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Compound Porcine Cerebroside and Ganglioside Injection (CPCGI) Attenuates Sevoflurane-Induced Nerve Cell Injury by Regulating the Phosphorylation of p38 MAP Kinase (p38MAPK)/ Nuclear Factor kappa B (NF-κB) Pathway

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 Haigang Song
BCD 2 Shining Xun
BD 2 Huali He
BC 2 Chongzhen Duan
AEFG 3 Qiang Li

1 Department of Anesthesiology, Xianyang Hospital of Yan'an University, Xianyang, Shaanxi, P.R China
2 Department of First Anesthesiology and Surgery, Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, P.R China
3 Department of Anesthesiology, The Fifth Medical Center of PLA General Hospital, Beijing, P.R. China

Corresponding Author: Qiang Li, e-mail: liqiang22081@163.com
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Background: Compound porcine cerebroside and ganglioside injection (CPCGI) has been widely applied in clinical practice in China to treat functional confusion caused by brain diseases. Sevoflurane, a frequently-used inhalational anesthetic, was discovered to have neurotoxicity that can cause neurological damage in patients. The present study was performed to investigate the protective effect of CPCGI on sevoflurane-induced nerve damage and to reveal the neuroprotective mechanisms of CPCGI.

Material/Methods: Firstly, the hippocampal neurons were separated from Sprague-Dawley embryonic rats, and were stimulated by 3% sevoflurane for different times (0, 2, 4, and 6 h). Then, cell viability and cell apoptosis were assessed by thiazolyl blue tetrazolium bromide (MTT) and flow cytometry (FCM), respectively. Western blot analysis was used to determine the apoptosis-related protein expression levels.

Results: The results demonstrated that 3% sevoflurane significantly inhibited cell viability but induced cell apoptosis in neurons in a time-dependent manner. Treatment with 3% sevoflurane also promoted the Bax [B cell leukemia/lymphoma 2 (Bcl2)-associated X protein] and cleaved caspase3 protein expressions, and suppressed Bcl-2 and pro-caspase3 expressions in hippocampal neurons. In addition, phosphorylated (p)-p38 and p-p65 expression and the ratio of p-p38/p38 and p-p65/p65 were upregulated in a time-dependent manner after 3% sevoflurane treatment. Further analysis indicated that all the effects of 3% sevoflurane on hippocampal neurons were reversed by CPCGI pre-treatment.

Conclusions: We demonstrated the neuroprotective role of CPCGI in sevoflurane-stimulated neuronal cell damage via regulation of the MAPK/NF-κB signaling pathway.

MeSH Keywords: Anesthesiology • MAP Kinase Kinase Kinases • Neurons • NF-kappa B

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Background

Sevoflurane, an inhalational anesthetic, is frequently used in clinical practice [1]. Because of its fast induction, aromatic taste, and recovery properties, sevoflurane has been widely used in various procedures, such as surgical operations, cesarean deliveries, and pediatric surgery [2,3]. Additionally, several reports have demonstrated that sevoflurane use in newborn rats resulted in neurocognitive dysfunction in the central nervous system, including behavioral and developmental disabilities [4]. Furthermore, increasing evidence has indicated that sevoflurane can trigger significant damage in many neuronal and non-neuronal cells and tissues [5]. Therefore, it is important to find effective strategies to protect neurons from sevoflurane-stimulated cell injury and to understand the pathomechanism of the neurotoxic effects of sevoflurane.

CPCGI (drug approval H22026472, China) has widespread clinical use for Alzheimer's disease, craniocerebral injury, spinal cord injury, and traumatic peripheral nerve injury therapy in China [6–8]. In addition, monosialotetrahexosyl ganglioside (GM-1) and hypoxanthine, the main components of CPCG, can promote the metabolism of brain tissue, are involved in neuron growth, differentiation, and regeneration, and accelerate the brain blood circulation [9–14]. The effective ingredients of CPCGI (polypeptide, GM-1, and hypoxanthine) have also been reported to possess a significant curative effect for stroke treatment in rats [6]. However, the protective response of CPCGI to neuronal damage stimulated by sevoflurane has not yet been described.

Cell apoptosis, also referred to as programmed cell death, can prevent dysfunctional cells from disturbing the balance of normal tissues, but abnormal apoptosis can lead to cell damage or death [15]. Multiple reports have shown that sevoflurane can accelerate the activation of cysteine aspartate-specific protease (caspase) and apoptosis signaling pathway [16]. Phosphorylation of p38 MAP kinase (p38MAPK) and nuclear translocation of NF-κB p65 are both involved in the signaling pathway, playing a vital role in cell growth and apoptosis [17]. Recent reports have suggested that sevoflurane neurotoxicity involves apoptosis-related elements, including caspase-3, B cell lymphoma-2 (Bcl-2), and Bcl-2-associated X (Bax) [18,19]. Therefore, we explored whether CPCGI participates in sevoflurane-induced neurons damage via the p38MAPK/NF-κB pathway.

We investigated the neuroprotective role of CPCGI in sevoflurane-stimulated hippocampal neurons, and sought to elucidate the underlying mechanism. Our goal was to provide new insights into the function of CPCGI and to develop a neuroprotective strategy for sevoflurane-induced neuronal injury. To test the hypothesis that miR-155 has a protective function in the macrophage immune response, we investigated miR-155-mediated CASP-3 mRNA modulation in LPS-activated RAW 264.7 cells.

Material and Methods

Nerve cell culture and sevoflurane treatment

We purchased pregnant (18 days) adult Sprague-Dawley rats from the Experimental Animal Center of Shanghai and housed them in standard surroundings (22–24°C, 55–65% humidity, 12-h light/dark cycle). The experiments with animals were conducted following the National Institutes of Health guidelines for the care and the use of laboratory animals and the study was approved by the Ethics Committee of the Experimental Animal Center of Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine. The adult rats were killed using cervical dislocation and soaked with alcohol. Then, we removed the embryos and separated the hippocampus tissues under aseptic conditions. Nerve cells were collected and grown in neurobasal medium (Gibco, USA) containing 2% B27, 10 mmol/l HEPES, and 0.5 mmol/l glutamine, at 37°C in an incubator containing 5% CO_2.

We used 3% sevoflurane to induce the nerve damage model [20]. Cells were first cultured in phosphate-buffered saline (PBS) or CPCGI for 6 h, then the cells were stimulated with 3% sevoflurane (Sigma-Aldrich, USA) for 6 h and assigned into 1 of 4 groups: control, sevoflurane, sevoflurane+PBS (vehicle), and sevoflurane+CPCGI. CPCGI was obtained from the Jilin Buchang Pharmaceutical Group (Jilin, China). For sevoflurane treatment, the cells were treated with 3% sevoflurane for indicated times.

MTT assay

The MTT assay was performed to evaluate cell viability. The nerve cells were cultivated in 96-well plates (BD Bioscience) and cultured in an incubator at 37°C with 5% CO_2. Then, the culture medium was removed and sevoflurane or CPCGI was added to treat cells for 0, 2, 4, and 6 h. After that, 10 µl MTT solution (Beyotime, Shanghai, China) was added for 4 h following the manufacturer's protocols. The formazan crystals were then solubilized with dimethylsulfoxide (DMSO). Finally, optical density (OD) at 490 nm was measured using a multifunctional plate reader (BD, USA).

Flow cytometry (FCM) analysis

The hippocampal neuronal cell apoptosis was assessed by annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) double-stain analysis (Beyotime, Shanghai, China) following the manufacturer's protocols. In this experiment, the cells were treated with 3% sevoflurane or CPCGI for indicated times. Then, cells were collected, centrifuged, and re-suspended in 100 µl binding buffer containing 5 µl annexin V/FITC and 2.5 µl PI. After co-incubation for 15 min in the dark, flow cytometry (Beckman

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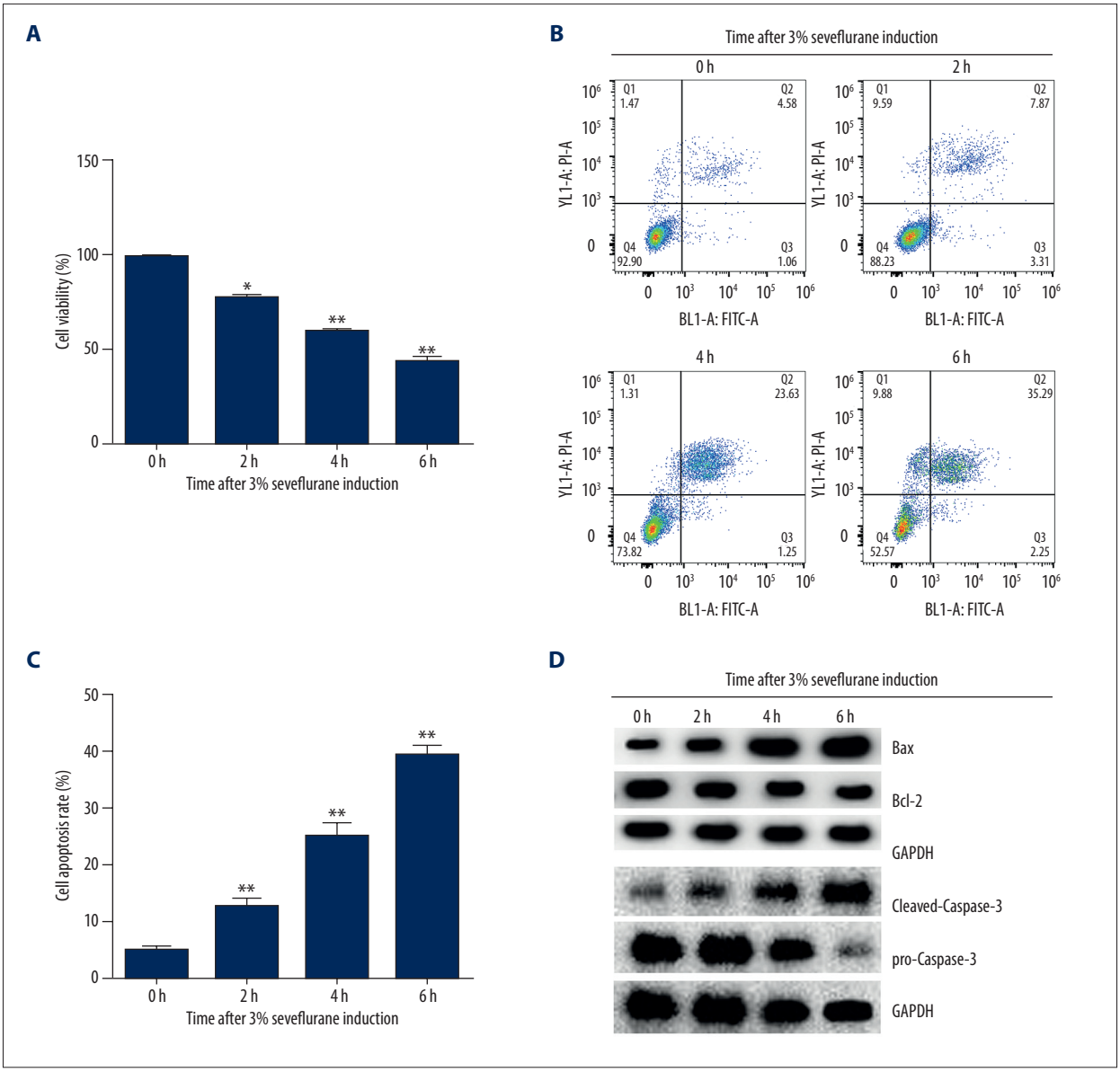

Figure 1. Sevoflurane suppressed hippocampal neurons cell growth and induced apoptosis. The hippocampal neurons cells were treated with 3% sevoflurane for indicated lengths of time (0, 2, 4, and 6 h). (**A**) MTT assay was performed to measure cell viability in different groups. (**B**) The FCM assay was carried out to evaluate the apoptosis rates of hippocampal neuronal cells. (**C**) The bar charts present the percentage of positive cells. (**D**) The apoptosis-related proteins expression levels were determined by Western blot assay. The data are presented as means±SD. * p<0.05, and ** p<0.01 compared to 0 h.

Coulter, USA) was performed to assess cell apoptosis. The data were analyzed using FlowJo software (version 7.6.1; FlowJo LLC).

Western blot assay

Western blot assay was used to evaluate the levels of apoptosis-related proteins (Bax, Bcl-2, pro-caspase3, and cleaved caspase3) and signaling pathway-related factors (p-p38, p38, p-p65, and p65). The neuronal cell total proteins were separated and quantified using the Bicinchoninic Acid (BCA) Protein Assay Kit (Sigma, USA). Then, proteins (30 μg/lane) were isolated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and blotted onto a polyvinylidene fluoride (PVDF) membrane. After incubating in 5% skim milk for 2 h at room temperature, the membranes were cultured at 4°C in specific primary antibodies (Abcam) at 1: 1000 dilution. GAPDH was used as internal control overnight. Subsequently, the membranes were cultured with secondary antibody (Abcam; 1: 2000 dilution) for 2 h. The protein bands were detected by enhanced chemiluminescence (ECL) detection system reagents

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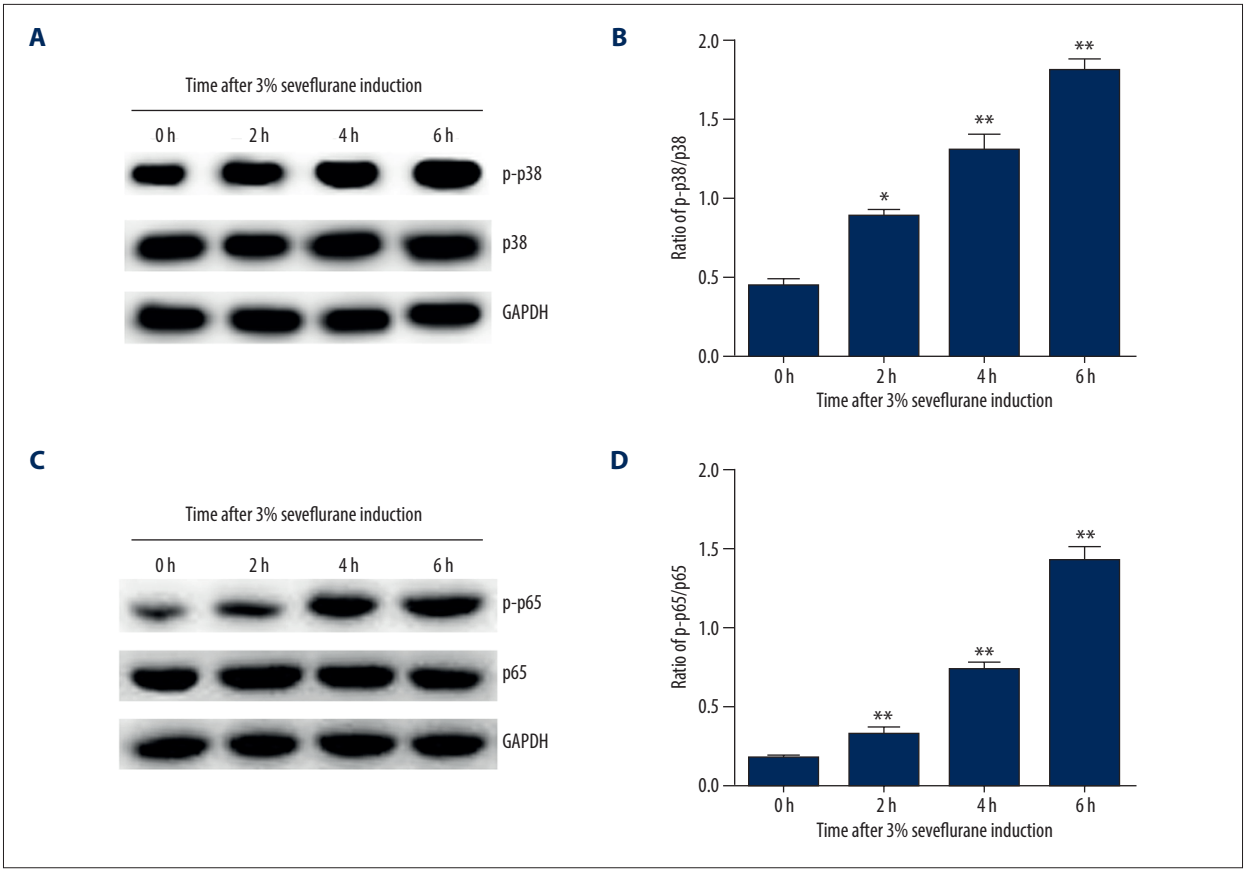

Figure 2. Sevoflurane regulated the MAPK/NF-κB signaling pathway in neurons. Hippocampal neuronal cells were treated with 3% sevoflurane for different lengths of time (0, 2, 4, and 6 h). (**A, C**) Western blot analysis was used to assess protein expression levels of p38, p65, p-p38, and p-p65. (**B, D**) The ratio of p-p38/p38 (**B**) and p-p65/p65 (**D**) were quantified using Image J software. The results are expressed as means±SD. * p<0.05, and ** p<0.01 compared to 0 h.

(GE Healthcare Life Sciences, UK) following the manufacturer's instructions. Proteins were quantified using ImageJ software (version 1.38X; National Institutes of Health).

Statistical analysis

The data were evaluated using GraphPad Prism 5 (San Diego, CA, USA) and are presented as the mean±standard deviation (SD). The statistical differences between groups were analyzed by *t* test or one-way analysis of variance (ANOVA). p<0.05 was considered to indicate a significantly significant difference.

Results

Sevoflurane inhibited cell viability and induced rat hippocampal neuronal cell apoptosis.

First, we assessed cell proliferation and apoptosis in sevoflurane-treated nerve cells by treating cells with 3% sevoflurane for specified lengths of time (0, 2, 4, and 6 h). MTT assay was carried out to determine hippocampal neural cell viability, and the neuronal cell apoptosis was detected by flow cytometry. Our results showed that 3% sevoflurane treatment significantly inhibited the cell viability of neurons in a time-dependent (2, 4, 6 h) manner compared to sevoflurane treatment for 0 h (Figure 1A). Sevoflurane treatment induced cultured neuronal cell apoptosis in a time-dependent manner compared with the 0 h treatment group (Figure 1B, 1C). Next, we assessed apoptosis in sevoflurane-induced neuronal cell injury, and Western blot assay was conducted to evaluate the expressions of apoptosis-related proteins (Bcl-2, Bax, pro-caspase3, and cleaved caspase3) in neuronal cells after sevoflurane treatment. The results from Western blot assay demonstrated that the Bcl-2 and pro-caspase3 protein levels were lower and the Bax and cleaved caspase3 levels were higher after sevoflurane treatment (Figure 1D). These results indicated the cytotoxicity effect of sevoflurane in hippocampal neuronal cells.

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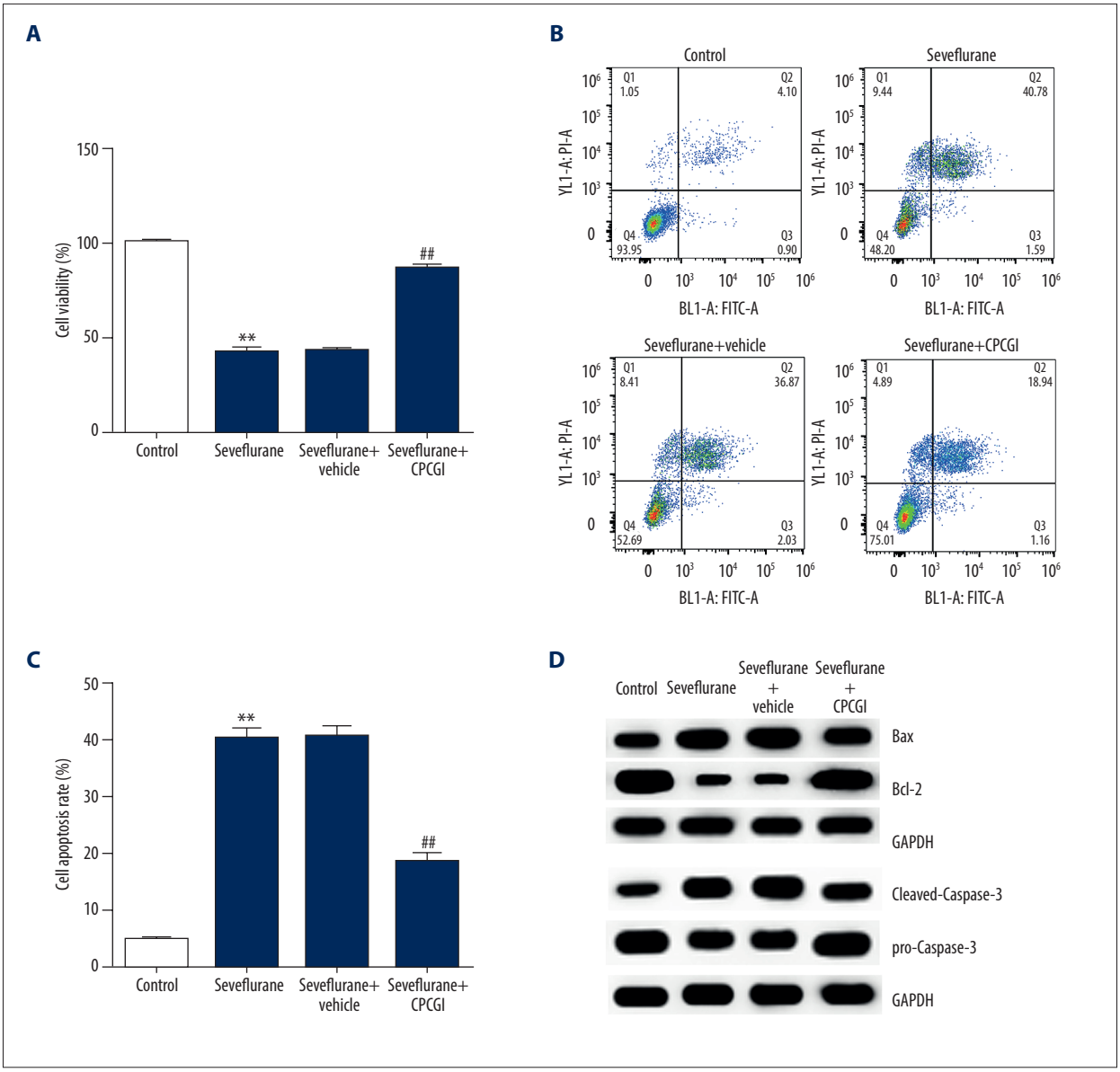

Figure 3. CPCGI blocked sevoflurane-stimulated hippocampal nerve injury. Hippocampal neurons were exposed to CPCGI or PBS for 6 h. Subsequently, the cells were treated with 3% sevoflurane for another 6 h. (**A**) Cell viability of hippocampal nerve cells was measured by MTT assay. (**B**) Flow cytometry assay was conducted to assess cell apoptosis. (**C**) Apoptosis was evaluated after sevoflurane or CPCGI treatment. (**D**) The apoptosis-related protein levels, including Bax, cleaved caspase3, Bcl-2, and pro-caspase3, were measured using Western blot assay in different groups. Results are shown as means±SD. ** $p<0.01$ compared with control group; ## $p<0.01$ compared with sevoflurane treatment groups.

Sevoflurane treatment activated the MAPK/NF-κB signaling pathway in hippocampal neuronal cells.

To further explore the potential mechanism of sevoflurane-induced hippocampal neuronal cell apoptosis, we investigated the MAPK/NF-κB signaling pathway. Western blot assay was carried out to measure the expression levels of major proteins in the MAPK/NF-κB signaling pathway, including p38, p-p38, p65, and p-p65. As shown in Figure 2A and 2C, the expression of p-p38 and p-p65 were increased in the sevoflurane treatment group in a time-dependent manner compared with the 0 h treatment group. The p-p38/p38 protein ratio (Figure 2B) and p-p65/p65 protein ratio (Figure 2D) were increased in 3% sevoflurane-treated hippocampal neuronal cells. These results suggested that sevoflurane activated the p38 MAPK/NF-κB signaling pathway in hippocampal neuronal cells.

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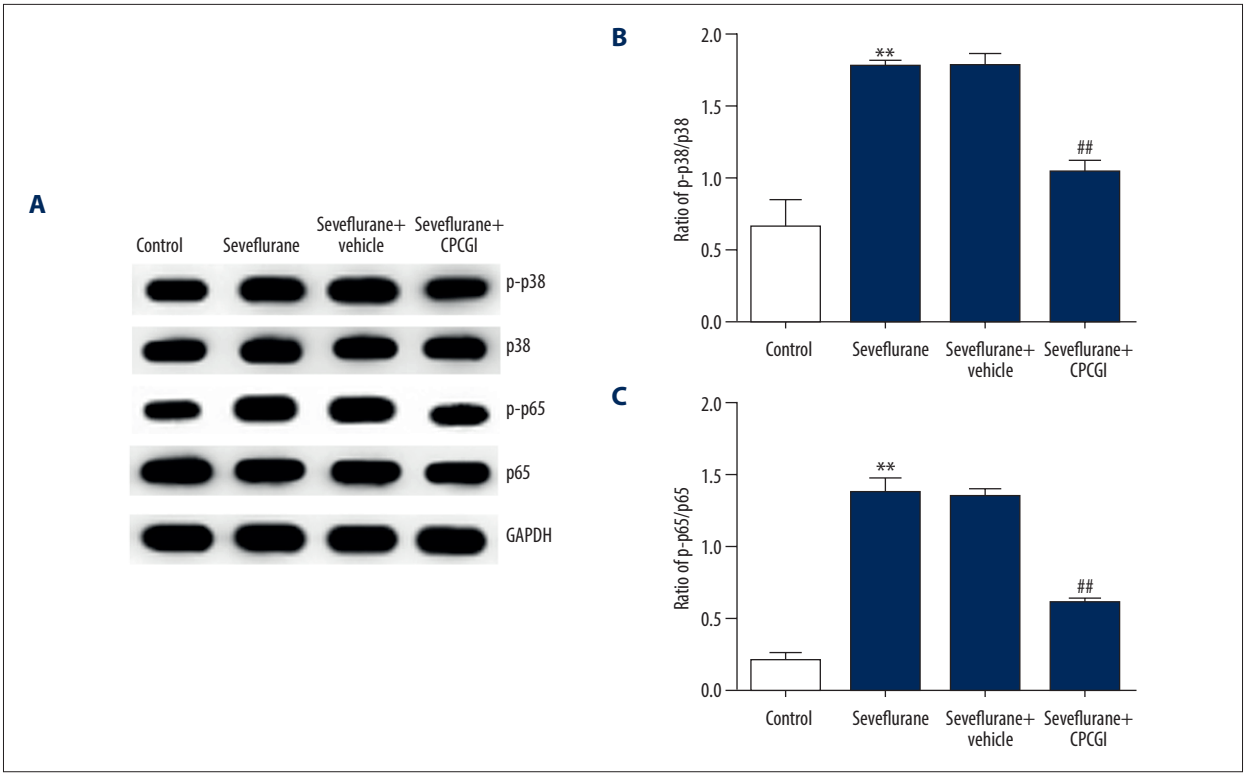

Figure 4. CPCGI inactivated the MAPK/NF-κB pathway in hippocampal neurons injured by sevoflurane. CPCGI or PBS was used to treat hippocampal neurons cells for 6 h, then the hippocampal neurons cells were treated with 3% sevoflurane for another 6 h. (**A**) The protein level of p38 and p65 and the phosphorylated levels of p38 and p65 were evaluated by Western blot assay in sevoflurane-treated hippocampal neuronal cells. (**B, C**) Ratios of p-p38/p38 (**B**) and p-p65/p65 (**C**) were analyzed using Image J software. Data are shown as the mean±SD. ** p<0.01 compared with the control group; ## p<0.01 compared with the sevoflurane treatment groups.

CPCGI rescued the sevoflurane-stimulated hippocampal neuronal cell damage

To determine whether CPCGI exhibited protective effects on sevoflurane-stimulated neuronal cell injury, hippocampal neurons were exposed to CPCGI or PBS for 6 h. Subsequently, the hippocampal neurons were treated with 3% sevoflurane for another 6 h. Our data revealed that sevoflurane significantly inhibited neuronal cell growth and promoted apoptosis compared to the control group (Figure 3A–3C), and these results were significantly reversed by CPCGI treatment. To further verify the regulatory function of CPCGI on sevoflurane-induced neuronal cell apoptosis, Western blot assay was used to evaluate Bax, Bcl-2, pro-caspase3 and cleaved caspase3 levels. Our results suggested that sevoflurane suppressed the Bcl-2 and pro-caspase3 levels and enhanced Bax and cleaved caspase3 protein levels compared to the control group (Figure 3D). However, CPCGI treatment increased Bcl-2 and pro-caspase3 expression, and decreased Bax and cleaved caspase3 expression levels in sevoflurane-injured neurons. These results show that CPCGI had an anti-apoptotic effect in sevoflurane-treated neuronal cells.

CPCGI suppressed activation of the p38MAPK/NF-κB pathway in neuronal cells after sevoflurane treatment.

To further investigate the effect of CPCGI on neuronal cell damage, the expression level of MAPK/NF-κB-related proteins was detected by Western blot assay in sevoflurane-treated neuronal cells. As presented in Figure 4A, p-p38 and p-p65 levels were significantly increased in the sevoflurane treatment groups compared to the control group, while these effects were reversed by CPCGI treatment, indicating that CPCGI participates in MAPK/NF-κB signaling pathway regulation. In addition, the ratio of p-p38/p38 protein (Figure 4B) and p-p65/p65 protein (Figure 4C) were increased in sevoflurane-treated neurons, which was reversed by CPCGI treatment. These results show that CPCGI protected against sevoflurane-induced neuronal cell injury by inhibiting nerve cell apoptosis through the MAPK/NF-κB signaling pathway.

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Discussion

Neurovirulence in many diseases has become an important research focus [21,22]. Sevoflurane, which is frequently used as an inhalation anesthetic in many surgeries, is reported to commonly lead to neurological injury and cognitive dysfunction [23,24]. Therefore, it is important to develop effective ways to prevent anesthetic-induced neurotoxicity. CPCGI, which is a potent treatment for many diseases, is involved in reducing cell apoptosis and enhancing synaptic functions. Previous studies have reported that CPCGI promotes neurogenesis by mediating cell apoptosis in hippocampal neurons, and its neuroprotective effects in MCAO-induced injury models have also been documented [6]. However, whether CPCGI participates in the progression of sevoflurane-stimulated injury in hippocampal neuronal cells has been unclear. Thus, in this study, we explored prevention of neuronal cell damage and revealed the underlying molecular mechanism of CPCGI.

In this study, we separated hippocampal neurons from Sprague-Dawley embryonic rats, and used 3% sevoflurane to induce a neuronal cell injury model [20]. We used 3% sevoflurane to stimulate the nerve injury, as previously described [20]. However, 3% sevoflurane is quite high and this level is rarely used in clinical practice, which is a limitation of the present study. MTT and FCM analysis were carried out to measure the effects of 3% sevoflurane on neuronal cell growth and apoptosis at 0, 2, 4, and 6 h. Consistent with a previous study [20], we found that 3% sevoflurane inhibited neuronal cell viability and promoted cell apoptosis in a time-dependent manner, so we chose to treat neuronal cells with 3% sevoflurane for 6 h in subsequent experiments. Abnormal apoptosis can also suppress cell growth, and sevoflurane neurotoxicity is associated with increased apoptosis, such as abnormal regulation of Bcl-2 family members and caspases [25,26]. Bcl-2 family members (Bax, Bcl-2, and Bcl-XL) participate in the regulation of apoptosis [27,28]. Caspase3, which belongs to the cysteine-aspartic proteases family, plays a vital role in mediating cellular components and promoting cellular apoptosis [29]. In accordance with previous reports, our results suggested that 3% sevoflurane remarkably enhanced the Bax and cleaved caspase3 protein expression levels, and inhibited the Bcl-2 and pro-caspase3 expression levels in neurons.

Recently, numerous studies have suggested that various pathways are involved in sevoflurane-stimulated injury in neurons, such as the p38MAPK, NF-κB p65, and PKA signaling pathways [30–32]. It was also reported that inhibition of MEK/ERK and mTOR phosphorylation is part of the mechanism of anesthetic-stimulated neurovirulence in the many diseases [33]. In the present study, we used Western blot assay to determine the relative protein expression level of MAPK/NF-κB. Guo et al. [30] suggested that sevoflurane treatment significantly activated the NF-κB pathway in rats. Lyu et al. [32] reported that phosphorylated-p38 MAP kinase (p38) protein expression was significantly enhanced in sevoflurane-treated newborn rats, indicating activation of the p38MAPK signaling pathway. Consistent with those results, our results demonstrated that the expressions of p-p38 and p-p65 were significantly enhanced by 3% sevoflurane treatment in hippocampal neurons, and the protein ratios of p-p38/p38 and p-p65/p65 were also increased. In summary, these results indicate that sevoflurane treatment inhibits hippocampal neuronal cell viability and promotes cell apoptosis by activating the MAPK/NF-κB signaling pathway.

We finally evaluated the protective response of CPCGI during sevoflurane-induced hippocampal neuronal cell damage. Neurons were first treated with CPCGI or PBS for 6 h, and then treated with 3% sevoflurane for another 6 h. The results from MTT and FCM analysis showed that treatment with CPCGI significantly reversed the effects of sevoflurane on neuronal cell viability and sevoflurane-induced apoptosis. Also, sevoflurane treatment enhanced protein levels of Bax and cleaved caspase3, and CPCGI treatment reversed the effects of sevoflurane treatment in reducing Bcl-2 and pro-caspase3 protein levels in neuronal cells. These results indicate that CPCGI can inhibit apoptosis in sevoflurane-induced neuronal cell injury. To better understand the potential mechanism underlying the role of CPCGI in neuronal cell damage induced by sevoflurane, we assessed the role of the MAPK/NF-κB pathway in sevoflurane-induced neuronal cell injury [30,32]. The results suggested that CPCGI protects neuronal cells against sevoflurane-stimulated neurotoxicity by inhibiting p-p38 and p-p65 protein expression levels, indicating that CPCGI inhibits the MAPK/NF-κB signaling pathway in sevoflurane-treated neuronal cells. However, we did not investigate the effect of CPCGI on nerve cells treated with sevoflurane *in vivo,* which is a limitation of this study, and we intend to assess this in future research.

Conclusions

Our results show that CPCGI can enhance cell viability and suppress apoptosis of hippocampal neurons injured by sevoflurane via regulating the p38MAPK/NF-κB pathway. This study provides a new strategy for preventing neuronal cell injury induced by anesthetics in clinical practice. The role of CPCGI in sevoflurane-stimulated behavioral variation *in vivo* needs to be further explored in the future.

Conflict of interest

None.

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References:

1. Ruan X, Jiang W, Cheng P et al: Volatile anesthetics sevoflurane targets leukemia stem/progenitor cells via Wnt/beta-catenin inhibition. Biomed Pharmacother, 2018; 107: 1294–301
2. Soubhia AF, Lauz S, Montero EF et al: Effects of the inhalational anesthetics halothane and sevoflurane on an experimental model of hepatic injury. Rev Bras Anestesiol, 2011; 61: 591–603, 324–30
3. Jiang J, Jiang H: Effect of the inhaled anesthetics isoflurane, sevoflurane and desflurane on the neuropathogenesis of Alzheimer's disease (review). Mol Med Rep, 2015; 12: 3–12
4. Huang H, Liu CM, Sun J et al: Repeated 2% sevoflurane administration in 7 and 60-day-old rats: Neurotoxicity and neurocognitive dysfunction. Anaesthesist, 2017; 66: 850–57
5. He H, Liu W, Zhou Y et al: Sevoflurane post-conditioning attenuates traumatic brain injury-induced neuronal apoptosis by promoting autophagy via the PI3K/AKT signaling pathway. Drug Des Devel Ther, 2018; 12: 629–38
6. Wang M, Zhang Y, Feng L et al: Compound porcine cerebroside and ganglioside injection attenuates cerebral ischemia-reperfusion injury in rats by targeting multiple cellular processes. Neuropsychiatr Dis Treat, 2017; 13: 927–35
7. Wang MY, Feng L, Fan SJ et al: Effect of compound porcine cerebroside and ganglioside injection on cerebral ischemia-reperfusion injury in rats. Chin J Rehabil Theory Pract, 2015; 22: 750–53
8. Wang H, Jiang HQ, Li J et al: [Clinical study of compound porcine cerebroside and ganglioside injection on Alzheimer disease.] Chinese Community Doctors, 2010; 12: 127–28 [in Chinese]
9. Posse de Chaves E, Sipione S: Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. FEBS Lett, 2010; 584: 1748–59
10. Zamfr AD: Neurological analyses: focus on gangliosides and mass spectrometry. Adv Exp Med Biol, 2014; 806: 153–204
11. Mo L, Ren Q, Duchemin AM et al: GM1 and ERK signaling in the aged brain. Brain Res, 2005; 1054: 125–34
12. Ohmi Y, Tajima O, Ohkawa Y et al: Gangliosides play pivotal roles in the regulation of complement systems and in the maintenance of integrity in nerve tissues. Proc Natl Acad Sci USA, 2009; 106: 22405–10
13. Nagao H, Nishizawa H, Tanaka Y et al: Hypoxanthine secretion from human adipose tissue and its increase in hypoxia. Obesity (Silver Spring), 2018; 26: 1168–78
14. Lee JS, Wang RX, Alexeev EE et al: Hypoxanthine is a checkpoint stress metabolite in colonic epithelial energy modulation and barrier function. J Biol Chem, 2018; 293: 6039–51
15. Hayashi Y, Yamamoto N, Nakagawa T, Ito J: Insulin-like growth factor 1 inhibits hair cell apoptosis and promotes the cell cycle of supporting cells by activating different downstream cascades after pharmacological hair cell injury in neonatal mice. Mol Cell Neurosci, 2013; 56: 29–38
16. Yang ZY, Yuan CX: IL-17A promotes the neuroinflammation and cognitive function in sevoflurane anesthetized aged rats via activation of NF-kappaB signaling pathway. BMC Anesthesiol, 2018; 18: 147
17. Hassanein E, Mohamed WR, Shalkami AS et al: Renoprotective effects of umbelliferone on methotrexate-induced renal injury through regulation of Nrf-2/Keap-1, P38MAPK/NF-kappaB, and apoptosis signaling pathways. Food Chem Toxicol, 2018; 116: 152–60
18. Xie SN, Ye H, Li JF, An LX: Sevoflurane neurotoxicity in neonatal rats is related to an increase in the GABAA R alpha1/GABAA R alpha2 ratio. J Neurosci Res, 2017; 95: 2367–75
19. Wang WY, Jia LJ, Luo Y et al: Location- and subunit-specific NMDA receptors determine the developmental sevoflurane neurotoxicity through ERK1/2 signaling. Mol Neurobiol, 2016; 53: 216–30
20. Xie Y, Wang X: Lycium barbarum polysaccharides attenuates the apoptosis of hippocampalneurons induced by sevoflurane. Exp Ther Med, 2018; 16: 1834–40
21. Dinnon IK, Gallichotte EN, Fritch EJ et al: Shortening of Zika virus CD-loop reduces neurovirulence while preserving antigenicity. PLoS Negl Trop Dis, 2019; 13: e7212
22. Cymerys J, Slonska A, Tucholska A et al: Influence of long-term equine herpesvirus type 1 (EHV-1) infection on primary murine neurons-the possible effects of the multiple passages of EHV-1 on its neurovirulence. Folia Microbiol (Praha), 2018; 63: 1–11
23. Palanca BJA, Avidan MS, Mashour GA: Human neural correlates of sevoflurane-induced unconsciousness. Br J Anaesth, 2017; 119: 573–82
24. Perez-Zoghbi JF, Zhu W, Grafe MR, Brambrink AM: Dexmedetomidine-mediated neuroprotection against sevoflurane-induced neurotoxicity extends to several brain regions in neonatal rats. Br J Anaesth, 2017; 119: 506–16
25. Choi J, Jo M, Lee E et al: Differential induction of autophagy by mTOR is associated with abnormal apoptosis in ovarian endometriotic cysts. Mol Hum Reprod, 2014; 20: 309–17
26. Kim SK, Yoon YD, Park YS et al: Involvement of the Fas-Fas ligand system and active caspase-3 in abnormal apoptosis in human testes with maturation arrest and Sertoli cell-only syndrome. Fertil Steril, 2007; 87: 547–53
27. Carpio MA, Katz SG: Methods to probe calcium regulation by BCL-2 family members. Methods Mol Biol, 2019; 1877: 173–83
28. Opferman JT, Kothari A: Anti-apoptotic BCL-2 family members in development. Cell Death Differ, 2018; 25: 37–45
29. Tengku DT, Abdul JM, Seeni A et al: The differential roles of caspase family members in mediating PF4-induced breast cancer apoptosis. Malays J Pathol, 2018; 40: 303–12
30. Guo L, Yu Y, Xin N et al: Clonidine protects against neurotoxicity induced by sevoflurane through NF-kappaB signaling inhibition and proinflammatory cytokine release in rats. J Mol Neurosci, 2018; 65: 507–13
31. Liu TJ, Zhang JC, Gao XZ et al: Effect of sevoflurane on the ATPase activity of hippocampal neurons in a rat model of cerebral ischemia-reperfusion injury via the cAMP-PKA signaling pathway. Kaohsiung J Med Sci, 2018; 34: 22–33
32. Lyu Z, Cao J, Wang J, Lian H: Protective effect of vitexin reduces sevoflurane-induced neuronal apoptosis through HIF-1alpha, VEGF and p38 MAPK signaling pathway *in vitro* and in newborn rats. Exp Ther Med, 2018; 15: 3117–23
33. Peng S, Yan HZ, Liu PR et al: Phosphodiesterase 4 inhibitor roflumilast protects rat hippocampal neurons from sevoflurane induced injury via modulation of MEK/ERK signaling pathway. Cell Physiol Biochem, 2018; 45: 2329–37

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