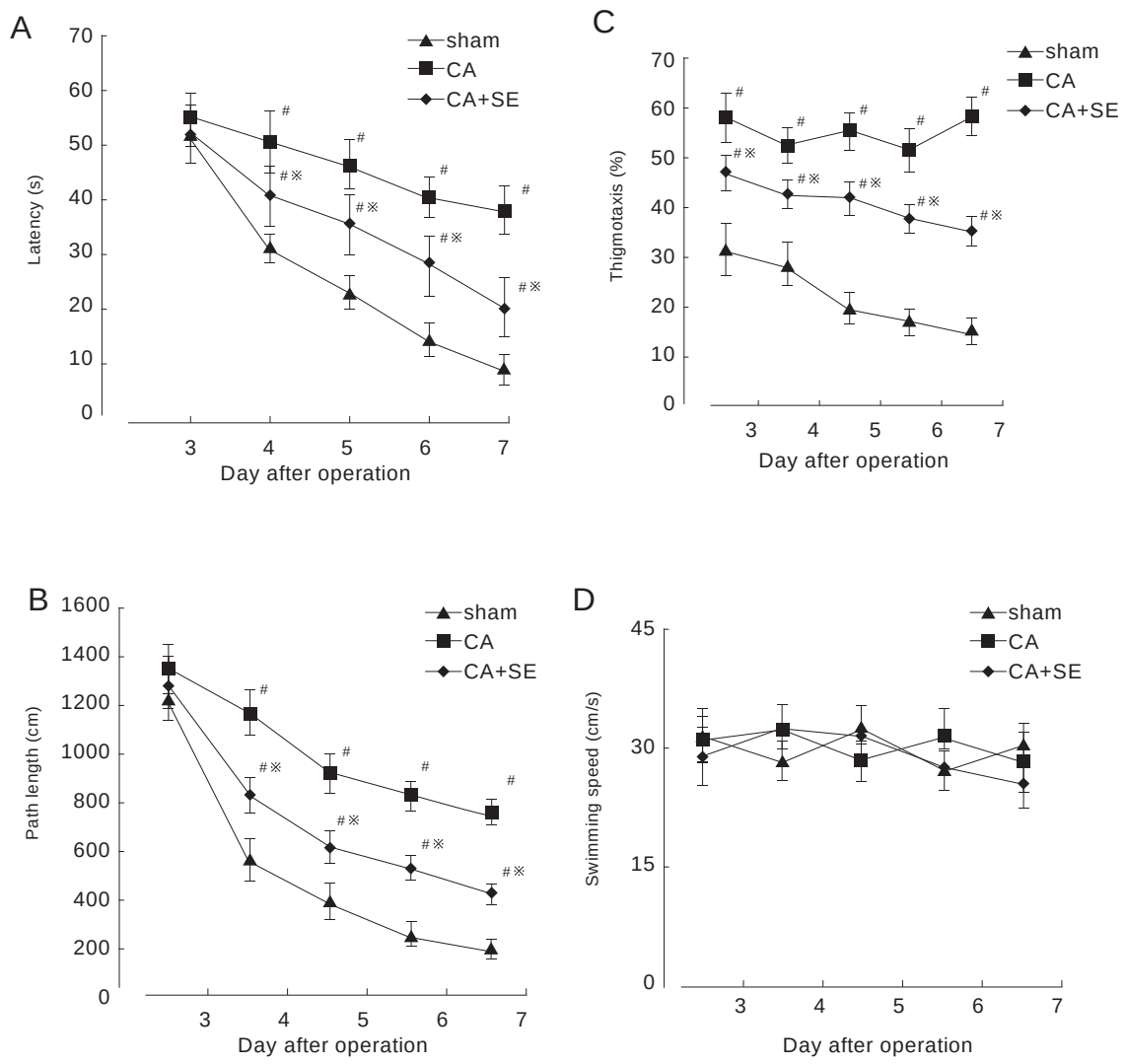

Figure 3. Acquisition Training in Morris Water Maze Test From the 3rd to 7th Day after Resuscitation.
A. Latency (seconds) to reach the platform; B. Path length (cm) to reach the platform; C. Thigmotaxis (%) in Morris water; D. Swimming speed (cm/s).
Data are expressed as means±SD. n=6-8, # P<0.05 vs. sham group, ※ P<0.05 vs. CA group.

increased survival rate compared with the CA group within 7 days after resuscitation (P< 0.05) (Figure 2).

Morris Water Maze Test

Three days after resuscitation, spatial learning and memory function was evaluated using the Morris water maze test. During the pre-training phase, all animals swam easily in all three groups. During the spatial acquisition phase, the mean latency of finding the platform and the path length to reach the platform declined progressively during the training period. However, rats in the CA group had more time of latency (P<0.01) (Figure 3A), longer distance of path length (P<0.05) (Figrue 3B) and higher proportion of thigmotaxis (P<0.05) (Figure 3C) when compared with the animals in the sham group. This prolongation of latency, path length and thigmotaxis were markedly suppressed by post-conditioning with sevoflurane (P<0.05) (Figure 3A-C). However, the swimming speed did not differ among three groups (Figure 3D).

The probe trial was made by removing the

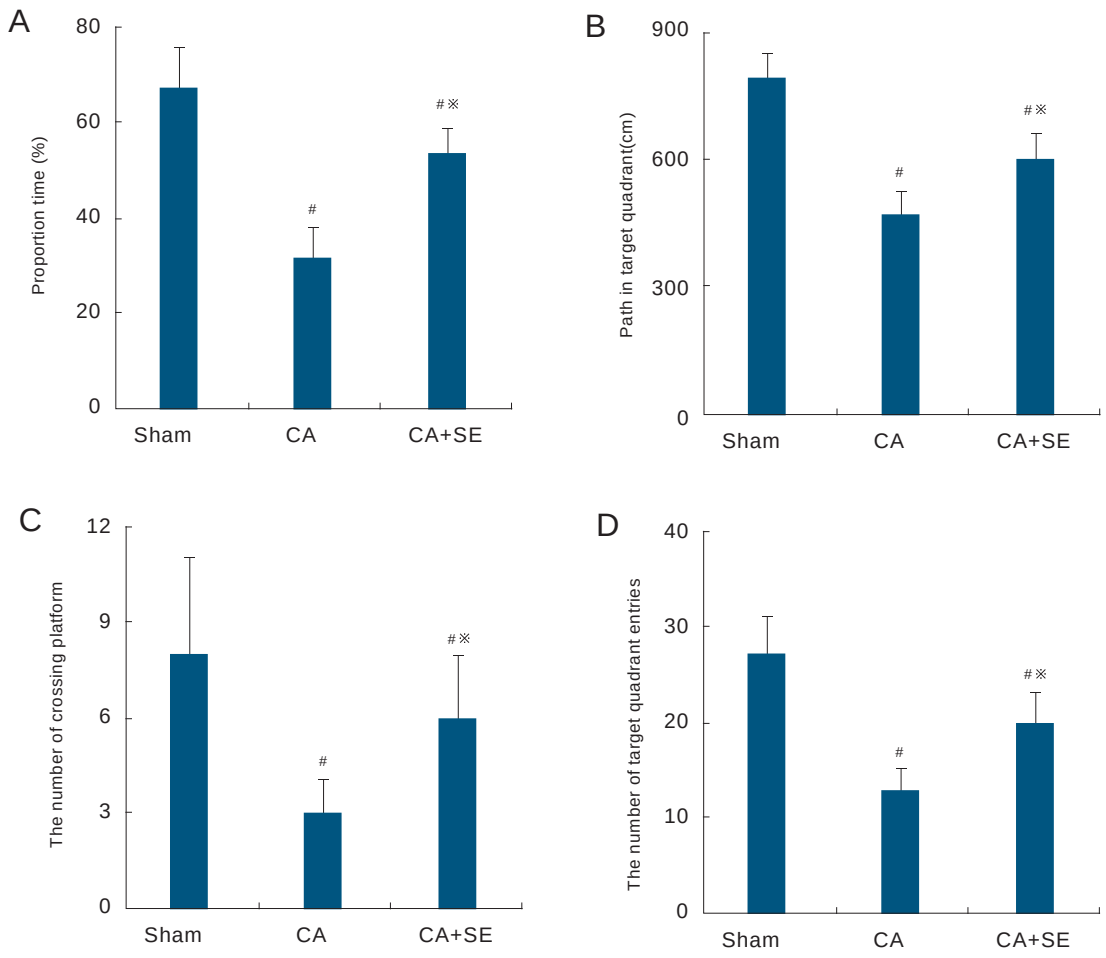

Figure 4. Probe Trial in Morris Water Maze Test on the 8th Day after resuscitation.
A. Proportion time (%) in target quadrant; B. Path in target quadrant (cm); C. The number of crossing platform during 60 seconds after removing the platform; D. The number of target quadrant entries during 60 seconds after removing the platform.
Data are expressed as means±SD. n=6-8, $^{\#}P<0.05$ vs. sham group, $^{※}P<0.05$ vs. CA group.

platform and allowing the rats to swim for 60 seconds in search of the platform. Proportion of time spent in the target quadrant, path in target quadrant, the number of platform crossings and the number of target quadrant entries were used to estimate retention performance. Animals in the CA group spent less proportions of time ($P<0.01$) (Figure 4A), swim path length ($P<0.01$) (Figure 4B) in the target quadrant, number of platform crossings ($P<0.01$) (Figure 4C) and number of target quadrant entries ($P<0.01$) (Figure 4D) than those in the sham group. These adverse results were reversed by sevoflurane postconditioning ($P<0.05$) (Figure 4A-D). The swimming speed and total path length of three groups did not differ significantly (data not shown).

Effect of Sevoflurane Postconditioning on Endogenous Antioxidant System

The activities of antioxidant enzymes (LPO, GSH, SOD and CAT), were measured at 4 hours, 1 day and 8 days after resuscitation (Figure 5A-D). SOD and CAT activities of hippocampal tissue were significantly higher in the CA + SE group than the control group at 4 hours and 1 day after resuscitation ($P<0.05$). However, there were no significant difference in the activities of SOD and CAT between the CA + SE and CA groups at 8 days after resuscitation (Figure 5B, C).

The effect of sevoflurane postconditioning on

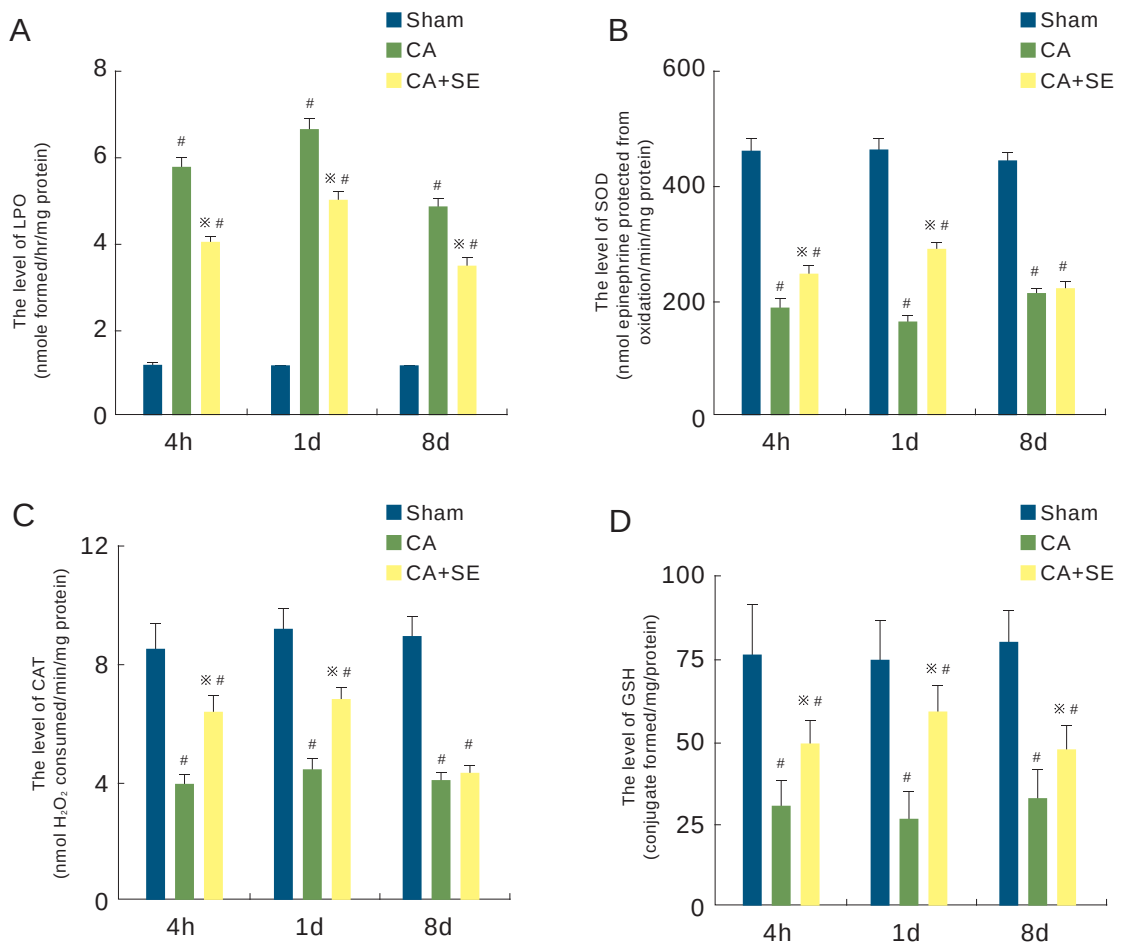

Figure 5. Antioxidase Activities of Hippocampal Tissue at 4 hours, 1 day and 8 days after Resuscitation.
Data are expressed as means±SD. n=6-8, #P<0.05 vs. sham group, ※P<0.05 vs. CA group.

TBARS level was measured to demonstrate the oxidative damage induced by LPO in hippocampus. A significantly increased level of TBARS was observed in the CA group compared with the sham group (P<0.05) at every time point after resuscitation. However, the CA + SE group has exhibited significant attenuation in TBARS level in hippocampus (P<0.05) as compared to CA rats. The level of GSH was depleted significantly in hippocampus in the CA group at every time point after resuscitation as compared to the sham group. Sevoflurane postconditioning had significantly more protection effect on the level of GSH in the CA+SE group than the CA group (P<0.05) (Figure 5D).

Effect of Sevoflurane Postconditioning on Hippocampal Memory Enhancement Proteins

The protein levels of GAP-43, PSD-95 and AP-1 in the hippocampus were very low in rats in the sham group but significantly increased in the CA and CA+SE groups (P<0.05), whereas the CA+SE group had much higher protein expression levels of GAP-43, PSD-95 and AP-1 than the CA group at 8 days after resuscitation (P<0.05) (Figure 6).

DISCUSSION

Although a continuous rise in the rate of ROSC restoration has been achieved in rats with CA, the final survival rate remains very low. Therefore, understanding the mechanisms responsible for brain injury due to global ischemia following CA and finding new neuroprotective strategies are critical and will be useful in clinical settings.

Oxidative stress induced by reperfusion after CA leads to the exhaustion of cerebral antioxi-

dant reserves and causes severe oxidative damage. Moreover, it has been reported that oxidative stress-induced lipid peroxidation alters the antioxidant defense system and plays an important role in the neurological damage occurring after cerebral ischemia (22, 23). Surplus amount of ROS generation is thought to be the key module of neuronal damage in the brain. ROS can induce lipid peroxidation and disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability (24, 25). Products of lipid peroxidation, such as MDA and unsaturated aldehydes, are capable of inactivating many cellular proteins by forming protein cross-linkages (26). Actually, the initial paper that showed postconditioning-induced brain protection by volatile anesthetics suggested that isoflurane applied immediately after the 15-min oxygen-glucose deprivation (OGD; to simulate ischemia in vitro) for 30 min dose-dependently reversed the OGD-induced injury (27). Sevoflurane, as a novel volatile anesthetic with minimal pungency, low solubility, and less toxicity, is widely used in anesthetic practice. Recently, our laboratory has already demonstrated that sevoflurane postconditioning could alleviate ischemic reperfusion injury in the brain (11, 28). However, the mechanism of sevoflurane protective effect on cerebral ischemia has not been well investigated.

In this present study, we found that TBARS level, which is an index of LPO, was significantly increased within 8 days after global ischemia following CA. Administration of sevoflurane after ROSC reversed the spike in TBARS levels, which was seen in the cerebral ischemic rats. Sevoflurane has been reported to inhibit lipid peroxidation and act against oxidative stress (29, 30). SOD and CAT along with GSH and other nonenzymatic antioxidant act in concert to protect brain cells against oxidative damage. SOD is a key constituent in oxidative stress. It is derived from various sources at different stages of reperfusion. SOD over-expression reduces ischemic damage from ischemia/reperfusion through inhibiting post-ischemic mitogen activated protein (31), BCL2 antagonist of cell death (BAD) cell death signaling pathway (32) and caspase activation (33), indicating a potential pro-apoptotic role for SOD in ischemia/reperfusion. CAT is located mainly in peroxisomes and elimination of hydrogen peroxide is critical to the efficacy of CAT in reducing oxidative stress (34). The activities of SOD and CAT in the CA+SE group were significantly higher than those in the CA group at 4 hours and 1 day after ROSC. However, we found that SOD and CAT activities at 8 days after ROSC did not differ from control animals. GSH is an endogenous antioxidant, which protects the cells from oxidative damage by scavenging free radicals (35). Furthermore, GSH plays a crucial role in the regulation of expression of several anti-inflammatory genes, whereas GSH inhibition in cerebral ischemia increases the susceptibility of plasma membranes towards peroxide attacks (36). There was a significant decrease in GSH level within 8 days after ROSC as compared with the sham-operated rats. Postconditining with sevoflurane increased GSH levels in ischemic rats, which may be indicative of neuroprotection. In the present study, we determined

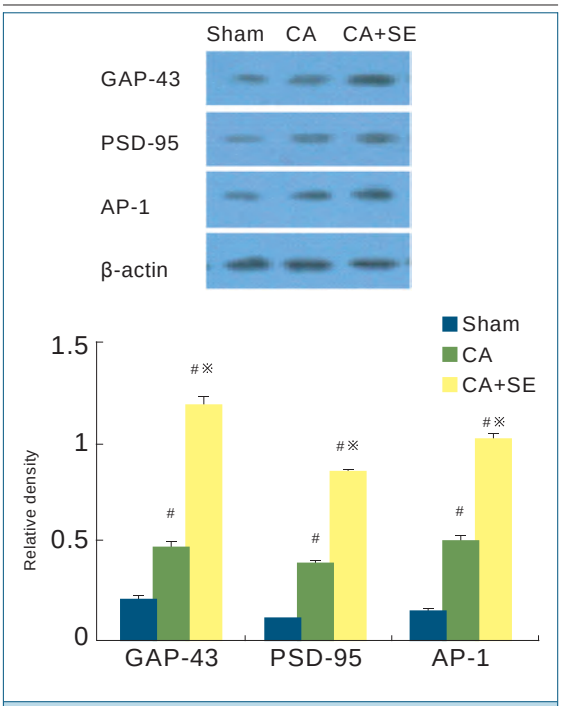

Figure 6. Effect of Sevoflurane Postconditioning on the Hippocampal Memory Enhancement Proteins.
Western blotting analysis for the hippocampal memory enhancement proteins at 8 days after resuscitation; Quantitative analysis of the level of hippocampal memory enhancement proteins.
Data are expressed as means±SD. n=6-8, # P< 0.05 vs. sham group, ※P<0.05 vs. CA group.

oxidation marker activities within 8 days after ROSC and found that sevoflurane postconditining significantly inhibited the content of TBARS and increased the activities of SOD, CAT and GSH. Consistent with our results, a previous study showed the protective effect of sevoflurane postconditioning against spinal cord ischemic reperfusion injury through the up-regulation of antioxidant enzymes in rabbits (29). Thus, the protective effects of sevoflurane postconditining might be largely due to the regulation of anti-oxidative activities.

On the other hand, global cerebral ischemia or hypoxic-ischemic injury following CA causes cognitive deficits including memory impairment (37). Result from previous findings suggested that oxidative damage correlates with cognitive deficits in rats with cerebral ischemia or hypoperfusion. The Morris water maze is a widely used test in behavioral neuroscience for studying the neural mechanisms of spatial learning and memory. Our results showed that cerebral ischemia following CA induced impairment in both spatial memory and reference memory. Sevoflurane postconditioning markedly improved deficits in spatial memory induced by cerebral ischemia. However, the mechanisms underlying the improvement of spatial learning and memory involved in sevoflurane postconditioning are not completely understood. Neurons in specific brain areas including cerebral cortex and hippocampus are known to play an important role in learning and memory processes (12, 13). Therefore, we further evaluated the expression of hippocampus proteins which could serve as memory enhancement biomarkers including c-jun/AP-1, PSD-95 and GAP-43. It is known that c-jun/AP-1 is critical for the control of progenitor cell viability and differentiation (38, 39). Previous studies have shown that c-jun/AP-1 is essential for memory retrieval in the probe trial of the Morris water maze and c-jun/AP-1 has specific roles in the formation of long-term potentiation (LTP) of synaptic transmission (39). PSD-95 is located in dendritic spines and is a key post-synaptic scaffold protein at excitatory synapses that directly promotes synapse maturation and exerts a major influence on synaptic strength and plasticity (40). Previous studies have shown that an increase in PSD-95 is correlated with enhanced hippocampal memory formation (16). Memory formation and neuronal adaptation also involve GAP-43 expressed in the hippocampus (41, 42). It has been shown that GAP-43 is closely associated with increasing learning and memory ability, whereas mutations in the GAP-43 gene have been linked to the impairment of memory and coordination (15). In current study, we showed that the expression of memory enhancement proteins including GAP-43, PSD-95 and c-jun/AP-1 in hippocampus was comparatively increased in sevoflurane-treated rats compared with rats in control group. These data suggested that sevoflurane postconditioning could ameliorate the ability of spatial learning and memory after global cerebral ischemia following CA in rats via regulating the expression of memory enhancement biomarker proteins in hippocampus.

In summary, sevoflurane postconditioning significantly increased survival rate and improved learning and memory deficits induced by global cerebral ischemia following CA in rats. These effects may involve a reduction in oxidative stress induced by ischemia and increased expression of hippocampal proteins that serve as memory enhancement biomarkers. Sevoflurane may be efficacious in treating ischemia-induced learning and memory deficits.

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