

Sevoflurane post-conditioning attenuates traumatic brain injury-induced neuronal apoptosis by promoting autophagy via the PI3K/AKT signaling pathway

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Background: Sevoflurane post-conditioning exerts nerve-protective effects through inhibiting caspase-dependent neuronal apoptosis after a traumatic brain injury (TBI). Autophagy that is induced by the endoplasmic reticulum stress plays an important role in the secondary neurological dysfunction after a TBI. However, the relationship between autophagy and caspase-dependent apoptosis as well as the underlying nerve protection mechanism that occurs with sevoflurane post-conditioning following a TBI remains unclear.

Methods: The Feeney TBI model was used to induce brain injury in rats. Evaluation of the modified neurological severity scores, measurement of brain water content, Nissl staining, and terminal deoxynucleotidyl transferase dUTP nick end labeling assay were used to determine the neuroprotective effects of the sevoflurane post-conditioning. Both immunofluorescence and Western blot analyses were used to detect the expression of autophagy-related proteins microtubule-associated protein 1 light chain 3-II and Beclin-1, pro-apoptotic factors, as well as the activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway within the lesioned cortex.

Results: Autophagy and neuronal apoptosis were activated in the lesioned cortex following the TBI. Sevoflurane post-conditioning enhanced early autophagy, suppressed neuronal apoptosis, and alleviated brain edema, which improved nerve function after a TBI (all $P < 0.05$). Sevoflurane post-conditioning induced the activation of PI3K/AKT signaling after the TBI ($P < 0.05$). The neuroprotective effects of sevoflurane post-conditioning were reversed through the autophagy inhibitor 3-methyladenine treatment.

Conclusion: Neuronal apoptosis and the activation of autophagy were involved in the secondary neurological injury following a TBI. Sevoflurane post-conditioning weakened the TBI-induced neuronal apoptosis by regulating autophagy via PI3K/AKT signaling.

Keywords: traumatic brain injury, sevoflurane post-conditioning, cell apoptosis, autophagy, PI3K/AKT signaling pathway

Introduction

Traumatic brain injury (TBI) is a major cause of disability and death in adolescence. Mitigating brain damage and promoting functional recovery following a TBI would alleviate the burden to patients and to society.¹ Pathophysiological changes following TBI such as secondary inflammation, production of oxygen radicals, and toxicity from excess excitatory amino acids would activate lysosomal enzymes in order to promote caspase-dependent neuronal apoptosis.^{2,3} This process involves the upregulation of

caspase-3, the pro-apoptotic factor B-cell lymphoma (Bcl)-2-associated X protein (Bax), and the inhibition of the anti-apoptotic protein Bcl-2.⁴ Suppressing caspase-dependent apoptosis after a TBI could reduce cortical tissue injury and promote nerve repair, which would improve the patient prognosis.^{1,4}

Secondary neurological injury following a TBI consists of complex pathophysiological changes that could result in abnormal aggregation of misfolded or unfolded proteins in the endoplasmic reticulum lumen, a process known as endoplasmic reticulum stress (ERS).⁵ ERS activates the autophagy pathway in order to eliminate these proteins via lysosomal degradation^{6,7} and to maintain cellular homeostasis.⁸ The continuous autophagy or hyperactivation of autophagy could lead to cell death under pathological conditions, which has been linked to a variety of diseases, including craniocerebral injury.^{3,9} The effects of autophagy remain controversial, and these effects are dependent on the degree and time interval following an injury, as well as the type of autophagy.^{10,11} Recent studies have shown that autophagy has neuroprotective effects that are exerted via inhibition of mitochondrial apoptosis or neuroinflammation in rat TBI models.^{3,4} The underlying mechanism remains unclear, particularly the relationship between neuronal apoptosis and autophagy after a TBI.

Sevoflurane is a volatile anesthetic that is fast acting, is easily controlled, and has minimal effects on intracranial pressure and cerebral oxygen metabolism rate. These factors make it a viable anesthetic for neurosurgery.¹²⁻¹⁴ Previous studies have shown that sevoflurane post-conditioning improves neurological function following ischemia reperfusion, as well as protects neurons by inhibiting the production of oxygen free radicals, which prevents intracellular calcium and excitatory amino acid overload, while stabilizing neuron membranes.¹³ Sevoflurane post-conditioning also increases nerve function by suppressing inflammation via protein kinase B (AKT) regulation.¹⁵ Lai et al¹⁶ demonstrated that sevoflurane post-conditioning displayed neuroprotective effects via the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway in neonatal hypoxia-ischemia-induced brain damage in rats.

Autophagy plays an important role in maintaining cellular homeostasis, which is closely controlled through PI3K/AKT signaling. Treatment with the PI3K inhibitors LY294002 and the 3-methyladenine (3-MA) block autophagosome formation.^{10,17} Activation of the PI3K/AKT pathway also suppresses apoptosis after a TBI.^{18,19} Although the neuroprotective effects of sevoflurane post-conditioning involve PI3K/AKT signaling, it is unclear if sevoflurane

post-conditioning could weaken the TBI-induced neuronal apoptosis by regulating autophagy.

Methods

Materials

The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) apoptosis detection kit and the NeuN antibodies were purchased from Boster Biotech (Wuhan, China). The cleaved caspase-3, Bax, Bcl-2, microtubule-associated protein 1 light chain 3 alpha (LC3), Beclin-1, PI3K, AKT, p-PI3K, and p-AKT antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). The autophagy inhibitor, 3-MA, was purchased from Sigma-Aldrich (St Louis, MO, USA). Sevoflurane was purchased from Abbott Laboratories (Chicago, IL, USA).

Animals

The experimental protocols in this study, including the surgical procedures and the animal procedures, were in accordance with the guidelines for the care and use of laboratory animals by the National Institutes of Health and were approved by the Fujian Medical University Experimental Animal Ethics Committee (No FMU10012). Adult male Sprague-Dawley rats weighing between 220 g and 250 g were purchased from the Fujian Medical University Laboratory Animal Center (Fujian, China). Rats were raised in a controlled environment, at a temperature range of 23°C ± 2°C on a 12:12-h light/dark cycle with free access to food and water.

TBI model

Rats were randomly assigned to sham, TBI, and TBI+Sevo (sevoflurane inhalation for 1 h following the TBI) groups. The groups were divided further into 1-, 3-, 7-, and 14-day subgroups (n = 12 each) following the TBI. The Feeney TBI model was used to induce the brain injury in the rats.¹ The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The scalp was then opened at 2 mm posterior to the right coronal suture and 2 mm from the midline. A 5 mm hole was drilled through the skull, although the dura mater was left intact. A 30 g hammer was dropped from a height of 20 cm to induce craniocerebral injury (impact force = 600 g/cm). The bone hole was sealed with wax and the scalp was sutured. Rats in the sham group underwent this surgical procedure without the hammer drop. Each rat was placed in an airtight anesthesia box (42 × 26 × 26 cm) with 1.5 cm diameter holes on two opposite sides for gas input and removal 30 min after the surgery. O₂ was delivered to the rats in the Sham and TBI groups. O₂ containing sevoflurane was delivered to the TBI+Sevo group. A gas analyzer monitored

the concentrations of sevoflurane, O₂, and CO₂. The sevoflurane concentration was kept at 1 MAC (2.4%) for 1 h. The rats were removed from the box after the 15 min additional oxygen inhalation. The 3-MA autophagy inhibitor specifically blocks autophagosome formation,^{10,17} and was used to solidify the role of the autophagy pathway in the neuroprotective effects of sevoflurane post-conditioning. The rat's right lateral ventricle was injected with 3-MA dissolved in 0.9% saline (600 nmol, diluted in 0.9% saline to a final volume of 5 µL) 0.5 h after the sevoflurane exposure.¹⁰ The remaining rats in each group were injected with 0.9% saline, as a control.

Evaluation of neurological function

Rat motion, sensation and reflex, muscle mass, abnormal behavior, vision, touch, and balance were evaluated with the Modified Neurological Severity Score (mNSS).¹ The mNSS test was graded on a scale of 0–18. A total score of 18 points indicated severe neurological deficits, and a score of 0 reflected a normal state. The higher the score, the greater the neurological damage. Neurological function was evaluated on day 1, 3, 7, and 14 post-TBI.

Measurement of brain water content

The neurological function of the rats was assessed and the rats were euthanized in order to remove the brain. The cerebral cortex (weighing 200 ± 20 mg at 2 mm from the bone window edge) was harvested, and the surface blood and cerebrospinal fluid were removed by blotting with filter paper. The wet weight was measured with an analytical balance. The tissue was baked at 100°C in an oven for 24 h in order to obtain the dry weight. The brain water content was calculated as % water = (wet weight – dry weight)/wet weight × 100%.

Nissl staining

Cortical tissues surrounding the lesioned areas were collected, fixed in formaldehyde, embedded in paraffin, and cut into 4 µm-thick sections that were de-paraffinized with xylene and then rehydrated in a graded series of alcohol. The samples were placed in a Nissl staining solution for 5 min. The damaged neurons shrank or contained vacuoles and the normal neurons had a relatively large and full soma, with round and large nuclei. Neurons in five randomly selected areas were counted under the microscope by experimenters who were blinded to the experimental group assignment.

TUNEL assay

Apoptotic cells were detected via a TUNEL assay kit (Boster Biotech), in accordance with the manufacturer's instructions. Cortical tissue from the lesioned area was incubated overnight at 4°C with an anti-neuronal nuclei antibody (1:100;

Boster Biotech). The samples were washed three times with phosphate-buffered saline and then incubated with the TUNEL reaction mixture for 1 h at 37°C. A double-blind approach was used for quantification. Five randomly selected areas that surrounded the injury site were used to count the TUNEL-positive neurons under an inverted fluorescence microscope at a high magnification (400×) (HB050; Zeiss, Hamburg, Germany).

Immunofluorescence analysis

The cortical tissues surrounding the lesioned areas were fixed in formaldehyde and embedded in paraffin. The tissue blocks were sectioned at a thickness of 3 µm. After deparaffinization with dimethyl benzene and dehydration in a series of graded alcohol, the antigen was obtained via the citric acid buffer/microwave method. The sections were blocked with goat serum and incubated overnight with primary antibodies for NeuN (1:100; Boster Biotech) and LC3 (1:200; Cell Signaling Technology). The samples were washed with phosphate-buffered saline and then incubated with a 1:200 dilution of fluorescein isothiocyanate-conjugated secondary antibody (goat anti-rabbit IgG-fluorescein isothiocyanate; Invitrogen, Grand Island, NY, USA) for 30 min, followed by 4',6-diamidino-2-phenylindole staining and observations with a fluorescence microscope.

Western blotting

The cortical tissue (30 mg) surrounding the lesioned area was lysed in lysis buffer, where the total protein level was quantified. The lysate was boiled for 8 min and centrifuged at 10,000 rpm for 5 min. The supernatant was stored at –20°C. A total of 25 µg of protein was separated by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane that was blocked in 10% skim milk at room temperature for 2 h. The solution was incubated at 4°C overnight with primary antibodies against the following proteins: LC3 (1:500), Beclin-1 (1:400), cleaved caspase-3 (1:400), Bax (1:400), Bcl-2 (1:500), PI3K (1:500), AKT (1:500), p-PI3K (1:400), p-AKT (1:400) (all from Cell Signaling Technology), and β-actin (1:5,000; Bioworld Technology, St Louis Park, MN, USA), followed by a horseradish peroxidase-conjugated secondary antibody (1:5,000; Cell Signaling Technology) at room temperature for 1 h. The protein bands were visualized by enhanced chemiluminescence. β-Actin served as a loading control.

Statistical analyses

SPSS v.18.0 software (SPSS Inc., Chicago, IL, USA) was used for the data analysis. Results are presented as

mean \pm standard deviation. The differences between groups were evaluated with a one-way ANOVA, and post hoc multiple comparisons were performed using Student-Newman-Keuls tests. Numerical data are presented as the absolute values and were compared with the χ^2 test. $P < 0.05$ was considered as statistically significant.

Results

Sevoflurane post-conditioning has neuroprotective effects following TBI

One rat in the TBI group died and was excluded. The average blood pressure was lower during sevoflurane post-conditioning than the baseline values throughout the TBI groups ($P < 0.05$). There were no other significant differences in arterial blood gas among the groups ($P > 0.05$) (data not shown).

Neurological function was evaluated with the mNSS.¹ There was no difference between the pre-operative and post-operative scores in the sham group. The neurological function was reduced at day 1 post-injury within the TBI group, but gradually improved until the end of the experimental period. The injured rats that were subjected to sevoflurane post-conditioning (TBI+Sevo group) had a lower mNSS from day 3 post-injury than groups that did not receive this treatment ($P < 0.05$) (Figure 1A).

Brain water content was greater in the TBI group than in the sham group and peaked on day 3 post-injury ($P < 0.05$). The rats that were subjected to sevoflurane post-conditioning had less brain water content on day 3 than the TBI group ($P < 0.05$) (Figure 1B).

The neuronal apoptosis in the lesioned cortex was investigated with Nissl staining.¹ There was a higher fraction

of apoptotic cells on days 3, 7, and 14 following the TBI than the sham group. The rats in the TBI+Sevo group had a lower fraction of apoptotic cells than the TBI group ($P < 0.05$) (Figure 2). These observations were confirmed with the TUNEL assay, which revealed that the TBI-induced increase in neuronal apoptosis on day 3 post-injury was nullified by the sevoflurane post-conditioning ($P < 0.05$) (Figure 3). These results demonstrate that sevoflurane post-conditioning has nerve-protective effects following a TBI.

Sevoflurane post-conditioning activates autophagy after TBI

Immunofluorescence analysis showed that the LC3 expression in neurons was upregulated following the TBI, where it increased further with the addition of sevoflurane post-conditioning in the lesioned cortex (Figure 4A). We also found that the expression of the autophagy-related proteins LC3-II and Beclin-1 was greater on day 3 post-injury in rats that were subjected to sevoflurane post-conditioning (TBI+Sevo group) than in rats in the TBI group, as determined by Western blotting (Figure 4B and C). These findings indicated that sevoflurane post-conditioning activated autophagy following a TBI.

3-MA treatment inhibits autophagy and reverses the protective effects of sevoflurane post-conditioning on neuronal apoptosis

3-MA is a specific inhibitor for autophagy that acts by blocking autophagosome formation.^{10,17} 3-MA was used to inhibit autophagy, in order to confirm the protective effects of

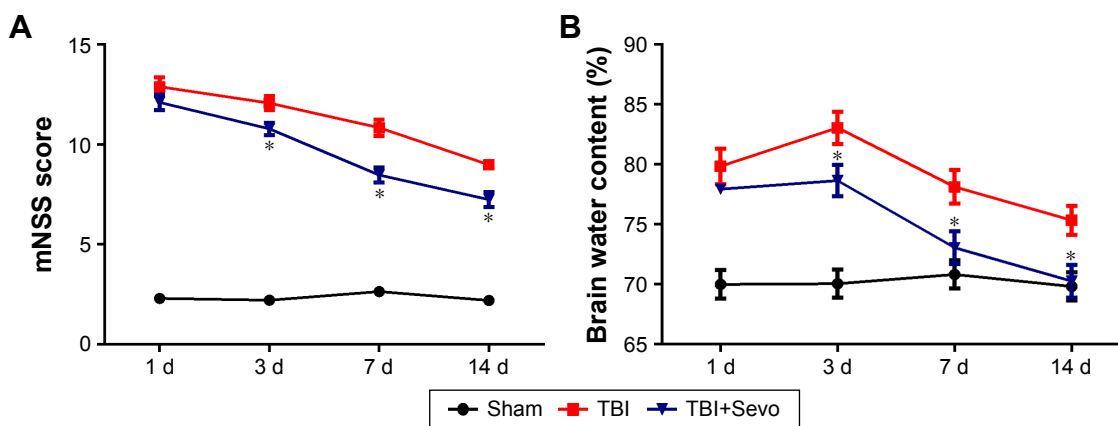


Figure 1 Sevoflurane post-conditioning reduced brain edema and improved neurological function following a TBI.

Notes: (A) There were no differences between the pre-operative and post-operative scores in the sham group. Sevoflurane post-conditioning (TBI+Sevo group) obtained a lower mNSS beginning at day 3 post-injury than the TBI group ($P < 0.05$). (B) Brain water content increased more in the TBI rats than in the sham controls and reached a peak on day 3 post-injury ($P < 0.05$). Rats subjected to sevoflurane post-conditioning had less brain water content on day 3 than the rats in the TBI group ($P < 0.05$). Data are represented as mean \pm SEM ($n = 6$). * $P < 0.05$ vs TBI group.

Abbreviations: mNSS, Modified Neurological Severity Score; SEM, standard error of the mean; Sevo, Sevoflurane; TBI, traumatic brain injury.

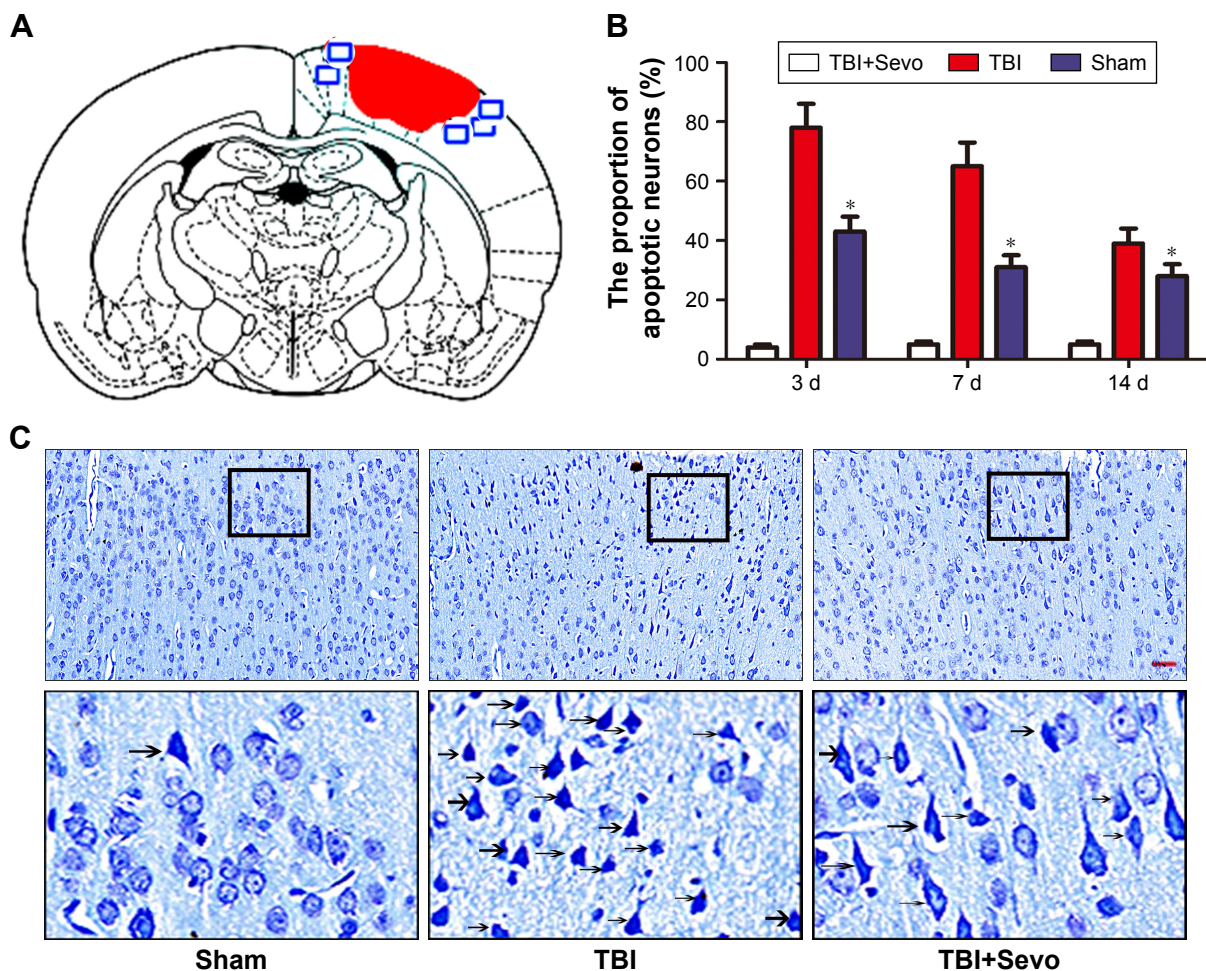


Figure 2 Sevoflurane post-conditioning reduced neuronal apoptosis in the lesioned cortex following the TBI.

Notes: (A) Schema of the brain sections following the TBI. Red and blue areas indicate the lesion sites and the sample points. (B) Compared to the TBI group, rats in the TBI+Sevo group had a lower fraction of apoptotic neurons on days 3, 7, and 14 after TBI, respectively. (C) Representative photomicrographs of Nissl-stained brain sections on day 3 after TBI; arrows indicate apoptotic neurons. Data are represented as mean \pm SEM (n = 6). * $P < 0.05$ vs TBI group; scale bars = 50 μ m; magnification $\times 200$.

Abbreviations: SEM, standard error of the mean; Sevo, Sevoflurane; TBI, traumatic brain injury.

sevoflurane post-conditioning. Compared with the TBI group, 3-MA treatment decreased the expression of LC3-II and Beclin-1 on day 3 post-injury in rats. Notably, the autophagy activation, induced by sevoflurane post-conditioning, was blocked by 3-MA. The expression of LC3-II and Beclin-1 relative to the TBI+Sevo group was also decreased ($P < 0.05$) (Figure 5).

The levels of the pro-apoptotic factors cleaved caspase-3 and Bax were downregulated and the levels of the anti-apoptotic factor Bcl-2 were upregulated in the TBI+Sevo group, as compared to the TBI group on day 3 after injury ($P < 0.05$). Again, these were determined by Western blot analysis. The 3-MA treatment weakened the anti-apoptotic effect of the sevoflurane post-conditioning ($P < 0.05$) (Figure 6), possibly by activating autophagy following the TBI.

The PI3K/AKT pathway plays an important role in mediating autophagy and in preventing apoptosis following a TBI.^{18,19} Inhibition of PI3K class I leads to the inhibition of Akt phosphorylation, a central molecule involved in many

arrays of intracellular cascades within the nervous system.¹⁷ We examined the role of PI3K/AKT signaling on sevoflurane post-conditioning-induced neuroprotection following a TBI. The sevoflurane treatment increased the expressions of p-PI3K and p-AKT more than the TBI group on day 3 after injury ($P < 0.05$). As a PI3K/AKT signaling pathway inhibitor, the 3-MA intervention effectively inhibited the expression of PI3K/AKT signaling pathway following the TBI ($P < 0.05$). The PI3K/AKT pathway activation that was induced by sevoflurane post-conditioning was blocked by the 3-MA treatment following the TBI ($P < 0.05$) (Figure 7). The results suggested that the PI3K/AKT signaling could mediate the autophagy activation induced by sevoflurane post-conditioning following a TBI.

Discussion

Intervention with inhalation anesthetics has been found to counter perioperative ischemic brain injury and improve the

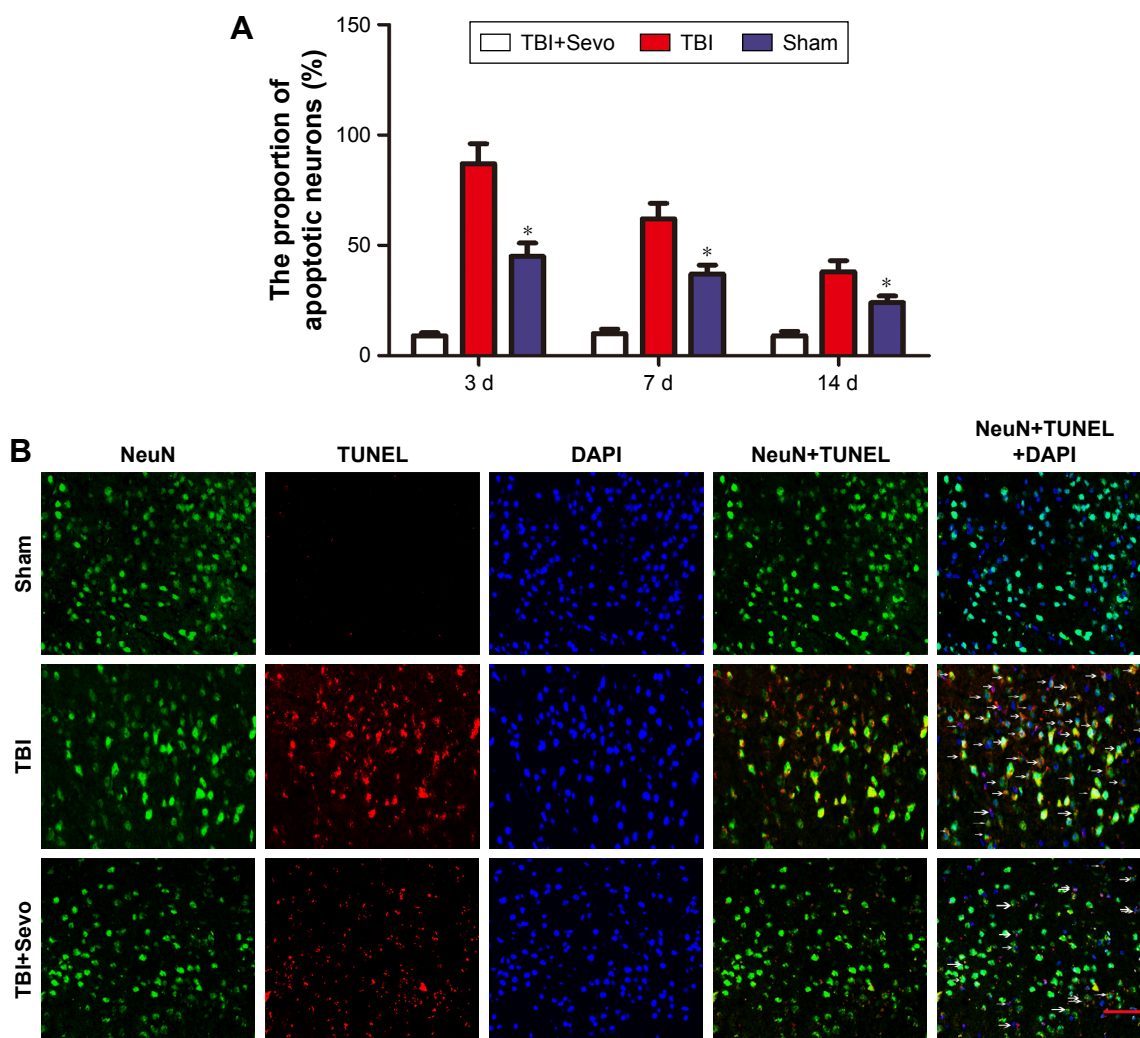


Figure 3 Sevoflurane post-conditioning inhibited neuronal apoptosis in the lesioned cortex following the TBI.

Notes: (A) Rats in the TBI+Sevo group had a lower fraction of apoptotic neurons on days 3, 7, and 14 after TBI, respectively ($P < 0.05$). (B) Representative images of TUNEL-positive neurons (arrows) on day 3 after TBI. Data are represented as mean \pm SEM ($n = 6$). * $P < 0.05$ vs TBI group. Scale bars = 50 μ m; magnification $\times 400$.

Abbreviations: DAPI, 4,6-Diamidino-2-phenylindole, dihydrochloride; SEM, standard error of the mean; Sevo, Sevoflurane; TBI, traumatic brain injury; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

patient's prognosis.²⁰ Sevoflurane is an ideal anesthetic for neurosurgery, since it has low blood gas distribution coefficient, rapid induction of awakening, and reduction of the cerebral oxygen metabolic rate.^{21–23} However, the neuroprotective mechanism underlying these effects following a TBI remains unknown. In this study, we confirmed that sevoflurane post-conditioning reduced brain edema and improved neurological scores in a rat TBI model. Data from the Nissl staining and the TUNEL assay demonstrated that sevoflurane post-conditioning protected the neurons and improved the neurological function by inhibiting TBI-induced neuronal apoptosis.

TBI-induced secondary injury is a complex pathophysiological process that includes abnormal mitochondrial activity, oxidative stress, and inflammatory cytokine release,

which promotes caspase-dependent neuronal apoptosis.^{17,24,25} Suppressing caspase-dependent apoptosis following a TBI could reduce cortical tissue injury and promote nerve repair, thereby improving the patient's prognosis.^{4,17} In this study, we found that intervention with sevoflurane post-conditioning decreased the expression of the pro-apoptotic factors cleaved caspase-3 and Bax, and upregulated the anti-apoptotic factor Bcl-2. These findings were consistent with the observation that sevoflurane post-conditioning reduced brain edema and neuronal apoptosis following a TBI.

Autophagy is a process in eukaryotic cells in which proteins or organelles are degraded by the lysosome²⁶ as an adaptation to stress, in order to maintain homeostasis in the organism.²⁷ LC3-II and Beclin-1 induce the formation of a pre-autophagosomal structure that promotes autophagic

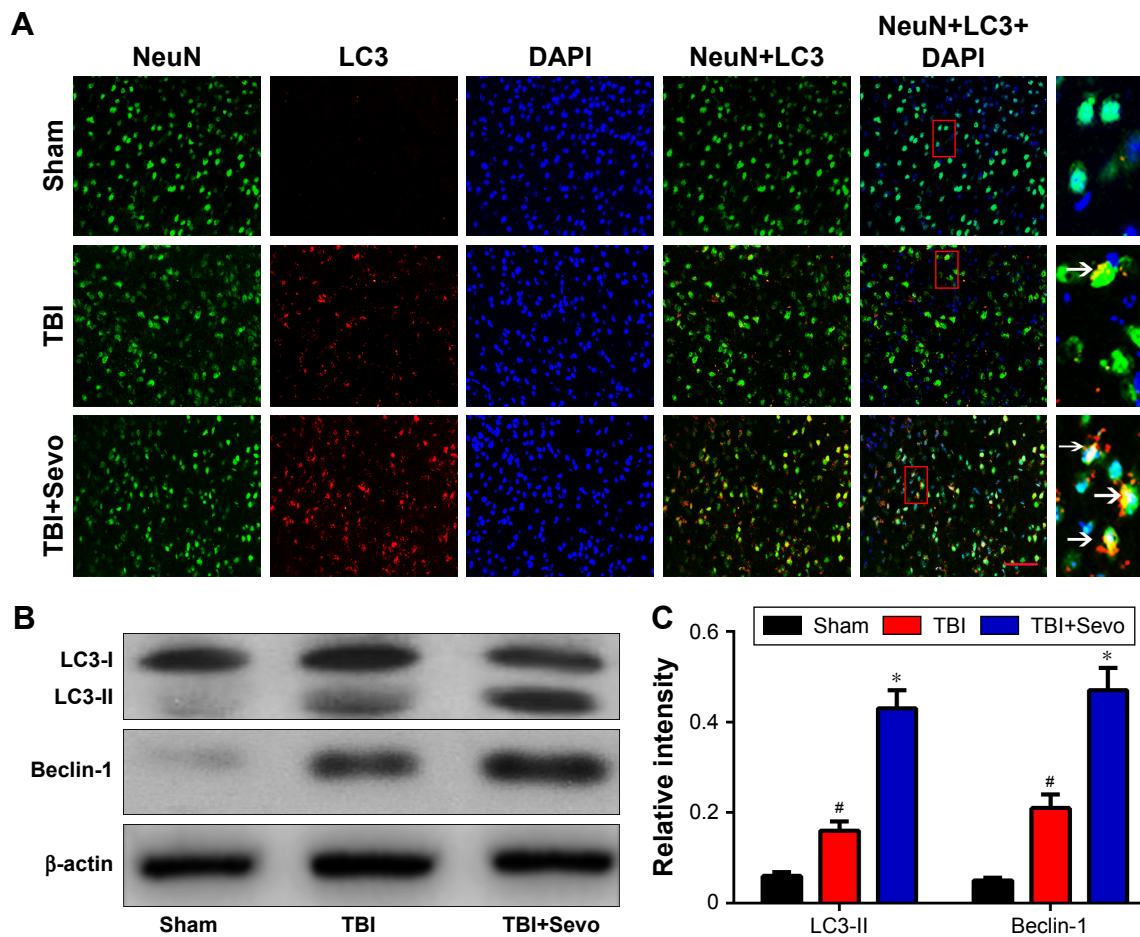


Figure 4 Sevoflurane post-conditioning activated autophagy in the lesioned cortex on day 3 following the TBI.

Notes: (A) Representative images of LC3-positive neurons (arrows) where LC3 expression in the neurons was upregulated after the TBI and increased further via sevoflurane post-conditioning. (B) Representative Western blot shows LC3-II and Beclin-1 levels where the LC3-II and the Beclin-1 levels increased via sevoflurane post-conditioning following the TBI. (C) Relative expression levels of the autophagy-related proteins, LC3-II and Beclin-1. Data are represented as mean \pm SEM (n = 6). [#]P < 0.05 vs Sham group, ^{*}P < 0.05 vs TBI group. Scale bars = 50 μ m; magnification \times 400.

Abbreviations: DAPI, 4,6-Diamidino-2-phenylindole, dihydrochloride; SEM, standard error of the mean; Sevo, Sevoflurane; TBI, traumatic brain injury; LC3, microtubule-associated protein 1 light chain 3 alpha.

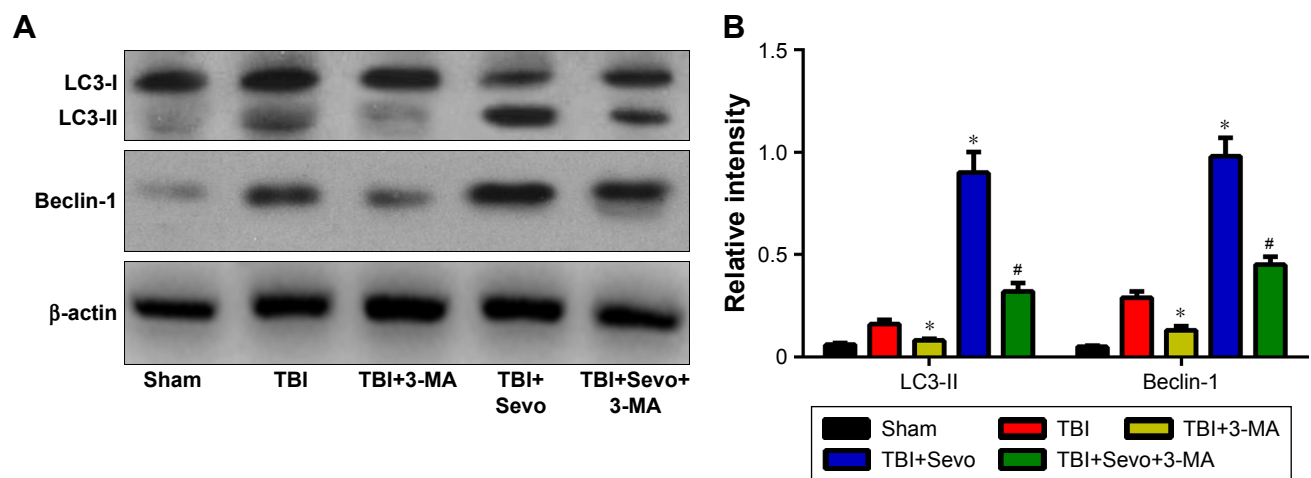


Figure 5 3-MA treatment inhibited autophagy induced by sevoflurane post-conditioning.

Notes: (A) 3-MA decreased the expression of LC3-II and Beclin-1 following the TBI. The autophagy activation, induced by sevoflurane post-conditioning, was blocked by 3-MA, which suppressed the expression of LC3-II and Beclin-1 more than the TBI+Sevo group. Representative Western blot displays the LC3-II and the Beclin-1 levels. (B) Relative expression levels of the autophagy-related proteins, LC3-II and Beclin-1. Data are represented as mean \pm SEM (n = 6). ^{*}P < 0.05 vs TBI group; [#]P < 0.05 vs TBI+Sevo group.

Abbreviations: 3-MA, 3-methyladenine; LC3, microtubule-associated protein 1 light chain 3 alpha; SEM, standard error of the mean; Sevo, Sevoflurane; TBI, traumatic brain injury.

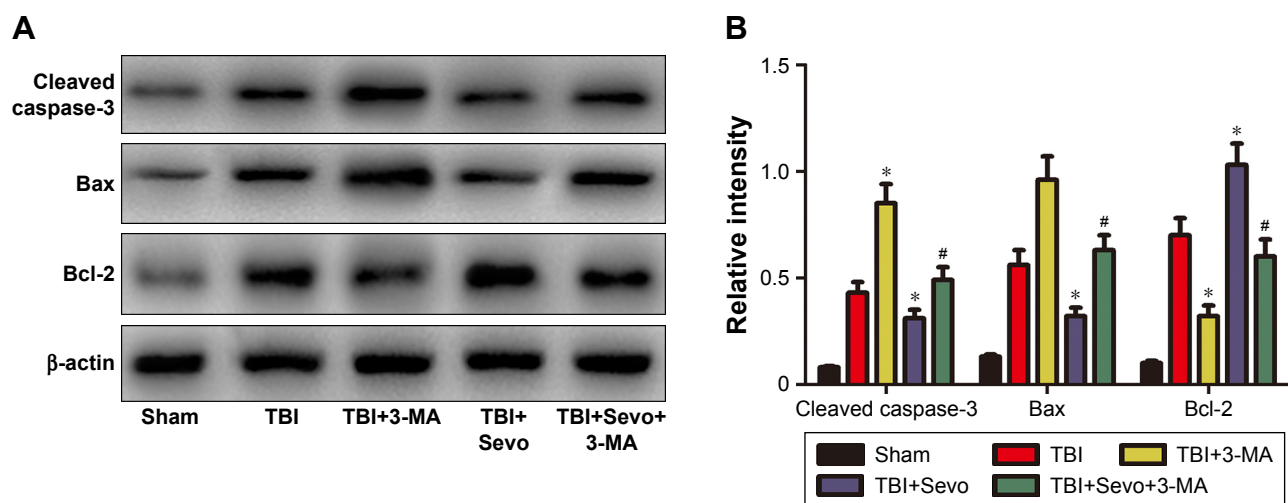


Figure 6 Sevoflurane post-conditioning suppressed the expression of pro-apoptotic proteins following the TBI.

Notes: (A) Protein expression of the cleaved caspase-3 and the Bax were downregulated, although the Bcl-2 expression was upregulated by the sevoflurane treatment on day 3 following the TBI. 3-MA treatment abolished the anti-apoptotic effect of the sevoflurane post-conditioning. Representative Western blot displays caspase-3, Bax, and Bcl-2 levels. (B) Relative expression levels of the apoptotic factors. Data are represented as mean \pm SEM (n = 6). * $P < 0.05$ vs TBI group; # $P < 0.05$ vs TBI+Sevo group.

Abbreviations: SEM, standard error of the mean; Sevo, Sevoflurane; TBI, traumatic brain injury; 3-MA, 3-methyladenine.

vacuole formation, making them markers of autophagy.^{28,29} 3-MA is a PI3K pathway inhibitor that is recognized as a specific autophagy inhibitor due to its ability to block autophagosome formation.^{10,17} 3-MA was used to illuminate the role of the autophagy pathway in the neuroprotective effects of sevoflurane post-conditioning. In the present study, LC3-II and Beclin-1 protein levels were upregulated following a TBI. The 3-MA treatment countered this increase in LC3-II and Beclin-1 expression, by decreasing the expression of the pro-apoptotic factors cleaved caspase-3 and Bax following

the TBI. This indicated that autophagy played a critical role in TBI-mediated neuronal apoptosis. Sevoflurane post-conditioning enhanced this early autophagy response, while inhibiting neuronal apoptosis after injury. Both of these were blocked by the 3-MA treatment. These results indicated that sevoflurane post-conditioning diminished the TBI-induced neuronal apoptosis by regulating autophagy.

The relationship between autophagy and apoptosis in neurologic diseases is complex and misunderstood. Emerging evidence has suggested that PI3K/AKT pathway activation

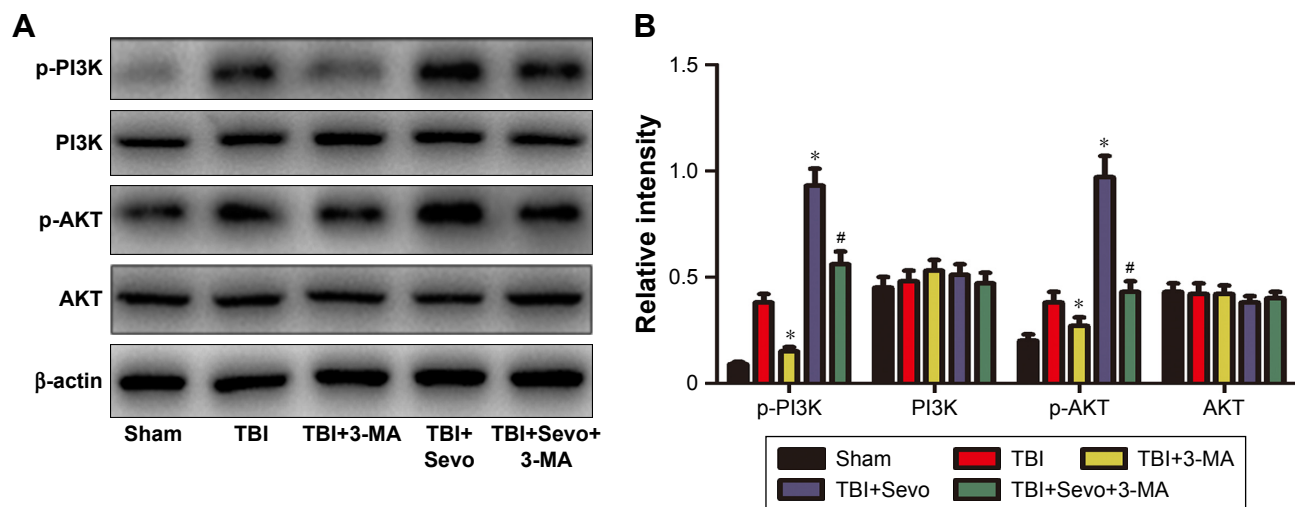


Figure 7 3-MA treatment inhibited the activation of PI3K/AKT signaling induced by sevoflurane post-conditioning following the TBI.

Notes: (A) Sevoflurane post-conditioning increased the expression of p-PI3K and p-AKT more than the TBI group on day 3 following the TBI. 3-MA intervention effectively inhibited the expression of PI3K/AKT signaling pathway following the TBI. The activation of the PI3K/AKT pathway induced by sevoflurane post-conditioning was blocked by the 3-MA treatment. Representative Western blot displays the p-PI3K and the p-AKT levels. (B) Relative expression levels of the PI3K/AKT pathway components. Data are represented as mean \pm SEM (n = 6). * $P < 0.05$ vs TBI group; # $P < 0.05$ vs TBI+Sevo group.

Abbreviations: 3-MA, 3-methyladenine; PI3K/AKT, phosphatidylinoside 3-kinase/protein kinase B; SEM, standard error of the mean; Sevo, Sevoflurane; TBI, traumatic brain injury; 3-MA, 3-methyladenine.

plays a critical neuroprotective role in TBI by regulating anti-apoptosis and autophagy responses.^{18,30} Akt phosphorylation weakens the expressions of neuronal apoptosis-related downstream molecules, such as cleaved caspase-3, Bcl-2, and Bax, resulting in the dissociation of the Bcl-2/Beclin-1 complexes.^{18,30} This process releases Beclin-1 and leads to an autophagy response. Treatment with the PI3K kinase inhibitors LY294002 and 3-MA blocks autophagosome formation.^{10,17} Activation of the PI3K/AKT pathway suppresses apoptosis following a TBI.^{18,19} We examined the role of PI3K/AKT signaling on sevoflurane post-conditioning-induced neuroprotection following a TBI. We found that sevoflurane treatment induced the activation of PI3K/AKT pathway, where the expressions of p-PI3K and p-AKT were increased more than the TBI group. 3-MA, a PI3K/AKT signaling pathway inhibitor, effectively inhibited the expression of the PI3K/AKT signaling pathway following the TBI. The PI3K/AKT pathway activation, induced by sevoflurane post-conditioning, was blocked by 3-MA treatment, which indicated that sevoflurane post-conditioning weakened the TBI-induced neuronal apoptosis by regulating autophagy induced by PI3K/AKT signaling. Further studies using PI3K knockout mice should be performed to clarify the mechanisms underlying sevoflurane post-conditioning-induced autophagy and consequent activation of the PI3K/AKT pathway.

Conclusion

Our results demonstrated that the neuronal apoptosis and the activation of autophagy were involved in the secondary neurological injury subsequent to a TBI. Sevoflurane post-conditioning diminished the TBI-induced neuronal apoptosis by regulating autophagy via PI3K/AKT signaling.

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Disclosure

The authors report no conflicts of interest in this work.

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