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Sevoflurane inhibits neuronal apoptosis and expressions of HIF-1 and HSP70 in brain tissues of rats with cerebral ischemia/reperfusion injury

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Abstract. – OBJECTIVE: To observe the influences of sevoflurane on neuronal apoptosis and expressions of hypoxia-inducible factor 1 (HIF-1), and heat-shock protein 70 (HSP70) in brain tissues of rats with cerebral ischemia/reperfusion (I/R) injury.

MATERIALS AND METHODS: A total of 60 Sprague-Dawley rats were selected and divided into sham-operation group (Sham group, n=20), cerebral I/R model group (Model group, n=20), and 3% sevoflurane treatment group (Sevoflurane group, n=20). The rats in each group received neurological scoring, and the blood and brain tissues were collected to detect the concentrations of serum K+, Na+ and glucose (Glu). Enzyme-linked immunosorbent assay (ELISA) was adopted to measure the levels of inflammatory factors [tumor necrosis factor-β (TNF-β) and interleukin-6 (IL-6)] and oxidative stress [catalase (CAT), malondialdehyde (MDA) and superoxide dismutase (SOD)]. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay was performed to determine the nerve cell apoptosis in the brain tissues. The gene and protein expressions of Caspase-3, HIF-1, and HSP70 in the brain tissues were measured *via* quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) and Western blotting.

RESULTS: In Sevoflurane group, the content of serum Glu and Na^+ was decreased markedly, that of K^+ was increased notably, and the levels of TNF-β, IL-1 and IL-6 were lowered remarkably compared with those in Model group ($p<0.05$). Moreover, the neurological score was reduced evidently ($p<0.05$). Model group had significantly strengthened the activity of MDA and CAT and decreased SOD content, while Sevoflurane group exhibited the opposite results. TUNEL staining showed that there were distinctly more apoptotic cells that were dominated by glial cells in Model group and fewer apoptotic cells in Sevoflurane group. It was indicated in gene assay that the messenger ribonucleic acid (mRNA) expression levels of HIF-1, HSP70, and Caspase-3 in Model group were remarkably higher than those in Sham group and Sevoflurane group ($p<0.05$). According to the results of Western blotting, the protein expressions of HIF-1 and HSP70 in Sevoflurane group were markedly lower than those in Model group.

CONCLUSIONS: Sevoflurane can reduce the content of inflammatory factors, inhibit apoptosis, and reduce the expressions of HIF-1 and HSP70 in the case of cerebral I/R injury, thus exerting protective effects on rats with cerebral I/R injury.

Key Words:
Sevoflurane, Cerebral ischemia/reperfusion injury, Rats, Neuronal apoptosis, HIF-1, Heat-shock protein 70.

Introduction

Cerebral ischemia refers to the loss or impairment of neuronal function in the brain, which is usually attributed to transient or permanent decrease in cerebral blood flow[1]. Currently, although ischemia/reperfusion (I/R) is still the standard therapy for recovering blood supply, it often causes reperfusion injury[2]. It has been proven that the I/R injury is the third major cause of death around the world[3]. Cerebral I/R injury is usually aggravated by the sudden increase in blood supply, which not only exacerbates brain damage and neurological impairment but also promotes revascularization and cerebral infarction[4]. The cerebral I/R injury includes the primary injury during ischemia period and the secondary injury during reperfusion period which can form cerebral edema. Cerebral edema is a harmful neuropathological state that can lead to deaths during serious disability and isch-

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emic stroke, thus aggravating the brain damage. The pathogeny of cerebral I/R injury varies from case to case, including cell membrane injury, excitatory amino acid toxicity, mitochondrial dysfunction, protein oxidation, and neutrophil activation[5,6]. The cerebral I/R injury is a complicated pathophysiological process[7]. Li et al[8] that the interruption and reperfusion of cerebral blood flow induced by brain cell injury is a rapid cascade involving numerous steps, such as acidosis, failure of blood-brain barrier, increased release of excitatory amino acids, formation of free radicals, and overexpression of apoptosis-related genes, which interact with each other to form a feedback loop, finally triggering the cell apoptosis or necrosis[9]. The neuroprotective effects of sevoflurane, one of the widely applied inhalation anesthetics in clinic, have been demonstrated. Deng et al[10] illustrated that the preprocessing with low-concentration sevoflurane has protective effects against ischemic injury in a relatively short time. The post-injury treatment is of important significance due to unpredictable cerebrovascular events. Therefore, as an efficacious therapeutic method, sevoflurane treatment has attracted more and more attention.

Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription complex composed of inducible HIF-1α subunit and constitutive HIF-1β subunit. HIF-1α will accumulate and cause cell death by activating different target genes under serious anoxic conditions, and it can bind to the pro-apoptotic members of the B-cell lymphoma-2 (Bcl-2) family, such as BNIP3, p53, and caspases[11]. As a crucial player in oxygen balance in cell bodies, HIF-1α is also proven to regulate the hypoxia-induced cell apoptosis *in vitro*[12]. Moreover, HIF-1α is a key component of hypoxia and ischemia which often result in severe brain damage in neonates. Hypoxic-ischemic brain damage (HIBD) generally induces cell death through necrosis or apoptosis. The mode of cell death depends on the severity of the damage. The cell apoptosis is closely associated with HIF-1α[13]. Heat-shock protein 70 (HSP70) is a HSP family member with the strongest inducing ability and potent apoptotic property. The induction of HSP70 is triggered within specific temporal-spatial parameters in the brain, so as to respond to ischemia, excitotoxicity, and other reactions[14]. HSP70 in the brain is classically expressed by neurons, which directly or indirectly participates in several critical cellular processes such as protein folding, translocation, and degradation[15]. Besides, it is always related to diseases or pathophysiological status at the whole-body level, e.g., ischemic damage[16]. However, the ischemic injury can be alleviated by decreasing the protein expressions in the myocardium of patients at a risk of acute ischemic stroke[17]. The influences of HIF-1 and HSP70 expressions on brain damage in cerebral I/R have not been systematically reported, and need to be further studied.

This research aims to observe the influences of sevoflurane on neuronal apoptosis and expressions of HIF-1 and HSP70 in the brain tissues of rats with cerebral I/R injury. After the intervention in the rat I/R model with sevoflurane was detected, the impacts on the cerebral I/R injury of rats were elaborated through *in-vivo* experiments: multiple molecular biological techniques, indexes of inflammation, oxidative stress and apoptosis, as well as gene and protein expressions of HIF-1 and HSP70. The protective effects of sevoflurane against the cerebral I/R injury in rats were observed, thus providing important experimental supports and theoretical references for the treatment of cerebral I/R injury with sevoflurane.

Materials and Methods

Reagents and Instruments

Sevoflurane and pentobarbital sodium (Nabot, Tokyo, Japan); TRIzol reagent, SuperScript III reverse transcription (RT) kit, and SYBR quantitative polymerase chain reaction (qPCR) Mix (ABI, Applied Biosystems, Foster City, CA, USA). Enzyme-linked immunosorbent assay (ELISA) kits for interleukin-1 (IL-1) and IL-6 (Hanbio Biotechnology Co., Ltd., Shanghai, China); radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China); fluorescence terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining kit (Roche, Basel, Switzerland); extracorporeal membrane oxygenator (Ohmeda, Boston, MA, USA); tissue homogenizer (FLUKO Equipment Shanghai Co., Ltd., Shanghai, China); 2500 gel imager and electrophoresis apparatus (Bio-Rad, Hercules, CA, USA); microplate reader (Thermo Fisher Scientific, Waltham, MA, USA); qPCR instrument (Applied Biosystems, Foster City, CA, USA); Loading Buffer, protease inhibitor, and bicinchoninic acid (BCA) protein assay kit (Biosharp, Hefei, China); β-actin and secondary antibodies (Boster Biological Technology Co., Ltd., Wuhan, China), and primary antibodies (Abcam, Cambridge, MA, USA).

Animal Modeling and Grouping

This investigation was approved by the Animal Ethics Committee of China Medical University Animal Center. Forty healthy male Sprague-Dawley rats were randomly selected, anesthetized by pentobarbital sodium, and disinfected. Then, an incision was made in the middle of the neck under sterile conditions to separate the right common carotid artery. Later, a labeled intraluminal thread was inserted from the right internal carotid artery into the middle cerebral artery. After continuous blocking for 120 min, perfusion was restored to establish the rat model of cerebral I/R injury. The rats were divided into cerebral I/R model group (Model group) and sevoflurane treatment group (Sevoflurane group), and they were given 3% sevoflurane using the extracorporeal membrane oxygenator for 5 consecutive days (30 min per day). Besides, another 20 rats were enrolled as sham-operation group (Sham group) which were treated by the same surgical methods as those in Model group except for the ischemia and reperfusion. After the experiment, blood and brain tissues were collected for subsequent studies.

Neurological Scoring on Rats in Each Group

Before the rats in each group were sacrificed, the neural functional defect was scored independently by three people using the blind method according to the 5-point Plesnila scale (Table I). The rats with the highest and lowest scores were discarded, and the ineligible rats were randomly supplemented by others during the experiment. The mean value of the scores given by the three people was calculated.

Detection of Serum Biochemical Indexes of Brain Damage

The content of blood glucose (Glu) and ion concentration will be changed in the case of cerebral I/R injury. Therefore, the indexes Glu, K^+, and Na^+ were detected to predict the incidence of brain damage in clinical practices in advance. The serum previously stored in a cryogenic refrigerator was taken out, thawed in gradient temperature, centrifuged, and then subpackaged in centrifuge tubes. The changes in the content of those indexes were determined using a full-automatic biochemistry analyzer with an operation procedure set.

Table I. Detailed scoring rules.

Score	Behavior
1	Walk autonomously, without neural functional defect
2	Rotate to the contralateral side of lesion under the condition of free movement
3	Rotate to the contralateral side of lesion with the tail grasped
4	Tumble to the contralateral side after the tail suspended
5	Unable to walk autonomously, with loss of consciousness

Detection of Content of Inflammatory Factors Via ELISA

The serum samples previously collected and frozen at –80°C were taken out, slowly thawed at 4°C, and centrifuged again at a low speed to harvest the supernatant. The ELISA kits and all the reagents were buffered at room temperature for 30 min to prepare the standard solution, followed by incubation, addition of biotin-labeled antibodies, incubation, and washing. After that, the changes in the indexes of inflammatory factors were examined in accordance with the practical situations and specific instructions. Finally, the absorbance of the inflammatory factors in each group was measured using the microplate reader.

Detection of Superoxide Dismutase (SOD), Malondialdehyde (MDA) and Catalase (CAT) Levels in Brain Tissues Via ELISA

The brain tissues (150 mg) stored in the refrigerator at –80°C were taken out, rapidly ground in a mortar, added with the lysis buffer, and centrifuged. The supernatant was separated to detect the changes in SOD, MDA, and CAT levels. At last, the absorbance of the indexes in each group was measured using the microplate reader, and the standard curves were plotted to analyze the changes in the content as per the specific instructions.

TUNEL Apoptosis Assay

The apoptosis assay kit (Roche, Basel, Switzerland) was utilized to measure the cell apoptosis in the paraffin-embedded sections as follows: the paraffin-embedded sections were deparaffinized, washed with PBS, added with proteinase K working solution, and soaked in blocking buffer, followed by fixation, rinsing, and permeation with 0.1% Triton X-100. Apoptotic deoxyribonucleic acid (DNA) fragments were subjected to fluorescein isothiocyanate (FITC)-end labeling *via* the TUNEL apoptosis assay kit, the images of FITC-labeled TUNEL-positive cells were

observed under a fluorescence microscope, and 10 fields of vision were selected to count the TUNEL-positive cells.

Detection of Expression of Relevant Genes Via RT-PCR

(1) The brain tissues were homogenized, and the TRIzol kit was applied to extract the total ribonucleic acid (RNA). Ultraviolet spectrophotometric assay and agarose gel electrophoresis were adopted to detect the concentration, purity, and integrity of the RNA, which were ensured to be qualified. After that, the messenger RNA (mRNA) was reversely transcribed into complementary DNA (cDNA) and then stored in the refrigerator at –80°C. (2) Primer amplification was performed using a 20 μL amplification system (2 μL of cDNA, 10 μL of qPCR Mix, 2 μL of primer, and 6 μL of ddH2O) for 40 cycles. Later, PCR amplification was conducted according to pre-denaturation at 95°C for 2 min, 94°C for 20 s, 60°C for 20 s, and 72°C for 30 s, 40 cycles in total. The primer sequences of target genes and internal reference glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed based on those on GenBank (Table II). The expression levels of target genes were determined *via* qRT-PCR. The relative expression levels of related genes in each group of brain tissues were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blotting Assay

The brain tissues were fetched and quickly frozen at –80°C. Then, the frozen samples were weighed and broken on ice. The protease inhibitor and modified RIPA buffer were added for incubation in the refrigerator, so that the tissues were lysed adequately to release the proteins, followed by centrifugation and collection of supernatant. The protein concentration was detected and calculated in accordance with the instructions of the BCA kit. Then, the Western blotting assay was carried out as follows: the proteins were loaded, separated on 12% gel, and transferred onto polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland). Next, the membrane was blocked in 5% skim milk at room temperature for 1.5 h and then incubated with primary and secondary antibodies (1:1000). Finally, the images were developed using a gel imaging system, the level of proteins to be detected was corrected *via* GAPDH, and the gray-scale of the protein bands was analyzed. The experiment was repeated three times.

Table II. Primer sequences of indexes for RT-PCR

Target gene	Primer sequences
GAPDH	F: 5'-TGACTTCAACAGCGACACCCA-3' R: 5'-CACCCTGTTGCTGTAGCCAAA-3'
Caspase-3	F: 5'-CTACCGCACCCGGTTACTAT-3' R: 5'-TTCCGGTTAACACGAGTGAG-3'
HIF-1	F: 5'-GCGGCGCGAACGACAAGAAAAAG-3' R: 5'-GAATGTGGCCTGTGCAGTGCAATAC-3'
HSP70	F: 5'-AGTGCTCTTGAGATCTCTGAG-3' R: 5'-TCATCGATCTTCAGAAGTCTC-3'

Statistical Analysis

The raw data recorded during experiments were processed using Statistical Product and Service Solutions (SPSS) 22.0 analysis software (IBM, Armonk, NY, USA), and the data were subjected to multiple comparisons. The experimental results obtained were presented as mean ± standard deviation ($\chi \pm SD$), and $p<0.05$ suggested statistically significant differences. Each experiment was repeated at least three times, and the histograms were plotted using GraphPad Prism 5.0 (La Jolla, CA, USA).

Results

Neurological Score of Rats in Each Group

Some of the rats in Model group were unable to walk and generally flexed to the contralateral side. In Sevoflurane group, the above-mentioned phenomena were alleviated notably ($p<0.05$), while there were no abnormalities in Sham group ($p<0.05$). The specific scores are shown in Figure 1.

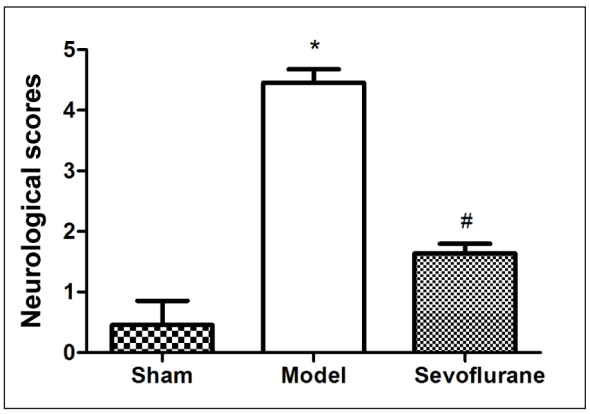

Figure 1. Neurological scores. Some of the rats in Model group are unable to walk and generally flex to the contralateral side ($p<0.05$). In Sevoflurane group, the above-mentioned phenomena are alleviated notably ($p<0.05$), while there are no abnormalities in Sham group. *$p<0.05$ *vs.* Sham group, $^{\#}p<0.05$ *vs.* Model group.

Results of Serum Test for Brain Damage

The detection results of such indexes as Glu, K^+, and Na^+ (Table III) showed that the levels of Glu and Na^+ were increased markedly, while the K^+ level was decreased notably in Model group, and the opposite changes in those levels were observed in Sevoflurane group ($p<0.05$), indicating that the disease occurs and progresses in favorable directions.

Content of Serum Tumor Necrosis Factor-β (TNF-β), IL-1, and IL-6

The content of IL-1, IL-6, and TNF-β was raised in Model group ($p<0.05$), but it was reduced in Sevoflurane group ($p<0.05$) (Table IV).

Measurement Results of CAT, MDA, and SOD Activity in Brain Tissues

Compared with Sham group, Model group had enhanced activity of CAT and MDA and weakened activity of SOD ($p<0.05$), and the opposite results were observed in Sevoflurane group ($p<0.05$) (Table V).

TUNEL Apoptosis Assay

According to Figure 2, there were no apparent positive cells in Sham group, while Model group exhibited a remarkably larger number of TUNEL-positive cells than Sham group, which were mainly distributed around the lesion site and dominated by glial cells. There were fewer apoptotic cells in Sevoflurane group, basically close to those in Sham group ($p<0.05$), illustrating that the cerebral I/R promotes the abnormal apoptosis of nerve cells.

Results of Related Genes in Brain Tissues Detected Via QRT-PCR

The mRNA expression levels of HIF-1, HSP70, and Caspase-3 rose evidently in Model group compared with those in Sham group ($p<0.05$), but they declined significantly in Sevoflurane group ($p<0.05$) (Figure 3).

Detection Results of Related Proteins i n Brain Tissues

The protein levels of HIF-1 and HSP70 were significantly higher in Model group than those in Sham group ($p<0.05$), but they were distinctly lower in Sevoflurane group ($p<0.05$) (Figure 4).

Table III. Changes in content of Glu, K^+ and Na^+.

Group	K^+ (mmol/L)	Glu (U/L)	Na^+ (mmol/L)
Sham group	29.2±5.2	8.2±2.8	98.9±2.7
Model group	8.1±1.5[a]	18.3±1.4[a]	218.6±6.3[a]
Sevoflurane group	24.6±2.0[b]	10.4±3.8[b]	113.8±5.8[b]

Note: The levels of Glu and Na^+ are increased markedly, while K^+ level is decreased notably in Model group ($p<0.05$). [a]$p<0.05$ *vs.* Sham group, [b]$p<0.05$ *vs.* Model group.

Table IV. Content of serum TNF-β, IL-1, and IL-6.

Group	TNF-β (fmol/mL)	IL-6 (mg/L)	IL-1 (mg/L)
Sham group	15.6±3.7	18.3±4.5	24.2±5.3
Model group	54.6±2.7[a]	91.5±4.8[a]	98.0±6.4[a]
Sevoflurane group	19.9±3.1[b]	26.1±3.4[b]	30.2±5.1[b]

Note: The content of IL-6, TNF-β and IL-1 is raised in Model group ($p<0.05$), but it is reduced in Sevoflurane group ($p<0.05$). [a]$p<0.05$ *vs.* Sham group, [b]$p<0.05$ *vs.* Model group.

Table V. Measurement of CAT, MDA and SOD activity in tissues.

Group	MDA (mmol/L)	SOD (U/mg)	CAT (IU/mL)
Sham group	2.1±1.0	39.5±0.1	12.5±0.5
Model group	14.7±1.6[a]	15.1±1.1[a]	55.8±0.2[a]
Sevoflurane group	4.1±1.5[b]	32.3±1.0[b]	20.9±0.8[b]

Note: Model group has enhanced CAT and MDA activity and weakened SOD activity ($p<0.05$). The opposite results are observed in Sevoflurane group ($p<0.05$). [a]$p<0.05$ *vs.* Sham group, [b]$p<0.05$ *vs.* Model group.

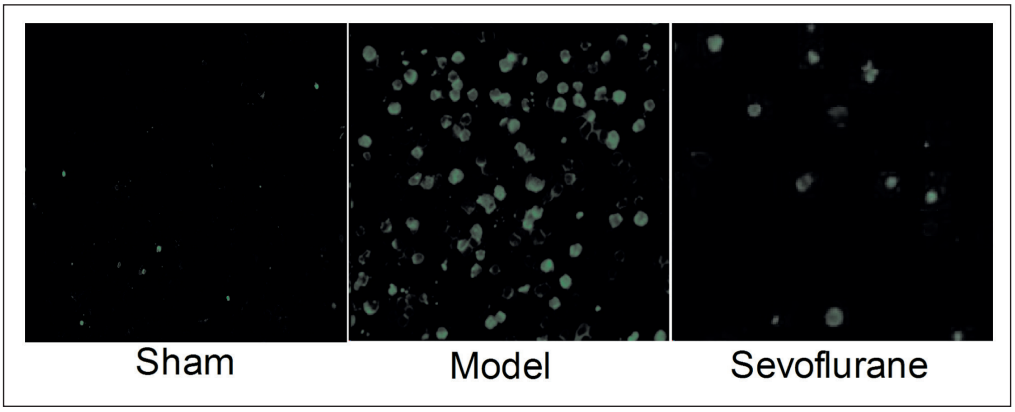

Figure 2. TUNEL staining. There are no apparent positive cells in Sham group, while Model group exhibits a remarkably larger number of TUNEL-positive cells than Sham group, which are mainly distributed around the lesion and dominated by neuroglia cells cells (magnification: 100×). There are fewer apoptotic cells in Sevoflurane group (p<0.05).

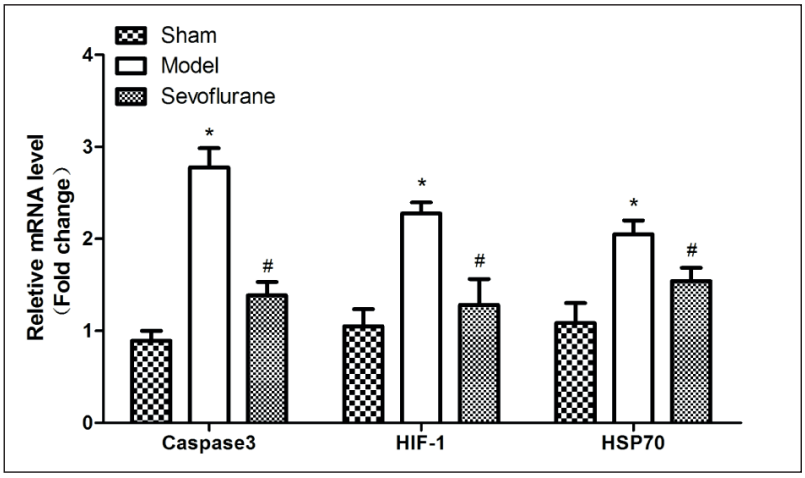

Figure 3. Results of related genes detected via qRT-PCR. The mRNA expression levels of HIF-1, HSP70, and Caspase-3 rise evidently in Model group compared with those in Sham group (p<0.05), but they decline significantly in Sevoflurane group (p<0.05). *p<0.05 *vs.* Sham group, $^{\#}p$<0.05 *vs.* Model group.

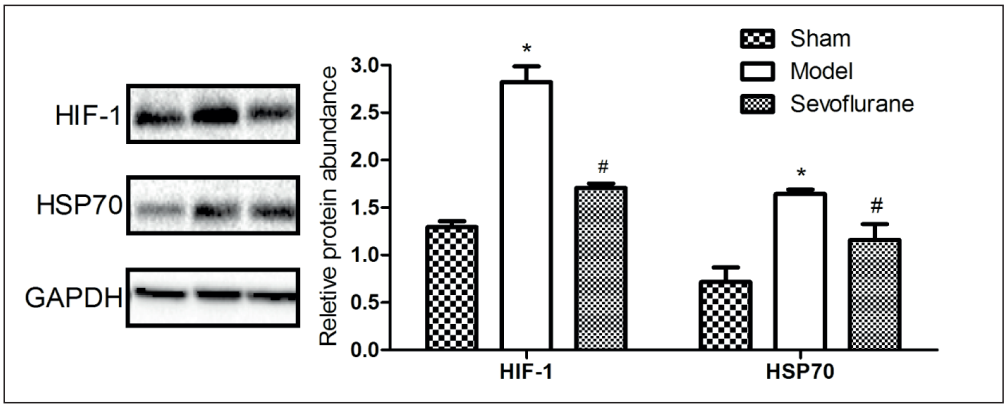

Figure 4. Protein detection results. The protein levels of HIF-1 and HSP70 are significantly higher in Model group than those in Sham group (p<0.05), but they are distinctly lower in Sevoflurane group (p<0.05). *p<0.05 *vs.* Sham group, $^{\#}p$<0.05 *vs.* Model group.

Discussion

With a fairly high morbidity rate in the world, cerebrovascular diseases have great negative impacts on the quality of life and public health[18]. The reperfusion of ischemic tissues increases the production of oxygen free radicals, destroys the balance between oxidants and anti-oxidants, and leads to uncontrolled brain damage[19]. The reperfusion injury is mediated by cell apoptosis, and the cell damage during cerebral ischemia is triggered by a series of events. Besides, serious blood stream obstruction induces the rapid death of central cells, which can result in death quickly if not treated in time[20]. The cerebral I/R injury consists of the primary injury during ischemia period and the secondary injury during reperfusion period which can form cerebral edema and other secondary diseases. Many studies have demonstrated that the pretreatment and post-treatment with inhalation anesthetics can reduce ischemic neuronal damage. Sevoflurane is often used for sedative and anesthetic management for hypoxic and ischemic encephalopathy. It was studied that the sevoflurane treatment is able to relieve the HIBD. In this research, sevoflurane was applied to treat the rats with cerebral I/R. A train of *in-vivo* experiments was utilized to further investigate the effects of sevoflurane on cerebral I/R injury. It was also found through the neurological scoring that the rats in Model group partially exhibited the disability to walk and general flexion to the contralateral side. However, these phenomena were alleviated remarkably in Sevoflurane group, but no abnormalities occurred in Sham group. The detection results of indexes Glu, K^+, and Na^+ indicated that Model group had markedly elevated levels of Glu and Na^+, and a distinctly lowered K^+ level, while Sevoflurane group manifested the opposite changes in those levels, signifying the preferable occurrence and development of the disease. The I/R injury is mediated by elements secreted by damaged cells and inflammatory cells. In addition, the inflammatory cells secret a large quantity of toxic cytokines[21]. In this research, it was discovered that the content of IL-1, IL-6, and TNF-β was increased in Model group but decreased in Sevoflurane group. The brain damage can trigger oxidative stress, and MDA and CAT are the metabolic products of lipid peroxidation, whose content reflects the damage degree of oxygen free radicals to the cells. As a crucial antioxidant enzyme, SOD can protect the organisms from oxidative stress injury[22]. The oxidant and anti-oxidant detection revealed that compared with Sham group, Model group exhibited increased activity of CAT and MDA and decreased activity of SOD, but the opposite results were observed in Sevoflurane group, which are similar to those in previous studies[23].

Apoptosis is capable of eliminating harmful substances in cells and rapidly initiating apoptotic response in the case of threats. Currently, apoptosis has become a hotspot of research. Apoptosis is regulated by apoptosis-related genes and proteins, including Bcl-2, Bax, and Caspase-3[24]. According to the TUNEL staining for apoptosis in this research, no distinctly positive cells were observed in Sham group, while Model group had clearly more TUNEL-positive cells than Sham group, which were mainly distributed around the lesion site and dominated by glial cells. There were fewer apoptotic cells in Sevoflurane group, basically close to those in Sham group, illustrating that the cerebral I/R facilitates the abnormal apoptosis of nerve cells. HSP70, a most frequently studied member of the HSP family, can respond to a variety of pathological conditions, including ischemia and heart failure-induced injury. HSPs have been gradually regarded as the ideal biomarkers of diseases, and HSP70 can participate in such processes as protein folding[25].

HIF-1α is a key component under pathophysiological conditions such as cell hypoxia and ischemia. When tissue hypoxia occurs, HIF-1α accumulation can induce angiogenesis, brain tissue injury, erythropoiesis or cell death[26,27]. The gene assay results displayed that the mRNA expression levels of HIF-1, HSP70, and Caspase-3 were raised evidently in Model group in comparison with those in Sham group, but they declined markedly in Sevoflurane group. Moreover, it was discovered in the protein assay results that Model group had notably higher protein levels of HIF-1 and HSP70 than Sham group, while Sevoflurane group manifested prominently lower levels, suggesting that sevoflurane can reduce the gene and protein expressions of HIF-1 and HSP70 in the case of cerebral I/R injury. The aforementioned investigations indicate that after the I/R rats are processed with sevoflurane, the apoptosis, inflammatory factors, and oxidative stress in the brain tissues are decreased, thereby exerting protective effects against the I/R injury.

Conclusions

In summary, it was discovered through a series of *in-vivo* experiments, as well as gene and protein assays, that sevoflurane may exert a protective effect against the cerebral I/R injury by repressing the apoptosis, inflammation, oxidative stress, and HIF-1 and HSP70 expressions. Therefore, sevoflurane can be applied to regulate the development of cerebral I/R injury. In a word, the results of this research provide an experimental basis and some theoretical bases for the treatment of cerebral I/R injury.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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