NEURAL REGENERATION RESEARCH
Volume 8, Issue 23, August 2013

www.nrronline.org

doi:10.3969/j.issn.1673-5374.2013.23.002 [http://www.nrronline.org; http://www.sjzsyj.org]
Qiu CW, Sheng B, Wang SR, Liu J. Is longer sevoflurane preconditioning neuroprotective in permanent focal cerebral ischemia? Neural Regen Res. 2013;8(23):2126-2133.

Is longer sevoflurane preconditioning neuroprotective in permanent focal cerebral ischemia?

Caiwei Qiu[1, 2], Bo Sheng[3], Shurong Wang[4], Jin Liu[2]

1 *West China School of Pharmacy, Sichuan University, Chengdu 610041, Sichuan Province, China*
2 *Neuroscience Research Center, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China*
3 *Department of Anesthesiology, Panzhihua Central Hospital, Panzhihua 617000, Sichuan Province, China*
4 *Department of Neurology, the 452 People's Liberation Army Hospital, Chengdu 610041, Sichuan Province, China*

Research Highlights

(1) In the current study, a rat model of permanent focal cerebral ischemia was used for the first time to demonstrate that 60-minute sevoflurane preconditioning has neuroprotective effects in rats with permanent cerebral ischemia.
(2) Long time (120 minutes) sevoflurane preconditioning had no obvious neuroprotective effects in rats with permanent cerebral ischemia.
(3) Sixty-minute sevoflurane preconditioning exerts the best neuroprotective effects in rats with permanent cerebral ischemia by inhibiting apoptosis.

Abstract

Sevoflurane preconditioning has neuroprotective effects in the cerebral ischemia/reperfusion model. However, its influence on permanent cerebral ischemia remains unclear. In the present study, the rats were exposed to sevoflurane for 15, 30, 60, and 120 minutes, followed by induction of permanent cerebral ischemia. Results demonstrated that 30- and 60-minute sevoflurane preconditioning significantly reduced the infarct volume at 24 hours after cerebral ischemia, and 60-minute lurane preconditioning additionally reduced the number of TUNEL- and caspase-3-positive cells in the ischemic penumbra. However, 120-minute sevoflurane preconditioning did not show evident neuroprotective effects. Moreover, 60-minute sevoflurane preconditioning significantly attenuated neurological deficits and infarct volume in rats at 4 days after cerebral ischemia. These findings indicated that 60-minute sevoflurane preconditioning can induce the best neuroprotective effects in rats with permanent cerebral ischemia through the inhibition of apoptosis.

Key Words

neural regeneration; brain injury; anesthesia; sevoflurane; preconditioning; cerebral ischemia; apoptosis; caspase-3; neuroregeneration

Caiwei Qiu, Ph.D.

Corresponding author: Jin Liu, M.D., Neuroscience Research Center, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China, scujinliu@gmail.com.

Received: 2013-04-07
Accepted: 2013-07-15
(N20120801001)

Acknowledgments: We would like to thank Chen F, Laboratory of Transplantation Immunity, Sichuan University, China for her help with immunostaining.

Author contributions: Qiu CW was responsible for the study conception, data analysis, and interpretation. Sheng B revised the manuscript. Wang SR was in charge of experimental design and revised the manuscript. Liu J was responsible for the experimental design, and manuscript revision. All authors approved the final version of the manuscript.

Conflicts of interest: None declared.

Ethical approval: All experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University, China.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application disputations.

Supplementary information: Supplementary data associated with this article can be found, in the online version, by visiting www.nrronline.org.

INTRODUCTION

Up to now, intravenous thrombolysis with tissue plasminogen activator has been the most effective treatment for acute ischemic stroke, but it has to be administered within 3 hours after vessel occlusion because the risk of hemorrhage increases with time[1]. Because of the narrow time window, this treatment is only applicable for less than 5% of patients with acute ischemic stroke in the United States (American Stroke Association). Most patients encounter permanent focal cerebral ischemia, which is more complicated in the pathological process and more difficult to treat than cerebral ischemia/reperfusion. Thus, a treatment that is able to confer neuroprotection in both transient and permanent brain ischemia may be beneficial.

Sevoflurane belongs to volatile anesthetics, and preconditioning with sevoflurane has been shown to exert protective effects against ischemic injury in the brain, heart[2-5], liver[6-7], and kidney[8]. In cerebral ischemia models, some studies reported that a higher concentration of sevoflurane pretreatment resulted in better neuroprotective effects than a lower concentration; however, others hold the opposite opinion[9-11]. The neuroprotection of sevoflurane preconditioning may be associated with antioxidant enzymes activation, reduced expression of apoptosis-regulating proteins, inhibition of apoptosis, activation of the extracellular signal-regulated protein kinase, anti-inflammation, mitoK (ATP) channel opening, protein kinase C-ε activation, and p38 MAPK phosphorylation[9, 12-26]. Although studies have shown the neuroprotective effects of sevoflurane preconditioning in the transient cerebral ischemia model, its effect in the permanent focal cerebral ischemia model remains unclear.

In the ischemic penumbra, apoptotic cell death is a common way of neuronal death[27]. Caspase-3, an apoptotic executor, plays an important role in ischemic neuronal injury. Increased caspase-3 expression has been reported in transient and permanent focal cerebral ischemia models[28-30]. It remains unknown whether caspase-3 expression and apoptosis may be inhibited by sevoflurane preconditioning in the permanent middle cerebral artery occlusion (MCAO) model, although sevoflurane preconditioning can attenuate apoptosis and caspase-3 expression in the transient MCAO model in rats[13].

Based on these results, we hypothesized that sevoflurane preconditioning could improve neurological function in the permanent MCAO model, reduce infarct volume, and that the protective effects of sevoflurane preconditioning might be associated with the reduction of caspase-3 expression and apoptosis. The aim of the current study was to verify this hypothesis.

RESULTS

Quantitative analysis of experimental animals

Ninety-two Sprague-Dawley rats were used. This study consisted of four parts. In the first part, the effects of 120-minute sevoflurane anesthesia on physiological parameters were assessed. Sixteen rats were randomly assigned to pentobarbital control and sevoflurane groups (n = 8 per group). The pentobarbital control group rats were anesthetized with pentobarbital and allowed inhalation of 30% oxygen. In the second part of the study, the protective effects of sevoflurane preconditioning on infarct volume and neurological deficits at 24 hours after MCAO were evaluated. Forty rats were randomly assigned to five groups: ischemia, 15-, 30-, 60-, and 120-minute sevoflurane preconditioning groups (n = 8 per group). The ischemia group was subjected to permanent MCAO alone. In the third part, the long-term neuroprotective effects of sevoflurane preconditioning on infarct volume and neurological deficits were investigated. Sixteen rats were randomly divided into ischemia and 60-minute sevoflurane preconditioning groups (n = 8 per group), and were sacrificed 4 days after stroke. Finally, we examined the anti-apoptotic effects of sevoflurane preconditioning. Fourteen ani-

mals were assigned to sham-surgery (n = 2), ischemia (n = 4), and 60- and 120-minute sevoflurane preconditioning groups (n = 4 per group). The sham-surgery group was subjected to a neck incision and craniotomy without artery occlusion. In the ischemia group, four rats (two from the ischemia group, and two from the 120-minute sevoflurane preconditioning group) died within 24 hours, and two rats in the 120-minute sevoflurane preconditioning group died within 4 days. Behavior test results included data collected from the rats that died.

Plasma glucose concentration increased following sevoflurane preconditioning

The physiological values in the period of sevoflurane anesthesia are presented in supplementary Table 1 online. During preconditioning, arterial oxygen tension (PaO_2) was not different between the sevoflurane and pentobarbital control groups (P > 0.05). At 60 and 120 minutes after sevoflurane anesthesia, potential hydrogen (pH) value was significantly higher (P < 0.01), while arterial carbon dioxide tension ($PaCO_2$) was significantly lower than in the pentobarbital control group (P < 0.01). However, the fluctuation of $PaCO_2$ and pH was within the physiological range. At 120 minutes after sevoflurane anesthesia, mean arterial pressure was significantly lower (P < 0.01) and plasma glucose concentration was significantly higher compared with the animals in the pentobarbital control group (P < 0.01).

Effects of sevoflurane preconditioning on neurological deficits and infarct volume

At 24 hours after MCAO, 30- and 60-minute sevoflurane preconditioning significantly reduced infarct volume compared with the ischemia group (P < 0.05, P < 0.01; Figure 1); however, sevoflurane preconditioning did not attenuate neurological deficits (P > 0.05; Figure 2).

At 4 days after MCAO, 60-minute sevoflurane preconditioning significantly attenuated neurological deficits (P < 0.01; Figure 3) and reduced infarct volume compared with the ischemia group (P < 0.05; Figure 4).

Sevoflurane preconditioning reduced cell apoptosis

At 24 hours after MCAO, TUNEL-positive cells were only found in the ischemic core and penumbra. The number of TUNEL-positive cells in the penumbra was significantly lower in the 60-minute sevoflurane preconditioning group compared with the ischemia group and the 120-minute sevoflurane preconditioning group (P < 0.01; Figure 5). Caspase-3-positive cells were only found in the penumbra, and the number of the cells was significantly lower in the 60-minute sevoflurane preconditioning group compared with the ischemia group and the 120-minute sevoflurane preconditioning group (P < 0.01; Figure 6).

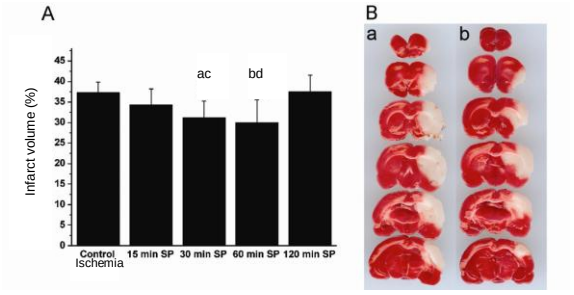

Figure 1 Sevoflurane preconditioning (SP) reduced infarct volume 24 hours after middle cerebral artery occlusion.

(A) Infarct volume at 24 h after middle cerebral artery occlusion (n = 8 per group). Data are presented as mean ± SD and were analyzed by two-sample t-test. [a]P < 0.05, [b]P < 0.01, *vs*. ischemia group; [c]P < 0.05, [d]P < 0.01, *vs*. 120-minute (min) SP group.

(B) Representative 2,3,5-triphenyltetrazolium chloride staining of the infarct volume at 24 hours after middle cerebral artery occlusion. White part is the cerebral infarction lesion. Infarct animal (a), 60-min SP animal (b).

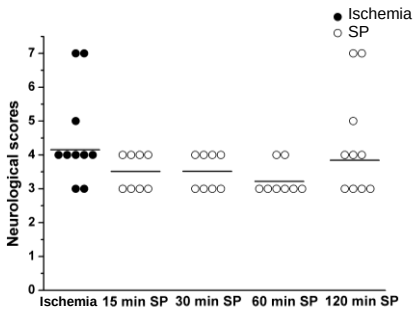

Figure 2 Effects of sevoflurane preconditioning (SP) on neurological deficit scores at 24 hours after middle cerebral artery occlusion.

Data are expressed as median (horizontal bars) with the individual values of animals (opened circles). n = 8–10 per group. Neurological deficit scores were analyzed using Kruskal-Wallis nonparametric analysis of variance followed by Mann-Whitney test for analysis of individual differences. min: Minutes.

DISCUSSION

The current study demonstrated that 60-minute sevoflurane preconditioning significantly reduced the infarct volume and the number of TUNEL-positive and caspase-3-positive cells in the penumbra at 24 hours after permanent MCAO. In addition, 60-minute sevoflurane preconditioning also attenuated neurological deficits and infarct volume at 4 days after MCAO. The optimal duration

of sevoflurane preconditioning was 60 minutes, at which infarct volume and apoptosis were significantly attenuated.

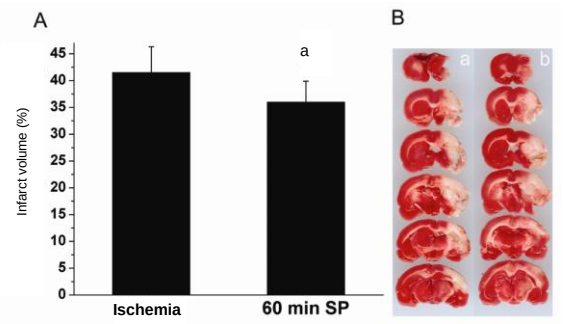

Figure 3 Sevoflurane preconditioning (SP) reduced infarct volume at 4 days after middle cerebral artery occlusion.

(A) Infarct volume at 4 days after middle cerebral artery occlusion (n = 8 per group). Data are presented as mean ± SD and were analyzed by two-sample t-test. $^aP < 0.05$, *vs*. ischemia group.

(B) Representative 2,3,5-triphenyltetrazolium chloride staining of the infarct volume at 4 days after middle cerebral artery occlusion. White part is the cerebral infarction lesion. Infarct animal (a), 60-minute (min) SP animal (b).

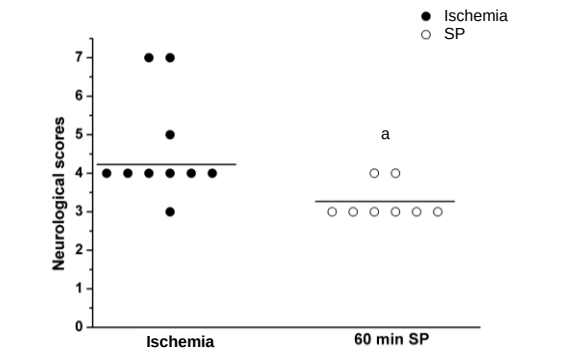

Figure 4 Sevoflurane preconditioning (SP) attenuated neurological deficit scores at 4 days after middle cerebral artery occlusion.

Data are expressed as median (horizontal bars) with the individual values of infarct animals (filled circles) and SP animals (opened circles). n = 8–10 per group. Neurological deficit scores were analyzed using Kruskal-Wallis nonparametric analysis of variance followed by Mann-Whitney test for analysis of individual differences. $^aP < 0.05$, *vs*. ischemia group.

The ischemic penumbra is an area directly in contact with the ischemic core where cells receive cerebral blood flow at a perfusion pressure of 18–23 mmHg, enough to survive but not to be functional. The blood flow in the area is so low that the Na^+/K^+ pump activity is inhibited because of the reduced adenosine 5'-triphosphate formation, which terminally induces caspases activation and apoptosis through the glutamate pathway. These cells may survive and restore function if the apoptotic process is prevented. Sevoflurane pretreatment has been shown to have neuroprotective effects by inhibiting caspase-3 activation and apoptosis in the penumbra in the transient MCAO model[13]. Our results also demonstrated that 60-minute sevoflurane preconditioning significantly reduced infarct volume and the density of TUNEL-positive and caspase-3-positive cells in the penumbra, supporting the hypothesis that the neuroprotection of sevoflurane preconditioning might be associated not only with the reduction of infarct size, but also with apoptosis attenuation and caspase-3 expression reduction in the permanent MCAO model.

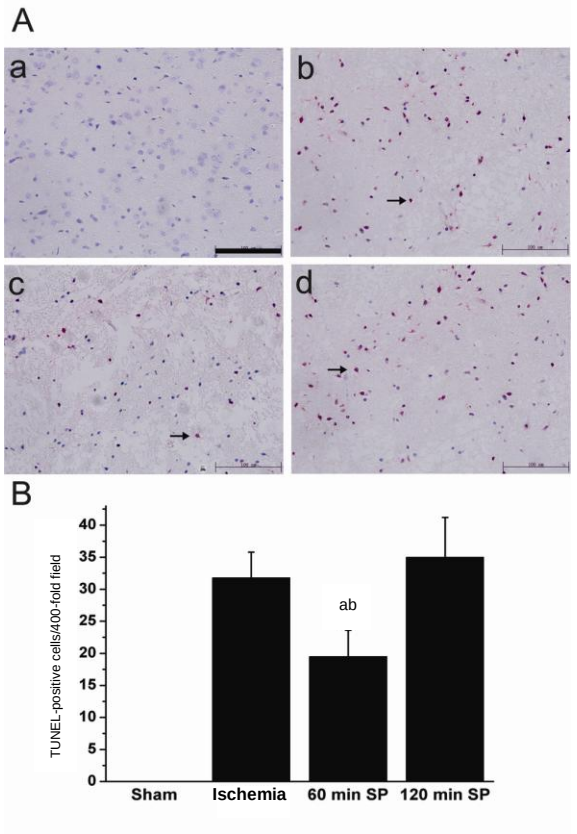

Figure 5 Sevoflurane preconditioning (SP) reduced TUNEL-positive cells 24 hours after middle cerebral artery occlusion.

(A) TUNEL-positive cells (TUNEL staining, × 400). Sham-surgery group (a) did not show TUNEL-positive cells. A large number of TUNEL-positive cells (red; marked with black arrows) were seen in the ischemia group (b) and the 120-min SP group (d). The number of TUNEL-positive cells was significantly lower in the 60-min SP group (c). Scale bar: 100 μm.

(B) Quantification of TUNEL-positive cells. The number of TUNEL-positive cells was significantly lower in 60-minute (min) SP animals. Data are presented as mean ± SD (n = 4 per group) and were analyzed using one-way analysis of variance followed by Bonferroni *post-hoc* test. $^aP < 0.01$, *vs*. ischemia group; $^bP < 0.01$, *vs*. 120-min SP group.

In our study, we found that the optimal duration of sevoflurane preconditioning was 60 minutes, which significantly reduced infarct volume, apoptosis, and caspase-3

expression. The mechanism through which the prolonged exposure abolishes the protective effect of sevoflurane preconditioning remains unclear.

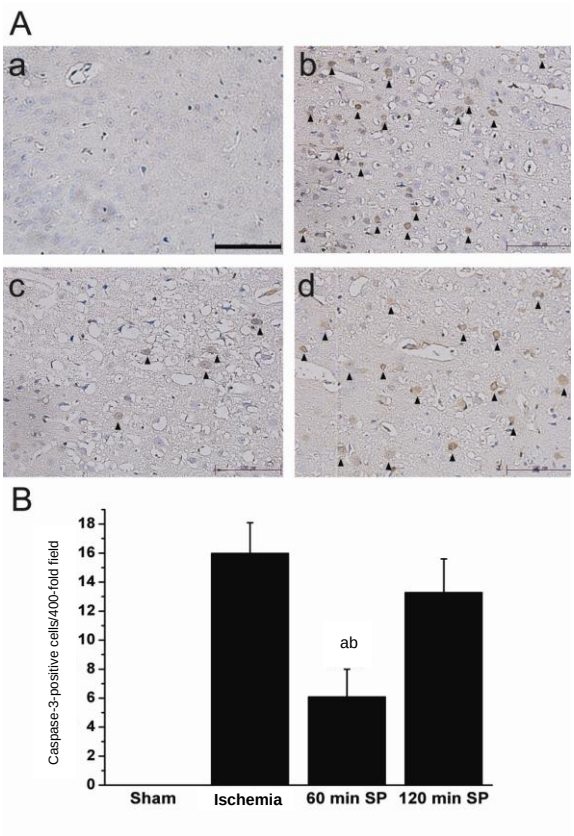

Figure 6 Sevoflurane preconditioning (SP) reduced caspase-3-positive cells 24 hours after middle cerebral artery occlusion (n = 4 per group).

(A) Caspase-3-positive cells (immunohistochemical staining, × 400). Sham-surgery group (a) did not show caspase-3-positive cells. A large number of caspase-3-positive cells (yellow brown; marked with black arrowheads) were seen in the ischemia group (b) and the 120-minute (min) SP group (d). The number of caspase-3-positive cells was significantly lower in the 60-min SP group (c). Scale bar: 100 μm.

(B) Quantification of caspase-3-positive cells. The number of caspase-3-positive cells was significantly lower in 60-min SP animals. Data are presented as mean ± SD and were analyzed using one-way analysis of variance followed by Bonferroni *post-hoc* test. [a]P < 0.01, *vs*. ischemia group; [b]P < 0.01, *vs*. 120-min SP group.

Some studies have shown that the prolonged exposure to sevoflurane causes neurotoxicity in naive mice. A study reported that 2.5% sevoflurane for 120 minutes induced caspase-3 activation and apoptosis in the naive mouse brain; another study showed that 3% sevoflurane for 6 hours induced a degree of active caspase-3-positive and TUNEL-positive cells in the brain tissue of neonatal naive mice[31-32]. Feng's[33] study in "adolescent" rats suggests that the neuronal loss caused by sevoflurane may be associated with decreased neuronal nitric oxide synthase. Although there is no evidence to prove that the long duration of sevoflurane inhalation causes obvious neuronal apoptosis or loss in adult rats, we cannot rule out the possibility that the prolonged exposure time may cause damage to the ultrastructure of neurons and gradually counteract the protective effects induced by sevoflurane. We also noticed that the mean arterial pressure was significantly lower at 120 minutes after sevoflurane anesthesia, but not at 60 minutes. As far as we know, hypotension leads to a reduced oxygen supply to the brain. Moreover, the plasma glucose concentration was significantly higher at 120 minutes after sevoflurane anesthesia, which involves decreasing insulin secretion and impairing glucose use[34-35]. It is also possible that the lack of oxygen-glucose is involved in the loss of the neuroprotective effects of 120-minute sevoflurane preconditioning.

Codaccioni *et al* [13] reported that sevoflurane preconditioning reduced the infarct volume at 3 days post stroke, but not at 7 days, in the transient MCAO model. Similarly, we found that 60 minutes of 1 minimum alveolar concentration sevoflurane preconditioning conferred neuroprotective effects 4 days after permanent MCAO. However, there is no evidence for growth of the ischemic lesion beyond 4 days after MCAO in rats, although infarcts mature 2 days after stroke in rats. Thus, we speculated that sevoflurane preconditioning-induced neuroprotection might be no longer detectable beyond 4 days; even so, sevoflurane preconditioning is still beneficial in stroke therapy. Furthermore, a previous study reported that 24 hours before MCAO, 3 hours of 1 minimum alveolar concentration isoflurane induced neuroprotection at 4 days after permanent MCAO[36]; in contrast to isoflurane preconditioning, sevoflurane preconditioning had a shorter induction duration and shorter interval between pretreatment and MCAO. In our preliminary study, the interval was set at 1.5, 6, 12, and 24 hours, while we found that only 1.5 hours had neuroprotective effects (data not shown).

In the present study, we used 2.4% sevoflurane based on the concentration-response relationship of sevoflurane[37]. Additionally, some reports showed that 4% sevoflurane provided better neuroprotection than 2% in the transient focal cerebral ischemia model and the global cerebral ischemia/reperfusion model[9-10]. Conversely, others showed that 2.8% sevoflurane caused severe hippocampal injury compared with 1.4% in the model of bilateral common carotid artery occlusion[11]. Thus, to investigate the neuroprotective effects of sevoflurane preconditioning, we used 2.4% sevoflurane to

prevent injury resulting from a high concentration of sevoflurane.

In conclusion, pretreatment with 2.4% sevoflurane for 60 minutes (the optimal duration) induced neuroprotective effects by attenuating cell apoptosis and caspase-3 expression in the permanent MCAO model in rats. In contrast to 60-minute sevoflurane preconditioning, the prolonged duration (120 minutes) of pretreatment had no neuroprotective effects. However, further studies are needed prior to clinical application to determine the optimal protocol for sevoflurane pretreatment.

MATERIALS AND METHODS

Design

A randomized, controlled animal study.

Time and setting

The experiments were conducted in the Neuroscience Research Center of Sichuan University, China from July 2011 to March 2012.

Materials

Animals

A total of 92 male specific pathogen-free Sprague-Dawley rats aged 2 months and weighing 250–300 g were provided by the Animal Centre of Sichuan Province (license No. SCXK (Chuan) 2008-24). Animals were raised in a room maintained at 24–26°C, and allowed free access to food and water. All experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University and carried out in accordance with the Guidelines laid down by the NIH in the US regarding the care and use of animals for experimental procedures.

Drugs

Sevoflurane ($C_4H_3F_7O$) was purchased from Shanghai Hengrui, Shanghai, China.

Methods

Sevoflurane preconditioning

In sevoflurane-preconditioned animals, anesthesia was induced with 6% sevoflurane in a box, and maintained through a mask at 2.4% concentration (1 minimal alveolar concentration) in a gas mixture of 30% oxygen and 70% nitrogen with a constant flow of 500 mL/minute[37]. The rectal temperature of each animal was maintained at 37 ± 0.5°C with a heated electric blanket. Plasma glucose concentration was measured using ACCU-CHEK Active (Roche, Basel, Switzerland) at three time points: immediately, 60 and 120 minutes after anesthesia. At 60 and 120 minutes after anesthesia, blood samples were collected from the right femoral artery for the measurement of pH, $PaCO_2$, and PaO_2 using ABL800 FLEX Blood Gas Analyzer (Radiometer America, Westlake, OH, USA). Mean arterial pressure was measured with a physiologic recorder (Biolap 420E+, Taimeng, Chengdu, China).

Establishment of the permanent MCAO model

After a 50-minute washout period (Figure 7), permanent MCAO model was established as previously described with minor modifications[38]. The animals were anesthetized with an intraperitoneal injection of pentobarbital (55–65 mg/kg). With the aid of an operating microscope, the right middle cerebral artery was cauterized using Sabre-2400 bipolar electrocauterizer (Conmed Co., Englewood, CO, USA) from the inferior margin of the olfactory tract to the inferior cerebral vein. The electric coagulation power was 3 W. The middle cerebral artery was cut below the rhinal fissure to avoid recanalization. The animals were allowed to recover at the ambient temperature of 24–26°C.

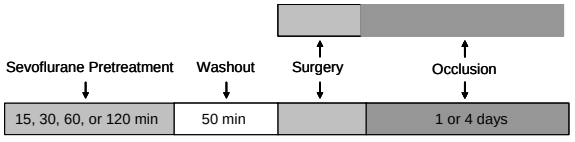

Figure 7 Schematic illustration of the experimental protocol.

The upper bar represents the ischemia group, the lower bar represents sevoflurane preconditioning animals. min: Minutes.

Neurological deficit score

Neurological deficit scoring was performed blindly 24 hours after MCAO according to the method proposed by Rogers[39]: 0, no apparent deficits; 1, failure to extend left forepaw fully; 2, decreased grip of the left forelimb when tail pulled; 3, spontaneous movement in all directions; left circling only if pulled by tail; 4, spontaneous left circling or walking; 5, walking only if stimulated; 6, no responsiveness to stimulation and with depressed level of consciousness; 7, dead (supplementary videos 1–3 online).

2,3,5-triphenyltetrazolium chloride staining for assessment of infarct volume

After scoring, animals were sacrificed under deep anesthesia to measure the infarct volume. The brains were removed and cut into six 2-mm-thick coronal slices using a Rat Brain Matrix (MyNeurolab, St. Louis, MO, USA). The sections were incubated in 2% phosphate-buffered

2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich, St. Louis, MO, USA) for 10 minutes at 37°C, followed by overnight immersion in 4% phosphate-buffered paraformaldehyde. After the brain slices were scanned, the images were analyzed blindly using Adobe Photoshop CS4 11.0.1 (Adobe Systems, San José, California, USA). The unstained area was defined as the infarction area. The infarct volume was calculated using Swanson's[40] method to correct for edema. The corrected infarct volume (%) = (volume of unleisioned side – volume of normal brain on leisioned side)/volume of unleisioned side × 100%.

Sample collection

Animals were deeply anesthetized and transcardially perfused with ice-cold saline, followed by ice-cold 4% phosphate-buffered paraformaldehyde. The brains were removed and post-fixed in the same fixative at 4°C for 36 hours. The brains were embedded in paraffin, and then 6-μm-thick sections were cut approximately 1.3 mm rostral to the bregma for staining[13].

TUNEL detection of cell apoptosis in brain tissues

TUNEL staining was performed according to the manufacturer's instructions (11684809910; Roche Diagnostics, Indianapolis, IN, USA). TUNEL-positive cells were counted in five to eight fields in the penumbra and were expressed as the average of all fields of each animal[13].

Immunohistochemistry for detection of caspase-3 expression in brain tissues

After deparaffinization, washing, incubation (3% H_2O_2 for 15 minutes) and antigen retrieval (95°C for 1 hour), the sections were incubated with the primary rabbit anti-caspase-3 polyclonal antibody (#9662, 1:800; Cell Signaling Technology, USA) overnight at 4°C. The sections were then incubated with the working fluid of the goat anti-rabbit secondary antibody (EnVision™ Detection Systems; K5007, Dako, Denmark) at 37°C for 1 hour. Caspase-3 staining was visualized using diaminobenzidine and sections were counterstained with hematoxylin. The penumbra was determined using a previously described method[41]. The sections were examined by light microscopy (Olympus). Caspase-3-positive cells were counted in five to eight fields in the penumbra and were expressed as the average of all fields of each animal[13].

Statistical analysis

Data were presented as mean ± SD. Physiological parameters and infarct volume were analyzed by two-sample *t*-test. The number of TUNEL-positive and caspase-3- positive cells was analyzed using one-way analysis of variance followed by Bonferroni *post-hoc* test. Neurological deficit scores were analyzed using Kruskal-Wallis nonparametric analysis of variance followed by Mann-Whitney test for analysis of individual differences. The value of $P < 0.05$ was considered statistically significant.

REFERENCES

[1] Hacke W, Brott T, Caplan L, et al. Thrombolysis in acute ischemic stroke: controlled trials and clinical experience. Neurology. 1999;53(7 Suppl 4):S3-14.

[2] Qiao S, Xie H, Wang C, et al. Delayed anesthetic preconditioning protects against myocardial infarction via activation of nuclear factor-κB and upregulation of autophagy. J Anesth. 2013;27(2):251-260.

[3] Xiao YY, Chang YT, Ran K, et al. Delayed preconditioning by sevoflurane elicits changes in the mitochondrial proteome in ischemia-reperfused rat hearts. Anesth Analg. 2011;113(2):224-232.

[4] Lu X, Moore PG, Liu H, et al. Phosphorylation of ARC is a critical element in the antiapoptotic effect of anesthetic preconditioning. Anesth Analg. 2011;112(3):525-531.

[5] Frädorf J, Huhn R, Weber NC, et al. Sevoflurane-induced preconditioning: impact of protocol and aprotinin administration on infarct size and endothelial nitric-oxide synthase phosphorylation in the rat heart in vivo. Anesthesiology. 2010;113(6):1289-1298.

[6] Minou AF, Dzyadzko AM, Shcherba AE, et al. The influence of pharmacological preconditioning with sevoflurane on incidence of early allograft dysfunction in liver transplant recipients. Anesthesiol Res Pract. 2012;2012:930487.

[7] Beck-Schimmer B, Breitenstein S, Urech S, et al. A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic. Ann Surg. 2008;248(6):909-918.

[8] Obal D, Dettwiler S, Favoccia C, et al. Effect of sevoflurane preconditioning on ischaemia/reperfusion injury in the rat kidney in vivo. Eur J Anaesthesiol. 2006;23(4):319-326.

[9] Yang Q, Dong H, Deng J, et al. Sevoflurane preconditioning induces neuroprotection through reactive oxygen species-mediated up-regulation of antioxidant enzymes in rats. Anesth Analg. 2011;112(4):931-937.

[10] Wang J, Lei B, Popp S, et al. Sevoflurane immediate preconditioning alters hypoxic membrane potential changes in rat hippocampal slices and improves recovery of CA1 pyramidal cells after hypoxia and global cerebral ischemia. Neuroscience. 2007;145(3):1097-1107.

[11] Engelhard K, Winkelheide U, Werner C, et al. Sevoflurane affects neurogenesis after forebrain ischemia in rats. Anesth Analg. 2007;104(4):898-903.

[12] Engelhard K, Werner C, Eberspächer E, et al. Sevoflurane and propofol influence the expression of apoptosis-regulating proteins after cerebral ischaemia and reperfusion in rats. Eur J Anaesthesiol. 2004;21(7):530- 537.

[13] Codaccioni JL, Velly LJ, Moubarik C, et al. Sevoflurane preconditioning against focal cerebral ischemia: inhibition of apoptosis in the face of transient improvement of neurological outcome. Anesthesiology. 2009;110(6):1271-1278.
[14] Sigaut S, Jannier V, Rouelle D, et al. The preconditioning effect of sevoflurane on the oxygen glucose-deprived hippocampal slice: the role of tyrosine kinases and duration of ischemia. Anesth Analg. 2009;108(2):601-608.
[15] Ye Z, Guo Q, Wang E, et al. Sevoflurane preconditioning induced delayed neuroprotection against focal cerebral ischemia in rats. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2009;34(2):152-157.
[16] McAuliffe JJ, Loepke AW, Miles L, et al. Desflurane, isoflurane, and sevoflurane provide limited neuroprotection against neonatal hypoxia-ischemia in a delayed preconditioning paradigm. Anesthesiology. 2009;111(3):533-546.
[17] Lin HF, Zhu ZR, Hu ZY. Protective effects of sevoflurane preconditioning on cerebral ischemia-reperfusion injury in rats. Zhonghua Yi Xue Za Zhi. 2009;89(41):2943-2945.
[18] Adamczyk S, Robin E, Simerabet M, et al. Sevoflurane pre- and post-conditioning protect the brain via the mitochondrial K ATP channel. Br J Anaesth. 2010;104(2):191-200.
[19] Ye Z, Guo Q, Wang E. Delayed neuroprotective effect of sevoflurane on the expression of NF-kappaB following transient focal ischemia-reperfusion in rats. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2010;35(3):262-266.
[20] Wang S, Dai ZG, Dong XW, et al. Duplicate preconditioning with sevoflurane in vitro improves neuroprotection in rat brain via activating the extracellular signal-regulated protein kinase. Neurosci Bull. 2010;26(6):437-444.
[21] Wang H, Lu S, Yu Q, et al. Sevoflurane preconditioning confers neuroprotection via anti-inflammatory effects. Front Biosci (Elite Ed). 2011;3:604-615.
[22] Yu Q, Chu M, Wang H, et al. Sevoflurane preconditioning protects blood-brain-barrier against brain ischemia. Front Biosci (Elite Ed). 2011;3:978-988.
[23] Ye Z, Huang YM, Wang E, et al. Sevoflurane-induced delayed neuroprotection involves mitoK(ATP) channel opening and PKC ε activation. Mol Biol Rep. 2012;39(5):5049-5057.
[24] Ye Z, Guo Q, Wang N, et al. Delayed neuroprotection induced by sevoflurane via opening mitochondrial ATP-sensitive potassium channels and p38 MAPK phosphorylation. Neurol Sci. 2012;33(2):239-249.
[25] Bedirli N, Bagriacik EU, Emmez H, et al. Sevoflurane and isoflurane preconditioning provides neuroprotection by inhibition of apoptosis-related mRNA expression in a rat model of focal cerebral ischemia. J Neurosurg Anesthesiol. 2012;24(4):336-344.
[26] Yang Q, Yan W, Li X, et al. Activation of canonical notch signaling pathway is involved in the ischemic tolerance induced by sevoflurane preconditioning in mice. Anesthesiology. 2012;117(5):996-1005.
[27] MacManus JP, Buchan AM, Hill IE, et al. Global ischemia can cause DNA fragmentation indicative of apoptosis in rat brain. Neurosci Lett. 1993;164(1-2):89-92.
[28] Bhat RV, DiRocco R, Marcy VR, et al. Increased expression of IL-1beta converting enzyme in hippocampus after ischemia: selective localization in microglia. J Neurosci. 1996;16(13):4146-4154.
[29] Hara H, Friedlander RM, Gagliardini V, et al. Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci U S A. 1997;94(5):2007-2012.
[30] Kitagawa H, Hayashi T, Mitsumoto Y, et al. Reduction of ischemic brain injury by topical application of glial cell line-derived neurotrophic factor after permanent middle cerebral artery occlusion in rats. Stroke. 1998;29(7):1417- 1422.
[31] Dong Y, Zhang G, Zhang B, et al. The common inhalational anesthetic sevoflurane induces apoptosis and increases beta-amyloid protein levels. Arch Neurol. 2009;66(5):620-631.
[32] Lu Y, Wu X, Dong Y, et al. Anesthetic sevoflurane causes neurotoxicity differently in neonatal naïve and Alzheimer disease transgenic mice. Anesthesiology. 2010;112(6):1404-1416.
[33] Feng X, Liu JJ, Zhou X, et al. Single sevoflurane exposure decreases neuronal nitric oxide synthase levels in the hippocampus of developing rats. Br J Anaesth. 2012;109(2):225-233.
[34] Saho S, Kadota Y, Sameshima T, et al. The effects of sevoflurane anesthesia on insulin secretion and glucose metabolism in pigs. Anesth Analg. 1997;84(6):1359-1365.
[35] Tanaka T, Nabatame H, Tanifuji Y. Insulin secretion and glucose utilization are impaired under general anesthesia with sevoflurane as well as isoflurane in a concentration-independent manner. J Anesth. 2005;19(4):277-281.
[36] Kapinya KJ, Löwl D, Fütterer C, et al. Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. Stroke. 2002;33(7):1889-1898.
[37] Kashimoto S, Furuya A, Nonaka A, et al. The minimum alveolar concentration of sevoflurane in rats. Eur J Anaesthesiol. 1997;14(4):359-361.
[38] Baker CJ, Onesti ST, Barth KN, et al. Hypothermic protection following middle cerebral artery occlusion in the rat. Surg Neurol. 1991;36(3):175-180.
[39] Rogers DC, Hunter AJ. Dissociation of effects of glutamate receptor antagonists on excitotoxic and hypoxic neuronal cell death in a novel rat cortical culture system. Brain Res Bull. 1997;44(2):131-139.
[40] Swanson RA, Morton MT, Tsao-Wu G, et al. A semiautomated method for measuring brain infarct volume. J Cereb Blood Flow Metab. 1990;10(2):290-293.
[41] Li Y, Powers C, Jiang N, et al. Intact, injured, necrotic and apoptotic cells after focal cerebral ischemia in the rat. J Neurol Sci. 1998;156(2):119-132.

(Reviewed by Lee-Kardashyan L, Wysong S, Wang RG, Liu M)
(Edited by Yu J, Su LL, Li CH, Song LP, Liu WJ, Zhao M)