

Sevoflurane preconditioning confers neuroprotection *via* anti-inflammatory effects

Hailian Wang¹, Shiduo Lu¹, Qiong Yu¹, Weimin Liang¹, Hui Gao¹, Peiying Li¹, Yu Gan^{1,2}, Jun Chen^{1,2}, Yanqin Gao^{1,2}

¹ State Key Laboratory of Medical Neurobiology and Department of Anesthesiology of Huashan Hospital, Fudan University, 138 Yixueyuan Road, Shanghai, China, 200032, ²Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and Methods
 - 3.1. Animal model of transient focal cerebral ischemia
 - 3.2. Measurement of infarct volume
 - 3.3. Assessment of neurological deficits
 - 3.4. Experimental groups
 - 3.5. Western blots
 - 3.6. ELISA
 - 3.7. Immunofluorescence staining
 - 3.8. RNA isolation
 - 3.9. Semi-quantitative real-time RT-PCR
 - 3.10. Intracerebroventricular injection
 - 3.11. Statistical analysis
4. Results
 - 4.1. Physiological variables
 - 4.2. Sevoflurane preconditioning induced tolerance against focal cerebral ischemia
 - 4.3. Sevoflurane preconditioning suppressed inflammatory factors after MCAO
 - 4.4. NF-kappa B was associated with sevoflurane preconditioning-mediated neuroprotection
 - 4.5. Sevoflurane preconditioning directly attenuated inflammation after MCAO
 - 4.6. p38 MAPK was involved in neuroprotection induced by sevoflurane preconditioning
5. Discussion
6. Acknowledgements
7. References

1. ABSTRACT

Neuroprotection afforded by volatile anesthetic preconditioning (APC) has been demonstrated in both *in vivo* and *in vitro* experiments, yet the underlying mechanism is poorly understood. We therefore investigated whether suppression of p38 MAPK, NF-kappa B and the downstream pro-inflammatory signaling cascade contribute to sevoflurane APC-induced neuroprotection. Male Sprague-Dawley rats were exposed for 30min/day on 4 consecutive days to ambient air or to sevoflurane (1.2% or 2.4%). Then rats were subjected to filament occlusion of the middle cerebral artery (MCAO) for 60 min, and euthanized 3 days after MCAO for measuring infarct volume. APC with sevoflurane markedly improved neurological performance of stroke rats, significantly decreased infarct volume, and robustly suppressed activation of NF-kappa B and p38 MAPK, and expression of inflammatory cytokines. Furthermore, APC with sevoflurane showed a direct inflammation-suppressing effect in rat brain receiving intracerebroventricular infusion of a dose of LPS that doesn't cause overt brain damage. Thus, the data suggest that APC with sevoflurane confers neuroprotection against focal ischemic brain injury, at least in part, by the anti-inflammatory effects of sevoflurane.

2. INTRODUCTION

The phenomenon whereby preconditioning with volatile anesthetics can mimic ischemic preconditioning was observed first in heart (1). Since then, the neuroprotective effect induced by volatile anesthetic preconditioning (APC) on cerebral ischemia has been demonstrated in both *in vivo* and *in vitro* settings (2–11). Volatile anesthetic preconditioning appears to confer biphasic protection in an early window (which occurs within minutes and subsides within hours) and a delayed window (which is manifested after 24 hours and is maintained for days). Edmands *et al.* reported that delayed preconditioning with isoflurane lasted 72–96 hours *in vitro* (12). Recently, studies have attempted to elucidate the mechanism underlying volatile anesthetic preconditioning, and it appears to involve attenuating neuronal apoptosis (2), opening of adenosine triphosphate-sensitive potassium channels (3), activation of adenosine A1 receptor (4), altering electrophysiological changes during energy deprivation (5), depending on inducible NO synthase (iNOS) and protein kinase C (6,7), and affecting the mitogen-activated protein kinase pathway (8–10) or activating the Akt pathway (11). However, the precise mechanism remains open to debate.

Inflammation contributes to ischemic brain injury. A critical role in modulating inflammatory gene expression is played by transcription factors, including nuclear factor kappa B (NF-kappa B), which induces several pro-inflammatory cytokines such as iNOS, cyclooxygenase-2 (COX2), tumor necrosis factor-alpha (TNF- α), interleukin-1 alpha (IL-1 α), interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6) (13). Furthermore, nuclear factor kappa B imposed a detrimental effect on rodents exposed to focal cerebral ischemia (14). Sevoflurane APC protected myocardium from ischemia and reperfusion by attenuating nuclear factor kappa B (15). p38, a stress-activated MAPK, plays an important role in the pathway of stress-related signal transduction *via* phosphorylating intracellular enzymes, transcription factors and cytosolic proteins involved in cell survival, apoptosis and inflammatory factor synthesis. Sustained activation of p38 MAPK has been shown to be associated with neuronal death/apoptosis, and selective p38 MAPK inhibitors can promote the survival of a variety of neurons *in vitro* (16). In the present study, we performed an *in vivo* investigation to determine the effects of preconditioning with sevoflurane on rats exposed to ischemic insult, and we hypothesized that nuclear factor kappa B and p38 MAPK were candidate molecular targets for the neuroprotective effect of sevoflurane-induced preconditioning.

3. MATERIALS AND METHODS

3.1. Animal model of transient focal cerebral ischemia

All animal experiments were performed in accordance with institutional guidelines and all efforts were made to minimize the number of animals. Transient focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) as previously described (17). Adult male Sprague-Dawley rats (SD, 250 g, 8 weeks old; Shanghai SLAC Laboratory Animal Co. Ltd., Shanghai, China) were anesthetized with 1.5% isoflurane (Forene, Abbott, Abbott Park, Illinois, U.S.A) in air and mechanically ventilated with an endotracheal tube. Rectal temperature was maintained at 37.0°C during and shortly after surgery with a temperature-regulated heat lamp. The left femoral artery and vein were cannulated and used for blood pressure monitoring, blood sampling and fluid administration. Arterial blood gas was analyzed 15 min after induction of ischemia and 15 min after reperfusion. The animals underwent left MCAO for 60 minutes and then reperfusion for the indicated duration. To confirm the induction of ischemia and successful reperfusion, changes in regional cerebral blood flow (rCBF) before, during, and after MCAO were evaluated in rats by laser Doppler flowmetry.

3.2. Measurement of infarct volume

At 72 hr after MCAO, animals were euthanized in a carbon dioxide chamber and the brains were removed and sliced into coronal sections (30 micrometers thick). Frozen slices then were prepared for cresyl violet staining, and infarct volumes measured with Leica image analysis software. The percent volume loss in the lesioned versus unlesioned hemisphere was determined for each animal by an investigator blinded to the experimental conditions using the following equation: (volume of unlesioned hemisphere-

volume of lesioned hemisphere)/(volume of unlesioned hemisphere).

3.3. Assessment of neurological deficits

All animals subjected to MCAO underwent neurological evaluation at 24 hr, 48 hr and 72 hr after ischemia. Each rat was assigned a score according to a five-point behavioral rating scale (11): 0, no deficit; 1, forelimb weakness, and torso turning to the ipsilateral side when held by tail; 2, circling to the affected side; 3, unable to bear weight on the affected side; and 4, no spontaneous locomotor activity or barrel rolling. Any animal without a deficit was excluded from the study. A single observer blinded to group assignment performed neurological testing.

3.4. Experimental groups

Rats were randomized into four experimental groups in experiment 1: sham-operated (not exposed to the anesthetic chamber, and branches of external cervical artery dissected but ischemia not induced); vehicle (exposed to ambient air); and sevoflurane (Baxter, Deerfield, Illinois, U.S.A) APC groups (n=8/sham, n=12/vehicle, n=10/sevo1, n=10/sevo2). In the preconditioning groups, the rats received 0.5 minimum alveolar concentration (MAC) sevoflurane (98.8% air/1.2% sevoflurane) or 1MAC sevoflurane (97.6% air/2.4% sevoflurane) in an anesthetic chamber for 30 min after 15 min equilibrium on 4 consecutive days. End tidal concentration of sevoflurane was monitored with a Datex-Ohmeda AS/3 monitoring device. Twenty-four hours after a four-cycle preconditioning with sevoflurane, rats were anesthetized, intubated, catheterized, and exposed to MCAO-induced transient focal cerebral ischemia.

Rats were assigned randomly to four groups (n=4 per group) in experiment 2: control (without any intervention), NS (intracerebroventricular injection of saline), LPS (intracerebroventricular injection of LPS diluted in saline), and sevoflurane + LPS (exposed to 2.4% sevoflurane repeatedly as mentioned above before intracerebroventricular injection of LPS).

3.5. Western blots

Rat cortical tissues were collected at the indicated time points after ischemia (n=4 per condition) and Western blot was performed as previously described (18). The blots were semi-quantified with gel densitometry and the BIO-RAD system. The primary antibodies used in this study were rabbit anti-NF-kappa B p65 subunit monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, California, U.S.A.), rabbit anti-COX2 polyclonal antibody (Cell Signaling Technology, Danvers, Massachusetts, U.S.A.), rabbit anti-iNOS antibody (Santa Cruz Biotechnology, Santa Cruz, California, U.S.A.), rabbit-anti-p38 and phospho-p38 antibody (Cell Signaling Technology, Danvers, Massachusetts, U.S.A.).

3.6. ELISA

NF-kappa B-DNA binding activity was detected using TransAM NF-kappa B p65 transcription factor assay

NF-kappa B is involved in sevoflurane preconditioning in brain

Table 1. List of primers

Name	Forward 5'-3'	Reverse 5'-3'
GAPDH	GTGAAGTCCGGTGTGAACGG	GTTCCTCCGTTGATGACCAG ;
iNOS	GGAAGTTTCTCTTCAGAGTC	CGATGGAGTCACATGCAGC ;
COX2	GACAGATCAGAAGCGAGGACCTG	GTAGATCATGTCTACCTGAGTG ;
TNF- α	GATCGGTCCCAACAAGGAGG	GCTGGTACCACCAAGTTGGTTG ;
IL1- α	CCAAAGTTCCTGACTTGTGTTG	GAAGGTGAAGGTGGACATC ;
IL1- β	CTGTCCCTGAACCTCAACTGTG	GTCCTCATCCTGGAAGCTCC ;
IL-6	CAGGGAGATCTTGGAAATGAG	GTGTTCTTCACAACTCC .

Table 2. Physiological variables during sevoflurane preconditioning

Physiological variables ¹	pH	PaCO ₂ mm Hg	PaO ₂ mm Hg	Rectal Temperature °C
Vehicle (n=6)	7.34±0.03	41.2±2.3	96.8±1.8	37.4±0.1
Sevo ² (n=6)	7.32±0.07	43.2±5.1	93.5±4.7	37.5±0.3

¹Values are mean±SD. ²Sevo indicates 1MAC sevoflurane pretreatment

kits (Active Motif, Carlsbad, California, U.S.A). The protocol is recommended by the manufacturer. Briefly, 20 microgram brain nuclear extracts were diluted to 20 micro liter by the complete lysis buffer per well, and absorbance was read on a spectrophotometer within 5 min at 450 nm with a reference wavelength of 655 nm.

3.7. Immunofluorescence staining

At the indicated time points after ischemia (n=3 per condition), rabbit anti-NF-kappa B p65 subunit monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, California, U.S.A.), mouse monoclonal anti-NeuN antibody (Millipore, Billerica, Massachusetts, U.S.A.), mouse monoclonal anti-GFAP antibody (Cell Signaling Technology, Danvers, Massachusetts, U.S.A.), rabbit anti-Iba-1 antibody (Wako, Osaka, Japan) were used as the primary antibodies. The procedures for immunohistochemistry were the same as described previously (19). For double-label immunofluorescence staining, sections were incubated simultaneously with two primary antibodies from different species at 37°C for 1 hr and then at 4°C overnight, followed by incubation for 1 hour at 37°C with DyLightTM 488-conjugated goat anti-rabbit and DyLightTM 549-conjugated goat anti-rat secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania, U.S.A.).

3.8. RNA isolation

Total RNA was isolated from the ipsilateral hemisphere with TRIzol. Brain tissues were treated with RNase-free DNase (Promega, Madison, Wisconsin, U.S.A) at 1 U/mcg of RNA at 37°C for 30 minutes, followed by phenol-chloroform extraction and ethanol precipitation. RNA quantity was determined by optical density measurement and electrophoresis.

3.9. Semi-quantitative real-time RT-PCR

Total RNA of brain samples from sham, vehicle, and sevoflurane-pretreated groups was extracted and stored at -70°C at 24 hr after ischemia. First-strand cDNA was synthesized with use of a Promega RT reagent kit, as recommended by the manufacturer. PCR analyses were conducted with gene-specific primers, and the endogenous control was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Real-time data were analyzed with a realplex analysis system (Eppendorf, Hamburg, Germany). All reactions were performed in duplicate. Each

run contained negative controls but no positive controls. The sequence-specific primers that were used are summarized in Table 1.

3.10. Intracerebroventricular injection

The rats were anesthetized and mounted in a stereotactic frame, and intracerebroventricular injections using a 10-microL Hamilton syringe were made at the following coordinates: 0.8 mm posterior to the bregma, 1.5 mm lateral, and 3.7 mm deep. The needle was kept in this position for an additional 20 min after injection and then withdrawn slowly from the brain. Lipopolysaccharide (LPS) serotype 055:B5 5 mcg (Sigma, St. Louis, Missouri, U.S.A) in 5 microL sterilized saline was used in place of MCAO (20).

3.11. Statistical analysis

Differences in infarction volumes, Western blotting, mRNA expression and DNA-NF-kappa B binding activity were determined with a Student nonpaired t-test. Neurologic deficit scores and physiological parameters were subjected to the Kruskal-Wallis test. It was considered statistically significant when the P value was equal to or less than 0.05.

4. RESULTS

4.1. Physiological variables

Physiological values of the pretreatment period are presented in Table 2. Sevoflurane was administered to the spontaneously breathing animals through a tightly fitting facemask with a constant flow of 2L/min. Blood samples were obtained immediately after cessation of pretreatment for 30 min. There were no significant differences in physiological variables between groups. Before, during and after MCAO, no significant differences in physiological variables were detected between sham-operated, vehicle, and sevoflurane-preconditioned groups as shown in Table 3.

4.2. Sevoflurane preconditioning induced tolerance against focal cerebral ischemia

Compared with the sham group, rats exposed to cerebral ischemic insults, whether sevoflurane APC or not, manifested significant histological lesions. The infarct volume was less in the 0.5 and 1 MAC sevoflurane APC groups (41.5±14.8 mm³ and 36.9±2.8 mm³, respectively,

Table 3. Physiological variables before, during, and after MCAO

Physiological variables ¹	pH	PaCO ₂ mm Hg	PaO ₂ mm Hg	MABP mm Hg	Rectal Temperature °C
Sham (n=6)	7.36±0.02	44.3±1.8	95.8±2.8	96.1±6.7	37.3±0.1
Vehicle (n=8)					
Pre-ischemia	7.35±0.07	46.6±10.7	96.0±28.0	84.0±6.6	37.3±0.2
Ischemia	7.33±0.03	46.9±7.4	86.7±6.8	79.6±3.5	37.6±0.3
Post-ischemia	7.33±0.03	40.9±14.9	93.7±19.9	87.8±12.3	37.7±0.4
Sevo ² (n=6)					
Pre-ischemia	7.30±0.07	44.5±6.7	89.9±2.8	85.0±4.5	37.6±0.3
Ischemia	7.32±0.05	49.7±8.1	84.3±5.3	74.4±9.4	37.5±0.2
Post-ischemia	7.30±0.09	48.2±8.4	89.5±6.7	79.2±10.0	37.6±0.3

¹Values are mean±SD. ²Sevo indicates 1MAC sevoflurane pretreatment

mean ± SD) than in vehicle group (133.5±10.5 mm³, ###p<0.001), but there were no significant differences between the 0.5 and 1 MAC sevoflurane APC groups (p>0.05). Similarly, sevoflurane preconditioning reduced the neurological deficit in rats 1, 2, and 3 days after reperfusion (Figure 1 A, B, C). Furthermore, sevoflurane pretreatment decreased tissue loss in the cerebral cortex and striatum (Figure 2). To rule out the possibility that sevoflurane might have altered rCBF and therefore infarct volume, we also monitored rCBF in both the vehicle and sevoflurane-preconditioned groups. As shown in Figure 1 D, no difference in rCBF change was detected between the 2 groups when induced with ischemia or after MCAO.

4.3. Sevoflurane preconditioning suppressed inflammatory factors after MCAO

To determine the effect of sevoflurane preconditioning on MCAO-induced inflammation, several inflammatory factors were measured at 0, 3, 6 and 24 hr after MCAO. Levels of iNOS and COX2 were significantly increased after MCAO (based on 4 different sets of experiments), all of which were reduced by sevoflurane preconditioning at 6 hr and 24 hr after reperfusion (Figure 3 A, C). Double-label immunofluorescent staining at 24 hours after MCAO showed iNOS was activated in neurons at cortex and striatum, and sevoflurane preconditioning diminished the activation of iNOS (Figure 3 B). The real-time PCR data show that sevoflurane preconditioning decreased expression of COX2, iNOS, TNF-α, IL-1α, IL-1β, and IL-6 significantly (p<0.05, Figure 3 D).

4.4. NF-kappa B was associated with sevoflurane preconditioning-mediated neuroprotection

NF-kappa B, which regulates proinflammatory cytokines, was activated (increased p65 subunit) in the cortex. Double-label immunofluorescent staining at 24 hours after MCAO demonstrated NF-kappa B activated in neurons, astrocytes and microglia at cortex and striatum, and this was decreased by sevoflurane preconditioning (Figure 4 A, B, C). Figure 4 D shows a representative Western blot of activated NF-kappa B in cortex at various time points after MCAO. Sevoflurane preconditioning reduced NF-kappa B activation at 3, 6, and 24 hr after MCAO. The graphs illustrate the semi-quantitative results from 4 independent experiments. The activity of NF-kappa B binding to DNA also was diminished by sevoflurane APC at 24 hours after MCAO, which was supported by Elisa assay (Figure 4 E).

4.5. Sevoflurane preconditioning directly attenuated inflammation after MCAO

To determine whether sevoflurane preconditioning has any direct inhibitory effects on cytokine expression, brain inflammation was induced by intracerebroventricular injection of LPS, an established model that induces a negligible amount of neuronal death in the brain. LPS significantly increased the levels of NF-kappa B and iNOS (based on 4 different sets of experiments), and both were reduced by sevoflurane preconditioning 24 hours after LPS injection (Figure 5 A, B).

4.6. p38 MAPK was involved in neuroprotection induced by sevoflurane preconditioning

In addition to NF-kappa B, p38 MAPK is also an important mediator in cerebral inflammation. Data indicate that an increase and activation of p38 MAPK in the cortex were significantly inhibited by sevoflurane preconditioning at 24 hours after MCAO. p-p38 was normalized to p38 with alpha-tubulin as reference (Figure 6 A, B, C).

5. DISCUSSION

In a well-established rat model of transient focal cerebral ischemia, we confirmed that repeated pretreatment with sevoflurane 24 hours before MCAO exerts a neuroprotective effect. Furthermore, the results indicate that suppression of the transcription factor NF-kappa B, p38 MAPK and downstream inflammatory factors contributed to sevoflurane preconditioning-induced tolerance against cerebral ischemic injury.

Volatile anesthetic preconditioning is shown to occur within a biphasic protective window: an early phase (early preconditioning), which occurs within minutes and subsides in less than 3 hours; and a delayed phase (late preconditioning), which is manifested after 24 hours and maintained for days (6). Our findings are consistent with other recent findings of neuroprotection induced by late volatile anesthetic preconditioning *in vivo* and *in vitro* (5-6, 8-11). However, the observed dose-effect curves of volatile anesthetic preconditioning vary. Pretreatment with 1,2 and 3 MAC desflurane or sevoflurane protected primary neuron cultures from insult induced by oxygen-glucose deprivation (OGD) in a dose-independent manner (2). However, several researchers have reported contrary data (3, 10, 21). The contradictory findings may be due to the diverse models (*in vivo* or *in vitro*), the various insults (ischemia, OGD, or hypoxia only), and different treatment timing

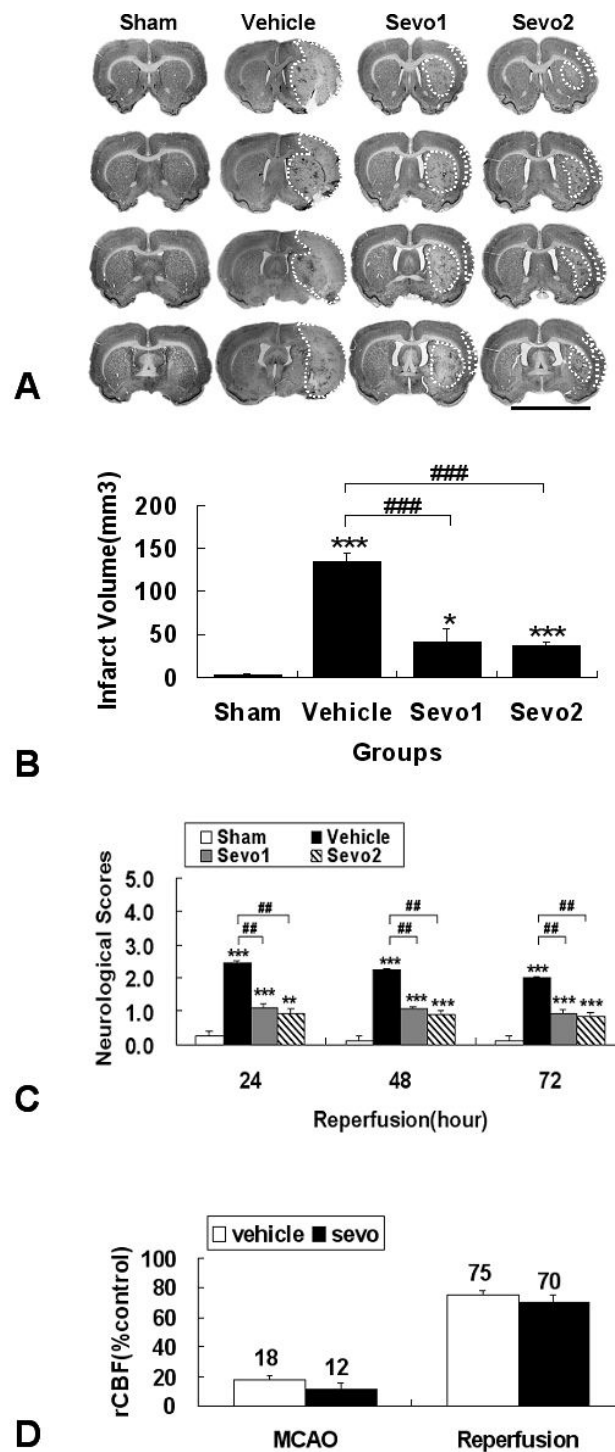


Figure 1. Effect of Sevoflurane Preconditioning on Neurological Deficit Scores and Infarct Volume. A, Representative images of CV-stained sections 72 hours after MCAO. Scale bar=1 cm. B, Effects of different doses of sevoflurane preconditioning on infarct volume. (n=8/sham,n=12/vehicle,n=10/sevo1,n=10/sevo2) C, Effect of different doses of sevoflurane preconditioning on neurological scores. Neurological function was tested in rats at 24, 48 and 72 hours after ischemia. D, Changes in rCBF were not different between vehicle-treated and sevoflurane-treated groups (n=8/vehicle, n=6/sevo) at the beginning and end of ischemia. Compared with the sham group, ***p<0.001, **p<0.01, *p<0.05; compared with the vehicle group, ###p<0.001, ##p<0.01. Sham, sham operation; Vehicle, exposed to vehicle gas (air); Sevo1, 0.5MAC sevoflurane preconditioning; Sevo2, 1MAC sevoflurane preconditioning.

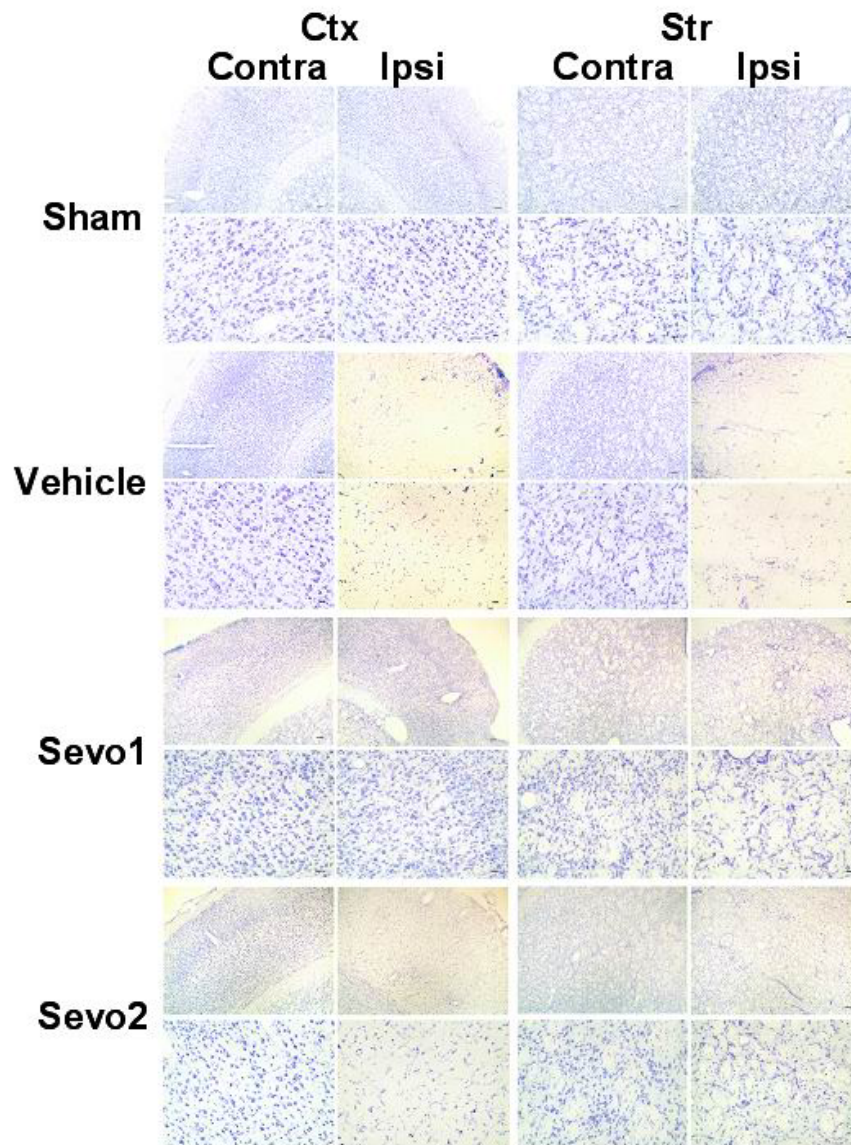


Figure 2. Effect of Sevoflurane Preconditioning on Tissue loss at the cerebral cortex and striatum. a, b, $\times 5$ and c, d $\times 20$; Contra=contralateral, Ipsi=ipsilateral, Ctx=cortex, Str=striatum, Scale bar=20 micrometers.

(early preconditioning or late preconditioning). We chose 0.5 MAC, a concentration that is subclinical but that attenuates the learning functions of rats (22), and 1 MAC, the most commonly used clinical concentration. We did not choose a higher concentration due to the undesirable effect of high concentrations of sevoflurane on respiratory and cardiovascular systems, which in turn blur the neuroprotection of volatile anesthetic pretreatment. Our data show that repeated preconditioning with 0.5 MAC sevoflurane produced neuroprotection in rats subjected to ischemia/reperfusion.

Inflammation plays an important role in ischemic brain injury. IL-1, IL-6, TNF- α , iNOS and COX2 have been suggested to be important mediators of inflammatory

reactions in cerebral ischemia (23). Anti-inflammatory effects of volatile anesthetics have been reported to induce protection in cardiac, pulmonary, renal and hepatic ischemia/reperfusion injuries (24-26). Furthermore, volatile anesthetic preconditioning appears to be protective against ischemia-induced injury in the heart and brain due to iNOS-dependent mechanisms. Pretreatment with isoflurane improved organ function after ischemia and increased iNOS expression and activity after the preconditioning of isoflurane; in addition, iNOS inhibitor abolished iNOS activation and protective effects of volatile anesthetics (6,27). However, the levels of iNOS activation after ischemic insult weren't detected in these studies. Our study suggests that there is an increment of iNOS after ischemia (immediately at the beginning of reperfusion) and a time-

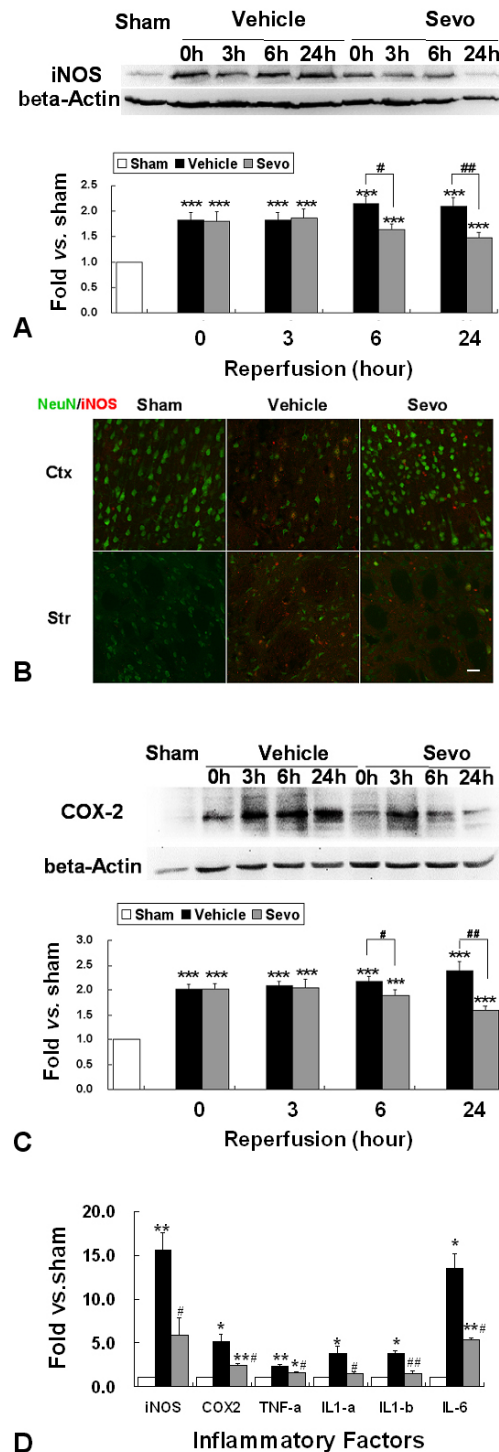


Figure 3. Sevoflurane Preconditioning Attenuated Inflammation Factors after Focal Cerebral Ischemia. A, C, Representative Western blot of iNOS and COX2 in cortex at various time points after MCAO. Graphs show semi-quantitative analysis of COX2 and iNOS (n=4/per group/per time point). Compared with sham group, ***p<0.001; compared with vehicle group, ##p<0.01, #p<0.05. B, Immunofluorescence photomicrographs of iNOS (red) and NeuN (green) within the infarct at the cerebral cortex and striatum 24 hr after MCAO, showing that iNOS is expressed by NeuN+ neurons. Scale bar=20 micrometers. D, Levels of iNOS, COX2, TNF-a, IL1-a, IL1-b and IL-6 mRNA, measured by semi-quantitative PCR and normalized to GAPDH (n=4/per group). Compared with the sham group, **p<0.01, *p<0.05; compared with the vehicle group, ##p<0.01, #p<0.05.

NF-kappa B is involved in sevoflurane preconditioning in brain

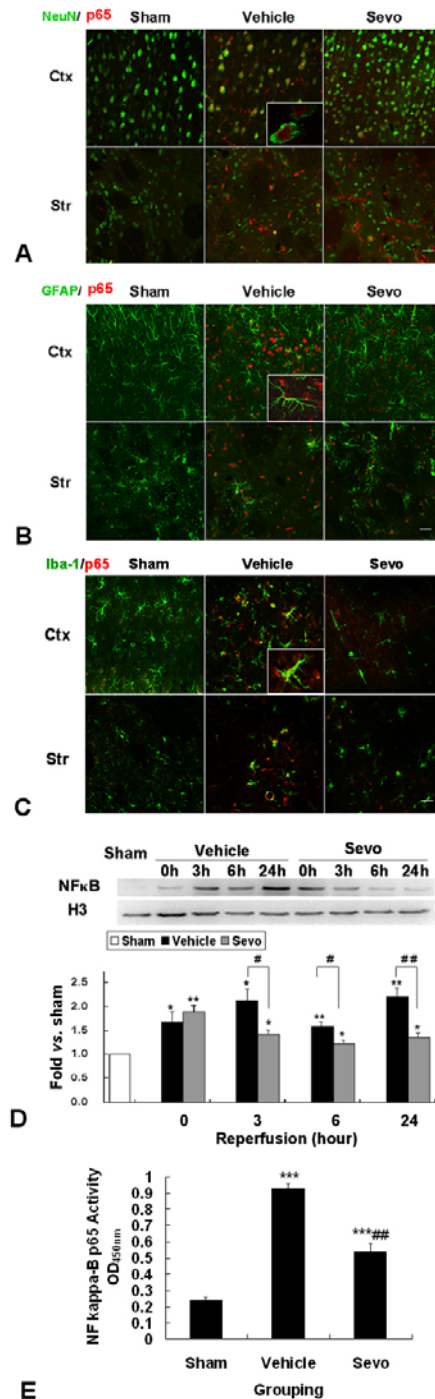


Figure 4. Sevoflurane Preconditioning Attenuated the Activation of NF-kappa B after MCAO. A, B, C, Immunofluorescence photomicrographs of NF-kappa B/p65 (red) and NeuN/GFAP/Iba-1 (green) within the infarction at the cerebral cortex and striatum 24 hr after MCAO, showing that NF-kappa B/p65 is expressed by neurons, astrocytes, or microglia. Scale bars=20 micrometers or 5 micrometers. D, Representative Western blots of NF-kappa B/p65 from nuclear protein in cortex at various time points after MCAO. Graph shows semi-quantitative analysis of NF-kappa B (n=4 /per group/per time point). Compared with the sham group, **p<0.01, *p<0.05; compared with the vehicle group, ##p<0.01, #p<0.05. E, Activity of NF-kappa B/p65-DNA binding, detected by ELISA assay (n=5/per group). Compared with the sham group, ***p<0.001; compared with the vehicle group, ##p<0.01.

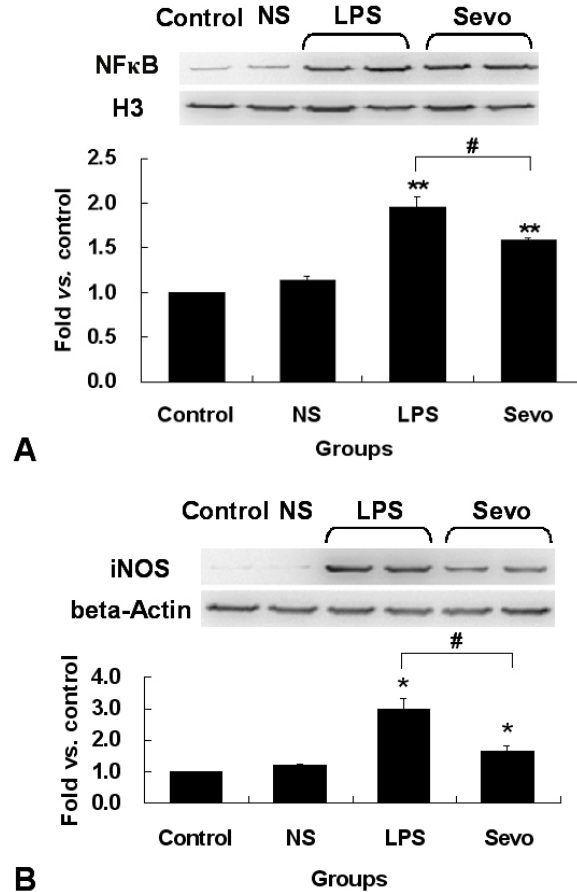


Figure 5. Sevoflurane Preconditioning Suppressed the Activation of NF-kappa B and iNOS after LPS Injection. A, B, Representative Western blots of NF-kappa B and iNOS in cortex at 24 hr after intracerebroventricular injection of LPS. Graphs show semi-quantitative analysis (n=4/per group). Compared with the control group, **p<0.01, *p<0.05; compared with the LPS group, #p<0.05.

dependent decline provoked by sevoflurane compared with vehicle-pretreated groups at the same time point after reperfusion.

NF-kappa B, a ubiquitous inducible transcription factor, is a key regulator of hundreds of genes involved in cell survival and inflammation, including those of inflammatory mediators as described above. Although it is well known that NF-kappa B is activated, mainly in neurons but also in astrocytes and microglia, by cerebral ischemia injury, the role of NF-kappa B in the brain is complex because it simultaneously regulates expression of antiapoptotic, proapoptotic, and proinflammatory genes (28). On one hand, NF-kappa B contributes to neuronal cell death if ischemia is prolonged and severe. On the other hand, it protects neurons from ischemic injury when the insult is transient and sublethal, and is responsible for the effect of ischemic preconditioning. Several studies have indicated that pharmacological inhibition of NF-kappa B provides protection against acute myocardial ischemia/reperfusion injury (29-31). One study found that sevoflurane preconditioning attenuated NF-kappa B activation and the subsequent down-regulation of NF-kappa

B-dependent inflammatory gene expression, such as tumor necrosis factor-alpha interleukin 1, intercellular adhesion molecule 1, and inducible nitric oxide synthase (29). This outcome is consistent with our findings that NF-kappa B was activated by focal ischemic insult in brain and that the activation of NF-kappa B was diminished by sevoflurane preconditioning. Using a simple inflammatory model without neuronal damage in place of MCAO, we detected significant inhibition of NF-kappa B and its downstream inflammatory effects. Consequently, we conclude that sevoflurane preconditioning suppressed inflammation triggered by cerebral ischemia directly, and that the attenuation of NF-kappa B and the down-regulation of inflammatory gene expression were shown to be putative mechanisms involved in the neuroprotection afforded by sevoflurane preconditioning. The suppression of NF-kappa B by sevoflurane APC was due to inhibition of I kappa B degradation or upregulation its content in intracellular (29,32).

P38 MAPK was also involved in mechanism of sevoflurane APC (33). Barone *et al.* reported a significant and prolonged increase in p38 activity in the ischemic

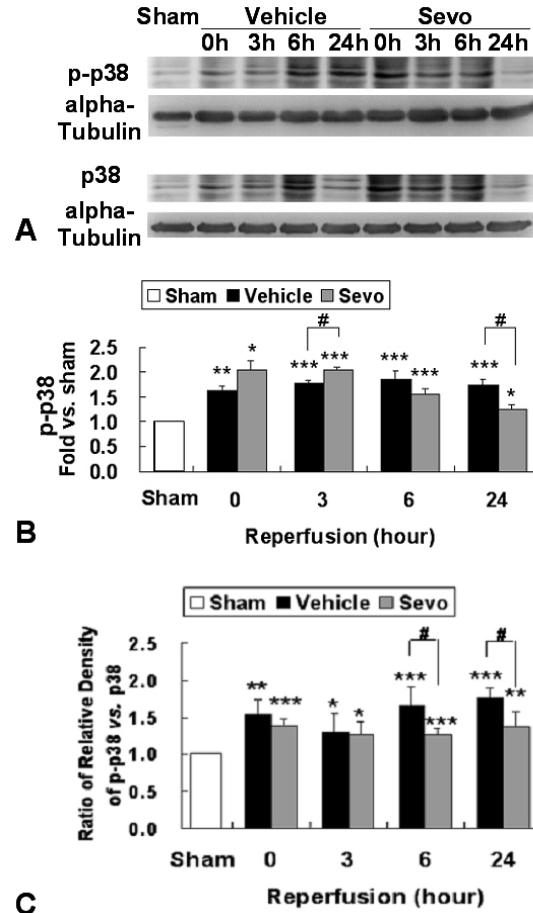


Figure 6. Sevoflurane Preconditioning Inhibited p38 MAPK after MCAO. A, Representative Western blot of p38 and p-p38. B, C, Graphs show semi-quantitative analysis (n=4 /per group/per time point). Compared with the sham group, ***p<0.001, **p<0.01, *p<0.05; compared with the vehicle group, #p<0.05.

brain. The time course of p38 MAPK activation was an early event, where p38 activity was seen at 1, 3 and 6 hr post-MCAO, and continued to be elevated for at least 24 hours (16). Our findings are in agreement with these. Zheng *et al.* reported that pretreatment with 2% isoflurane provided neuroprotection against permanent MCAO, and detected elevation of p-p38 immediately after isoflurane preconditioning but before cerebral ischemia, which was abolished by inhibitor of p38 (34). The authors didn't explore the level of p38 after ischemia. The inhibitor of p38 also increased I kappa B level, which correlates with decreased NF- kappa B activation (35). Desflurane preconditioning inhibits NF- kappa B activation *via* inhibiting I kappa B phosphorylation and degradation. Simultaneously, it also suppressed p38 phosphorylation after anoxia insults combined with inflammatory stimulus (36). Based on these studies, we infer that transient moderate ischemia (such as ischemic preconditioning or chemical agent preconditioning, including inhaled anesthetics) caused the increase in p38 MAPK, and that preconditioning inhibited elevation of p38 MAPK induced by prolonged severe ischemia.

In conclusion, preconditioning with sevoflurane decreased infarct volume, reduced deficiency of neurological function and suppressed inflammatory factors along with upstream NF-kappa B and p38 MAPK in rats exposed to focal cerebral ischemia. Furthermore, the anti-inflammatory effect of sevoflurane pretreatment was direct, not secondary to reduced infarct volume. However, how sevoflurane inhibits the activities of NF-kappa B and p38 deserves further exploration.

6. ACKNOWLEDGEMENTS

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- Abbreviations:** APC: volatile anesthetic preconditioning; iNOS: inducible NO synthase; COX2: cyclooxygenase-2; TNF- α : tumor necrosis factor-alpha; IL-1 α : interleukin-1 alpha; IL-1 β : interleukin-1 beta; IL-6: interleukin-6; MCAO: middle cerebral artery occlusion; rCBF: regional cerebral blood flow; MAC: minimum alveolar concentration; OGD: oxygen-glucose deprivation; ANOVA: one-way analysis of variance; MAPK: mitogen activated protein kinase.
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- Send correspondence to:** Yanqin Gao, State Key Laboratory of Medical Neurobiology and Institutes of Brain Science, Fudan University, 138 Yixueyuan Road, Shanghai, China, 200032 Tel: 086-021-54237395, Fax, 086-021-64224778, E-mail: yanqin.gao@gmail.com
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