Submitted: 14 January, 2025 Accepted: 13 March, 2025 Published: 08 April, 2025 DOI:10.22514/sv.2025.059

Open Access
Signa Vitae

ORIGINAL RESEARCH

Clematichinenoside AR improves isoflurane-stimulated cognitive dysfunction in aged mice by ameliorating neurotoxicity through ERK-CREB pathway

Jingjing Hu[1], Yuanfang Li[1], Guoqiang Fei[1,*]

[1]Department of Neurology, Zhongshan Hospital (Xiamen), Fudan University, 361000 Xiamen, Fujian, China

***Correspondence**
Feiguoqiang886@163.com
(Guoqiang Fei)

Abstract

Background: Postoperative cognitive dysfunction (POCD) can result in decreased memory and information processing speed after anesthesia. Clematichinenoside AR (AR) has recently been recognized for its multiple biological activities, including anti-inflammatory and anti-apoptotic properties. However, its effectiveness in alleviating symptoms of POCD is not yet well understood. Our objective is to evaluate and explore the role of AR in addressing POCD. **Methods**: Mice were divided into 5 groups: sham, isoflurane, isoflurane + AR (8 mg/kg), isoflurane + AR (16 mg/kg) and isoflurane + AR (32 mg/kg). The Morris Water Maze (MWM) test and Fear Conditioning Test (FCT) were conducted to evaluate the role of AR in mice with POCD. Subsequently, Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and Immunoblotting were performed to confirm the effects of AR on cell apoptosis. Furthermore, Quantitative Polymerase Chain Reaction (qPCR) and Enzyme-Linked Immunosorbent Assay (ELISA) were utilized to verify the impact of AR on inflammation. The morphology of hippocampal neurons and protein expression were examined through Hematoxylin and Eosin (H&E) staining and western blot. **Results**: The results indicated that AR can enhance isoflurane-stimulated cognitive dysfunction in mice. In addition, AR exhibits anti-apoptotic and anti-inflammatory effects in POCD mice, highlighting its neuroprotective effects. Additionally, AR activates the Extracellular Signal-Regulated Kinase—cAMP Response Element-Binding Protein (ERK-CREB) signaling pathway. **Conclusions**: AR improves isoflurane-stimulated cognitive dysfunction in aged mice by ameliorating neurotoxicity.

Keywords

Clematichinenoside AR; Postoperative cognitive dysfunction (POCD); Isoflurane; ERK; CREB

1. Introduction

POCD is a significant neurological complication characterized by a decline in memory following anesthesia [1–3]. This condition is relatively common among the elderly [4]. However, the precise etiology of POCD remains unclear.

Neuroinflammation is a key factor in the progression of POCD [4]. Anti-inflammatory therapies have the potential to alleviate the symptoms of POCD [5–7]. In the elderly brain, microglia cells become overactive, leading to inflammation and impaired cognitive function [5, 6]. Research has shown that volatile anesthetics can trigger increased inflammatory responses in the brains of aging rats [7]. In addition, anesthesia may induce apoptosis in neurons, which could further contribute to the development of POCD [8, 9]. It has been reported that anesthesia can induce rat hippocampus apoptosis, possibly rupturing mitochondrial membranes [10]. Therefore, it is essential to address neuroinflammation and neuronal apoptosis in this context. Clematichinenoside AR (AR) is an active compound isolated from

Clematischinensis Osbeck is a common traditional Chinese medicine widely used to treat patients suffering from ischemic stroke or myocardial infarction. AR has demonstrated various pharmacological effects related to cardiovascular diseases and nerve injury. For example, AR plays a significant role in the myocardium by regulating mitochondria-mediated signaling pathway and reducing hypoxia/reoxygenation-stimulated apoptosis of H9C2 cardiomyocytes [11]. Furthermore, AR inhibits the Notch/Nuclear Factor-Kappa B (NF-κB) axis, thereby mitigating inflammatory response, enhancing neuronal survival and promoting function recovery of motor and neuronal functions following cerebral ischemic injury [12]. Therefore, we hypothesized that AR could alleviate cognitive dysfunction caused by anesthetics.

In this study, our results showed that Clematichinenoside

AR suppressed neuronal apoptosis, neuroinflammatory response and activated microglia cells in mice, thereby alleviating cognitive impairment caused by the anesthetic isoflurane.

2. Materials and methods

2.1 Establishment of isoflurane animal model

Male C57BL/6 mice, aged seven to eight months, were acquired from BIORAY LABORATORIES. The experiments were carried out in accordance with the Animal Care and Use Guidelines [13].

Thirty mice were divided into 5 groups: sham, isoflurane, isoflurane + AR (8 mg/kg, GC46698, GlpBio, Montclair, CA, USA), isoflurane + AR (16 mg/kg) and isoflurane + AR (32 mg/kg). Mice in the isoflurane-stimulated groups were subjected to a specified protocol [14]. Specifically, the mice were placed in an anesthesia tank and injected with isoflurane and oxygen, and the isoflurane concentration was maintained at 1.5% for 4 hours.

2.2 Drug administration

On the first day after isoflurane treatment, isoflurane + AR (8 mg/kg), isoflurane + AR (16 mg/kg) and isoflurane + AR (32 mg/kg) groups received AR administration.

2.3 Fear conditioning test

14 days after isoflurane anesthesia and drug administration, the animals underwent a fear conditioning test (FCT) based on established protocol [15]. Each mouse was placed in a dark room and subjected to tone-foot shock at 1-minute intervals. After two hours, the mice were transferred to a different, well-lit room, where they experienced three cycles of tone and shock accompanied by a smell cue.

2.4 Morris water maze test

The Morris water maze (MWM) test was conducted 2 days after the FCT was completed according to established protocol [16]. During each trial, each mouse was placed in the water facing the maze wall in a quadrant without a platform. A camera connected to a computer video system recorded the mouse's swimming behavior. On the 6th day, the mice were subjected to a probe trial (120 s) without the platform. Memory performance was quantified by the percentage of time spent in the target quadrant, the number of target zone transitions, and freezing time in the fear conditioning context test.

2.5 TUNEL staining

The hippocampus sections were treated with TUNEL (ab66108, Abcam, Cambridge, UK) to elucidate the effects on cell apoptosis.

2.6 Western blot

The proteins extracted from brain tissues were separated using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), transferred to Polyvinylidene Fluoride (PVDF) membranes (Millipore), and then blocked with 5% non-fat milk for 1 h. The proteins was identified by antibodies, including Caspase-3 (ab4051, 1:1200; Abcam, Cambridge, UK), Bax (ab53154, 1:3000; Abcam, Cambridge, UK), Bcl-2 (ab182858, 1:1200; Abcam, Cambridge, UK), Tumor Necrosis Factor-Alpha (TNF-α) (ab205587, 1:3000; Abcam, Cambridge, UK), Interleukin-6 (IL-6) (AF506, 1:2500; R&D Systems, Minneapolis, MN, USA), IL-1β (ab205924, 1:1200; Abcam, Cambridge, UK), p-NF-κB p65 (ab86299, 1:1500; Abcam, Cambridge, UK), NF-κB p65 (ab16502, 1:1500; Abcam, Cambridge, UK), Ionized Calcium Binding Adaptor Molecule 1 (Iba1) (ab153696, 1:2500; Abcam, Cambridge, UK), p-ERK (ab201015, 1:3000; Abcam, Cambridge, UK), ERK (ab17942, 1:1200; Abcam, Cambridge, UK), p-p90RSK (ab32413, 1:3000; Abcam, Cambridge, UK), p90RSK (ab32114, 1:1200; Abcam, Cambridge, UK), p-CREB (ab10564, 1:1200; Abcam, Cambridge, UK), CREB (ab178322, 1:1200; Abcam, Cambridge, UK) and β-actin (ab8227, 1:1200; Abcam, Cambridge, UK) overnight at 4 °C. Then, the membranes were incubated with Horseradish Peroxidase (HRP)-conjugated goat anti-rabbit Immunoglobulin G (IgG) secondary antibody (ab205718, 1:2000; Abcam, Cambridge, UK), and the bands on the membranes were visualized by the Enhanced Chemiluminescence (ECL) reagent (P0018, Beyotime, Beijing, China).

2.7 qPCR

Total RNA was extracted using Trizol reagent (15596026, Invitrogen, Carlsbad, CA, USA). The mRNA levels of TNF-α, IL-6, IL-1β, CD86 and CD206 was detected through Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) by the SYBR Premix EX Taq (RR420A, Takara, Kusatsu, Japan) and analyzed by the $2^{-\Delta\Delta CT}$ method. Primer sequences are shown in Table 1.

2.8 Histopathological analysis

Hippocampus tissues were fixed in 4% (v/v) Paraformaldehyde (PFA) and embedded in paraffin. Next, the tissues were cut into 3 μm sections and stained with hematoxylin and eosin solution (HHS32, Sigma-Aldrich, St. Louis, MO, USA). Lastly, they were observed under a microscope (BX53, Olympus, Tokyo, Japan).

2.9 Statistical analysis

The data were analyzed with GraphPad 5.0 software (GraphPad Software, San Diego, CA, USA). Error bars represent the means $\pm$ Standard Errors of the Mean (SEMs). Statistical significance between two groups was determined via an unpaired Student's *t*-test. One-way Analysis of Variance (ANOVA) with Tukey's *post hoc* test was applied for multiple comparisons. A $p < 0.05$ was considered statistically significant.

3. Results

TABLE 1. Primers for *TNF-α*, *IL-6*, *IL-1β*, *CD86*, *CD206* and reference genes.

Gene	Primer	Sequence (5′→3′)
TNF-α		
	Forward	GCCACCACGCTCTTCTGTCTAC
	Reverse	GGGTCTGGGCCATAGAACTGAT
IL-6		
	Forward	CACATGTTCTCTGGGAAATCG
	Reverse	TTGTATCTCTGGAAGTTTCAGATTGTT
IL-1β		
	Forward	ACCTTCCAGGATGAGGACATGA
	Reverse	CTAATGGGAACGTCACACACCA
CD86		
	Forward	ATGGGACTGAGTAACATTCTCTTTGTGATGGCCT
	Reverse	CTCGAGTTAAAAACATGTATCACTTTTGTCGCATGA
CD206		
	Forward	TTCGGACACCCATCGGAATTT
	Reverse	CACAAGCGCTGCGTGGAT
β-actin		
	Forward	GTGACGTTGACATCCGTAAAGA
	Reverse	GCCGGACTCATCGTACTCC

TNF: Tumor Necrosis Factor; IL: Interleukin.

3.1 Clematichinenoside AR improves isoflurane-stimulated cognitive dysfunction in mice

The animal POCD model was established, and behavioral tests were conducted to evaluate the behavioral performance following isoflurane administration. The MWM test was first performed to evaluate POCD (Fig. 1A). The results indicated that mice treated with isoflurane required more time to locate the hidden platform compared to mice in the control group, suggesting that isoflurane impairs spatial learning in the aged mice (Fig. 1B). Furthermore, the mice treated with isoflurane spent less time in the target quadrant. They demonstrated a reduced frequency of transitions to the target zone (Fig. 1C,D). In contrast, treatment with AR improved the time taken to find the hidden platform and increased the time spent in the target quadrant, indicating that AR can mitigate the cognitive deficits caused by isoflurane in aged mice.

Subsequently, FCT was conducted to assess POCD. The FCT assay revealed that mice treated with isoflurane exhibited decreased freezing times compared to mice in the sham group, indicating that isoflurane impaired associative learning and memory in aged mice. However, AR treatment improved isoflurane-stimulated short freezing times in the mice, suggesting that AR effectively rescued the isoflurane-stimulated declines in learning and memory in aged mice (Fig. 1E). AR, therefore, can improve isoflurane-stimulated cognitive dysfunction in mice.

3.2 Clematichinenoside AR can inhibit isoflurane-stimulated apoptosis in mice hippocampus

Isoflurane treatment substantially increased the number of TUNEL-positive neurons, whereas AR treatment resulted in a notable decrease in TUNEL-positive neurons, demonstrating significant inhibitory effects (Fig. 2A). Additionally, isoflurane treatment markedly upregulated the expression levels of Bax and cleaved caspase-3, while downregulating Bcl-2. However, the expression of Bax and cleaved caspase-3 were dramatically reduced while Bcl-2 was enhanced by AR treatment in the isoflurane administration group (Fig. 2B). Therefore, AR can inhibit isoflurane stimulated apoptosis in mice hippocampus.

3.3 Clematichinenoside AR can reduce isoflurane-stimulated inflammatory response in mice

The qPCR and western blot analyses showed that the isoflurane group's mRNA and protein expression levels of TNF-α, IL-6 and IL-1β were significantly increased. However, AR markedly reduced the levels of these inflammatory factors in the isoflurane administration group. Furthermore, the inflammatory factors stimulated by isoflurane administration showed a dose-dependent change (Fig. 3A,B). The phosphorylation levels of NF-κB p65 were also detected by western blot. The phosphorylation of NF-κB p65 was notably increased in the isoflurane administration group, but it was dramatically reduced after AR administration (Fig. 3C). Therefore, AR has

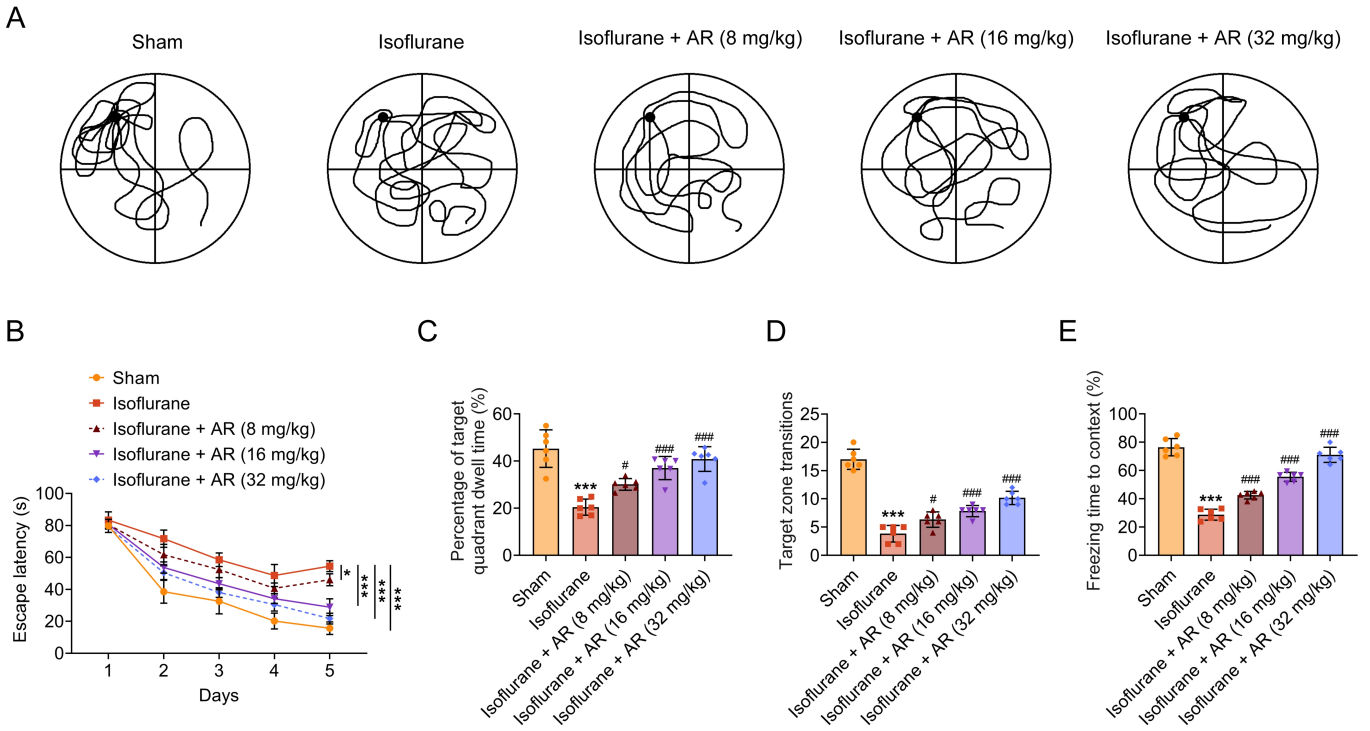

FIGURE 1. Clematichinenoside AR can improve isoflurane-stimulated cognitive dysfunction in mice. (A) Representative swim paths in the MWM. (B) Escape latency in the MWM. (C) The percentage of time spent in the target quadrant in the probe trial of the MWM. (D) The number of target zone transitions in the probe trial of the MWM. (E) Freezing time in the fear conditioning context test. Data were presented as the mean ± SD with three independent experiments. $^{*}p < 0.05$, $^{***}p < 0.001$, versus sham group, $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$, and versus isoflurane group. AR: Clematichinenoside AR; MWM: Morris Water Maze.

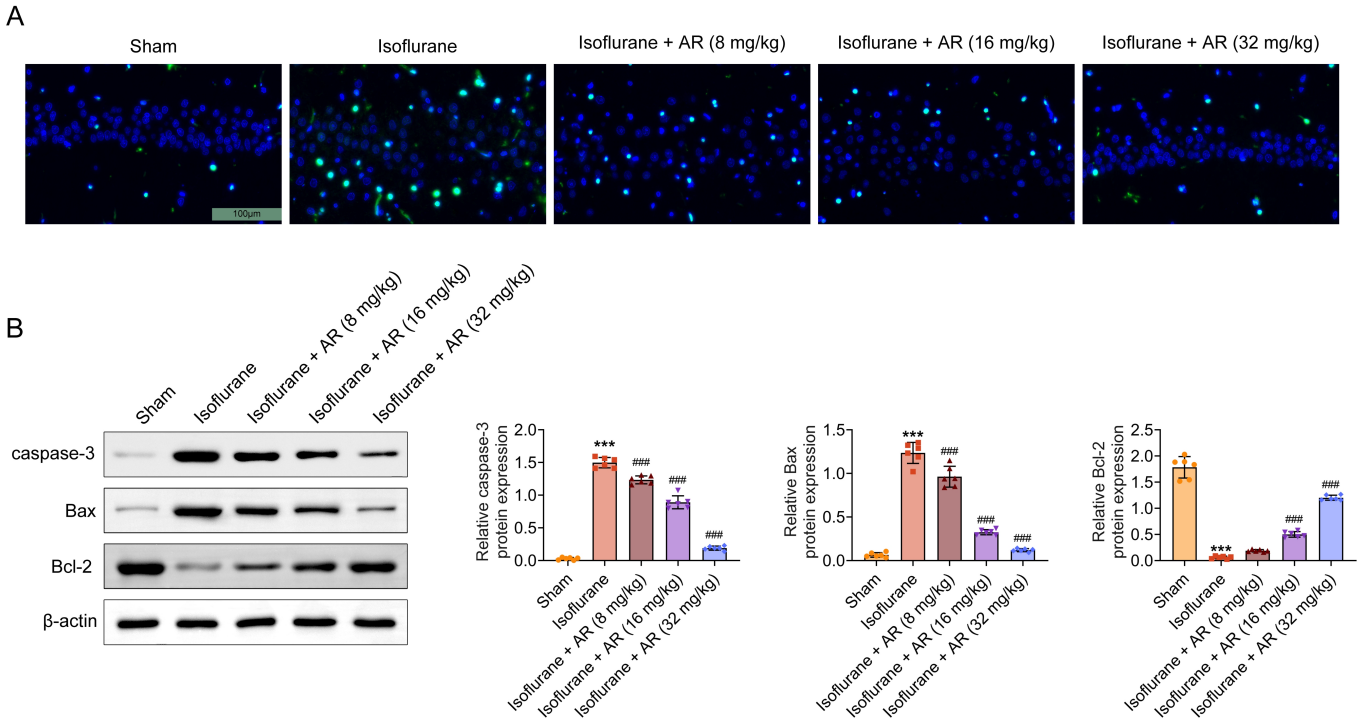

FIGURE 2. Clematichinenoside AR can inhibit isoflurane-stimulated apoptosis in mice hippocampus. (A) TUNEL assay was conducted to determine cell apoptosis for each group. (B) Western blot was conducted to detect cell apoptosis-related proteins for each group. Relatively quantitative results were determined by Image J and shown as a histogram. Data were presented as the mean ± SD with three independent experiments. $^{***}p < 0.001$ versus sham group, $^{\#\#\#}p < 0.001$ versus isoflurane group. AR: Clematichinenoside AR; Bcl-2: B-cell lymphoma 2.

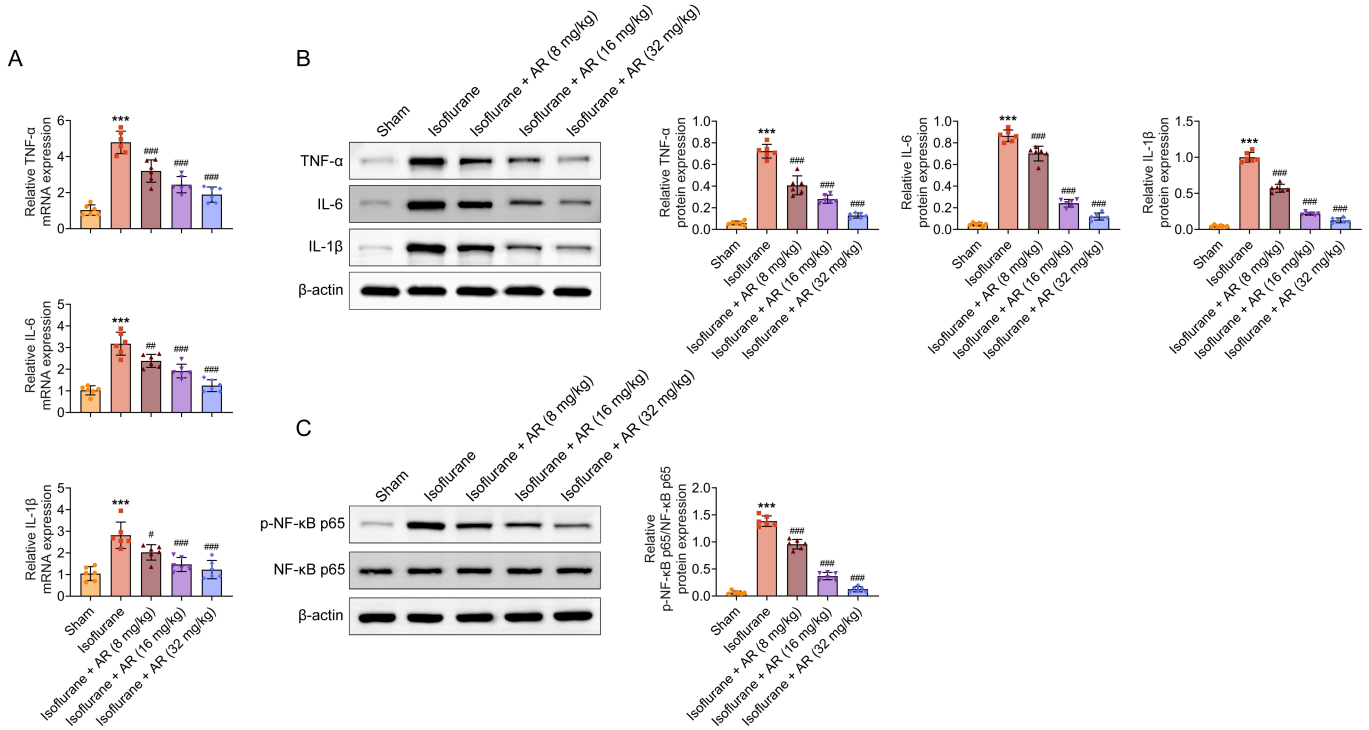

FIGURE 3. Clematichinenoside AR can reduce isoflurane-stimulated inflammatory response in mice. (A) The mRNA expression levels of TNF-α, IL-6 and IL-1β in mice in each group. (B) The protein expression levels of TNF-α, IL-6 and IL-1β in mice in each group. (C) The protein expression levels of NF-κB p65 in mice in each group. Data were presented as the mean $\pm$ SD with three independent experiments. ***$p < 0.001$ versus sham group, $^{\#}p < 0.05$, and $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus isoflurane group. AR: Clematichinenoside AR; TNF: Tumor Necrosis Factor; IL: Interleukin; NF-κB: Nuclear Factor-Kappa B.

a significant anti-inflammatory effect on the isoflurane-treated mice.

3.4 Clematichinenoside AR can inhibit isoflurane-stimulated activation of microglia cells in mice hippocampus

H&E staining and western blot analysis were conducted on the hippocampus of mice in each group to investigate the neuroprotective effect of AR in POCD mice. The H&E staining results indicated that the nuclei of hippocampal neurons of mice in the isoflurane group were shrunken and damaged. However, mice in the isoflurane administration group treated with AR showed preserved nuclear structures within their hippocampal neuron (Fig. 4A). Furthermore, mice in the isoflurane administration group showed elevated expression levels of Iba1, indicating an increased number of activated microglia cells (Fig. 4B). Following AR treatment in isoflurane administration group, the expression levels of Iba1 markedly reduced. The mRNA expression levels of CD86 were significantly increased, while the levels of CD206 profoundly decreased after isoflurane treatment, which revealed that an increasing number of microglia cells became activated in response to the isoflurane administration. After AR treatment, the mRNA expression levels of CD86 and CD206 were reversed, which indicated that microglia cells in the hippocampus of mice were gradually shifted from M1 to M2 phenotype (Fig. 4C). Therefore, AR can induce neuroprotective effects in isoflurane-treated mice.

3.5 Clematichinenoside AR can activate the ERK-CREB pathway

Western blot analysis was conducted to investigate the mechanism by which AR attenuates isoflurane-stimulated cognitive dysfunction in aged mice by ameliorating neurotoxicity through the ERK-CREB pathway. The phosphorylation levels of ERK1/2, p90RSK and CREB were dramatically downregulated in the hippocampus after isoflurane administration. However, the phosphorylation levels of ERK1/2, p90RSK and CREB were reversed in the isoflurane administration group after AR treatment, indicating that AR could activate ERK1/2, p90RSK and CREB axis (Fig. 5).

4. Discussion

Despite notable advances in neuroscience, the precise mechanism underlying POCD remains poorly understood. Clematichinenoside AR (AR) is essential for its anti-inflammatory and anti-apoptotic properties [17]. However, it is infrequently studied concerning POCD. In addition, the role of AR in influencing the outcomes associated with POCD is still not fully elucidated.

AR is a triterpene saponin derived from *Clematischinensis Osbeck*, a traditional Chinese medicine commonly used to treat ischemic stroke or myocardial infarction patients. This compound exhibits anti-inflammatory and anti-apoptotic properties [18], significantly alleviating symptoms associated with cardiovascular diseases, nerve injuries, and other medical condi-

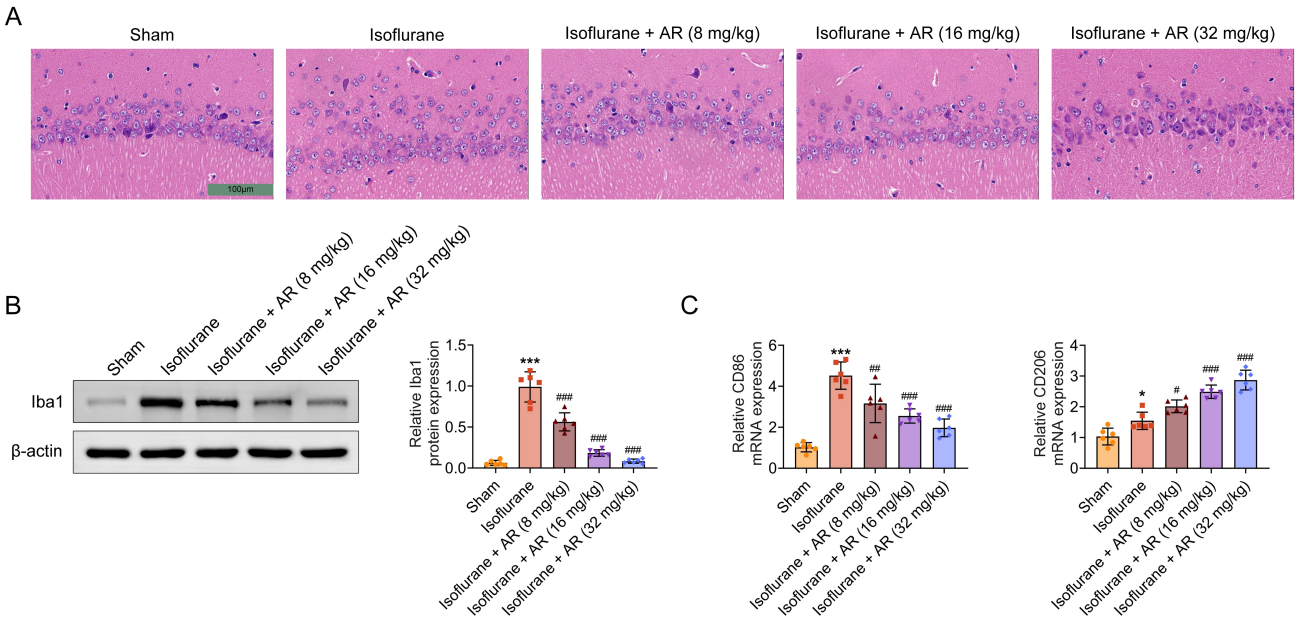

FIGURE 4. Clematichinenoside AR can inhibit isoflurane-stimulated activation of microglia cells in mice hippocampus. (A) H&E staining of the hippocampus of mice in each group. (B) The protein expression levels of Iba1 in the hippocampus of mice in each group. Relatively quantitative results were determined by Image J and shown as a histogram. (C) The mRNA expression levels of CD86 and CD206 in mice in each group. Data were presented as the mean ± SD with three independent experiments. $^{*}p < 0.05$, $^{***}p < 0.001$ versus sham group, $^{\#}p < 0.05$, and $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus isoflurane group. AR: Clematichinenoside AR; Iba1: Ionized Calcium Binding Adaptor Molecule 1.

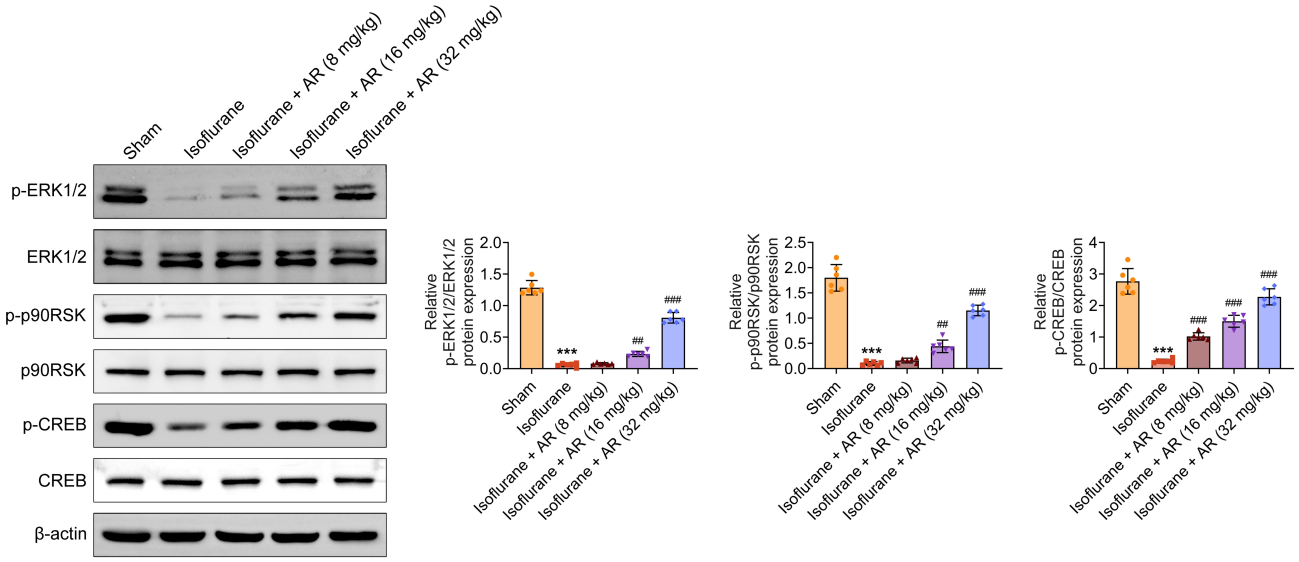

FIGURE 5. Clematichinenoside AR can activate the ERK-CREB pathway. The protein levels of p-ERK1/2, ERK1/2, p-p90RSK, p90RSK, p-CREB and CREB in each group. Relatively quantitative results were determined by Image J and shown as a histogram. Data were presented as the mean ± SD with three independent experiments. $^{***}p < 0.001$ versus sham group, $^{\#\#\#}p < 0.001$, and $^{\#\#}p < 0.01$ versus isoflurane group. AR: Clematichinenoside AR; ERK: Extracellular Signal-Regulated Kinase; p90RSK: p90 Ribosomal S6 Kinase; CREB: cAMP Response Element-Binding Protein.

tions [12]. Notably, AR has the potential to mitigate the effects of myocardial infarction in instances of ischemia/reperfusion injury [19]. Recent research has established a relationship between AR and human tumor necrosis factor-α, indicating its ability to prevent inflammation and cytotoxicity [20, 21]. Moreover, AR was found to serve as a neuroprotective agent against ischemic stroke via ERK1/2 and Calcium-Dependent Protein Kinase C (cPKC) pathways [13]. Due to its multi-targeting actions, AR holds promise for the development of new and safe therapeutic agents as a natural product. Studies have demonstrated that AR can enhance cognitive function in mice experiencing isoflurane-stimulated cognitive dysfunction. In addition, AR exhibits anti-inflammatory effects and inhibits cell apoptosis in POCD mice. AR can also inhibit

isoflurane-stimulated activation of microglia cells in mice hippocampus. As a result, AR may play a significant role in alleviating symptoms of POCD. However, it is essential to note that, despite numerous literature reports that AR can restore neurological function [12], no literature has yet reported whether AR can cross the blood-brain barrier (BBB). Therefore, future studies need to further explore whether AR directly inhibits isoflurane-stimulated activation of microglia cells in mice hippocampus across the BBB.

AR exerts its functions by regulating mRNA levels. AR has been demonstrated to protect the blood-brain barrier from ischemic stroke by upregulating A20 [22]. Furthermore, Notoginsenoside R1 (NGR1) has been shown to inhibit the expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1) in endothelial cells through the mediation of the IκB kinase/NF-κB axis [23]. NGR1 also provides protection to renal epithelial cells against hypoxia/reoxygenation injury via the Nuclear Factor Erythroid 2-Related Factor 2/Heme Oxygenase-1 (Nrf2/HO-1) pathway [24]. Our research indicates that AR regulates the expression of ERK, p90RSK and CREB proteins. ERK is a signaling protein that transmits information from receptors to DNA and is involved in a wide range of cellular activities. p90RSK serves as a protein kinase that facilitates signal transduction, while CREB functions as a transcription factor that regulates brain activity, circadian rhythms, and the manifestation of various diseases. Notably, our findings revealed that the expression levels of ERK, p90RSK and CREB proteins stimulated by isoflurane administration were gradually changed with increasing doses of AR, indicating that AR may alleviate symptoms of POCD by activating the ERK-CREB pathway.

Additionally, numerous studies have reported that ERK-CREB signaling is associated with anti-inflammatory effects. In particular, it has been found that Silibinin can reduce the inflammatory response of retinal ganglion cells by mediating the Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Kinase (MEK)/ERK/CREB signaling pathway [25]. Recently, it has been demonstrated that renal tubular cell inflammation is regulated by the Mitogen-Activated Protein Kinase (MAPK)-ERK-CREB signaling pathway [26]. Therefore, future studies should pay more attention to whether AR can directly achieve anti-inflammatory effects through the ERK-CREB signaling pathway. Although our findings suggest an effect of AR on the ERK pathway, we cannot yet make a definitive conclusion regarding the influence of this pathway on the POCD process. Additional research involving ERK inhibitors in POCD mice is necessary to clarify the underlying mechanisms.

Despite our findings suggesting that AR may alleviate POCD symptoms through the ERK-CREB signaling pathway, several limitations should be acknowledged. First, although AR has demonstrated neuroprotective and anti-inflammatory effects, its ability to cross the blood-brain barrier (BBB) remains uncertain, necessitating further pharmacokinetic studies. Second, while our data indicate that AR modulates ERK, p90RSK and CREB protein expression, we have not yet confirmed whether these effects are directly responsible for the cognitive improvements observed. Future studies using ERK inhibitors or gene knockout models are required to establish a causal relationship. Lastly, our study primarily focused on a mouse model of POCD, and the translational potential of AR in human subjects remains to be explored. Clinical studies are needed to determine the efficacy and safety of AR in treating POCD in patients undergoing surgery and anesthesia.

5. Conclusions

In conclusion, the findings indicate that AR enhances isoflurane-stimulated cognitive dysfunction in mice. AR effectively inhibits isoflurane-stimulated apoptosis in mice hippocampus and mitigates isoflurane-stimulated inflammatory response in mice. Moreover, AR inhibits isoflurane-stimulated activation of microglia cells in mice hippocampus. Finally, mechanistic studies showed that AR improves symptoms of POCD.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

JJH—designed the study and carried them out. JJH, YFL, GQF—supervised the data collection, analyzed the data, interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics committee of Ethics committee of Zhongshan Hospital, Fudan University (Approval no. 20221109-002).

ACKNOWLEDGMENT

Not applicable.

FUNDING

This work was supported by Incubation Program of Zhongshan Hospital (Xiamen), Fudan University (Grant no. 2020ZSXMYS07).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Gallagher E, Li S, Lee H, Xu H, Lee VM, Mach RH, *et al*. Noninvasive detection of oxidative stress in a mouse model of 4R tauopathy via

positron emission tomography with [^{18}F]ROStrace. International Journal of Molecular Sciences. 2025; 26: 1845.

[2] Mu DL, Li LH, Wang DX, Li N, Shan GJ, Li J, *et al*. High postoperative serum cortisol level is associated with increased risk of cognitive dysfunction early after coronary artery bypass graft surgery: a prospective cohort study. PLOS ONE. 2013; 8: e77637.

[3] Steinmetz J, Christensen KB, Lund T, Lohse N, Rasmussen LS; ISPOCD Group. Long-term consequences of postoperative cognitive dysfunction. Anesthesiology. 2009; 110: 548–555.

[4] Devinney MJ, Spector AR, Wright MC, Thomas J, Avasarala P, Moretti EW, *et al*. The role of sleep apnea in postoperative neurocognitive disorders among older noncardiac surgery patients: a prospective cohort study. Anesthesia & Analgesia. 2025; 140: 99–109.

[5] Hovens IB, van Leeuwen BL, Nyakas C, Heineman E, van der Zee EA, Schoemaker RG. Postoperative cognitive dysfunction and microglial activation in associated brain regions in old rats. Neurobiology of Learning and Memory. 2015; 118: 74–79.

[6] Fujinami Y, Saito M, Ono Y, Akashi M, Inoue S, Kotani J. Malocclusion worsens survival following sepsis due to the disruption of innate and acquired immunity. International Journal of Molecular Sciences. 2025; 26: 1894.

[7] Wang Z, Meng S, Cao L, Chen Y, Zuo Z, Peng S. Critical role of NLRP3-caspase-1 pathway in age-dependent isoflurane-stimulated microglial inflammatory response and cognitive impairment. Journal of Neuroinflammation. 2018; 15: 109.

[8] Zhang X, Dong H, Li N, Zhang S, Sun J, Zhang S, *et al*. Activated brain mast cells contribute to postoperative cognitive dysfunction by evoking microglia activation and neuronal apoptosis. Journal of Neuroinflammation. 2016; 13: 127.

[9] Guo M, Zhu X, Xu H, Li J, Yang S, Zuo Z, *et al*. Ulinastatin improves isoflurane-stimulated cognitive dysfunction in aged rats by inhibiting neuroinflammation and β-amyloid peptide expression in the brain. Neurological Research. 2019; 41: 923–929.

[10] Li Y, Zhang Y, He X, Guo Z, Yang N, Bai G, *et al*. The mitochondrial blueprint: unlocking secondary metabolite production. Metabolites. 2024; 14: 711.

[11] Ding H, Han R, Chen X, Fang W, Liu M, Wang X, *et al*. Clematichinenoside (AR) improves hypoxia/reoxygenation-stimulated H9c2 Cardiomyocyte Apoptosis via a mitochondria-mediated signaling pathway. Molecules. 2016; 21: 683.

[12] Xu D, Xia N, Hou K, Li F, Chen S, Hu Y, *et al*. Clematichinenoside facilitates recovery of neurological and motor function in rats after cerebral ischemic injury through inhibiting notch/NF-κB pathway. Journal of Stroke & Cerebrovascular Diseases. 2019; 28: 104288.

[13] National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals. 8th edn. National Academies Press (US): Washington (DC). 2011.

[14] Abdelghaffar R, Hamed M, Basiony M, Algyar MM, Fargaly OS, Shawky MA. Opioid-free anesthesia for upper limb surgery in obese patients as a day case surgery: a prospective observational study. Anesthesia and Pain Medicine. 2025; 14: e150997.

[15] Milad MR, Igoe S, Orr SP. Fear conditioning in rodents and humans. In Raber J (ed.) Animal models of behavioral analysis (pp. 111–132). 2011 edn. Humana Press: Totowa, NJ. 2011.

[16] Meng TY, Zhang X, Zhao JL, Xue H, Yu LH. Acetate and propionate *vs*. iTBS as a novel method for cognitive dysfunction and anxiety symptoms in delayed encephalopathy after acute carbon monoxide poisoning rat. Frontiers in Pharmacology. 2025; 16: 1520988.

[17] Diao Y. Clematichinenoside AR alleviates foam cell formation and the inflammatory response in Ox-LDL-stimulated RAW264.7 cells by activating autophagy. Inflammation. 2021; 44: 758–768.

[18] Zhong H, Hao L, Li X, Wang C, Wu X. Anti-inflammatory role of Trilobatin on lipopolysaccharide-stimulated acute lung injury through activation of AMPK/GSK3β-Nrf2 pathway. Signa Vitae. 2020; 16: 160–166.

[19] Zhang R, Fang W, Han D, Sha L, Wei J, Liu L, *et al*. Clematichinenoside improves myocardial infarction in ischemia/reperfusion injury both *in vivo* and *in vitro*. Planta Medica. 2013; 79: 1289–1297.

[20] Xiong Y, Ma Y, Kodithuwakku ND, Fang W, Liu L, Li F, *et al*. Protective effects of Clematichinenoside AR against inflammation and cytotoxicity stimulated by human tumor necrosis factor-α. International Immunopharmacology. 2019; 75: 105563.

[21] Chen F, Wang W, Cai X, Yu H, Qu C, Zhang X, *et al*. Methyl jasmonate reduces the inflammation and apoptosis of HK-2 cells stimulated by LPS by regulating the NF-κB pathway. Signa Vitae. 2021; 17: 218–224.

[22] Han D, Fang W, Zhang R, Wei J, Kodithuwakku ND, Ma W, *et al*. Clematichinenoside protects blood brain barrier against ischemic stroke superimposed on systemic inflammatory challenges through up-regulating A20. Brain, Behavior, and Immunity. 2016; 51: 56–69.

[23] Yan S, Zhang X, Zheng H, Hu D, Zhang Y, Guan Q, *et al*. Clematichinenoside inhibits VCAM-1 and ICAM-1 expression in TNF-α-treated endothelial cells via NADPH oxidase-dependent IκB kinase/NF-κB pathway. Free Radical Biology and Medicine. 2015; 78: 190–201.

[24] Feng J, Kong R, Xie L, Lu W, Zhang Y, Dong H, *et al*. Clemaichinenoside protects renal tubular epithelial cells from hypoxia/reoxygenation injury *in vitro* through activating the Nrf2/HO-1 signalling pathway. Clinical and Experimental Pharmacology and Physiology. 2020; 47: 495–502.

[25] Shen Y, Zhao H, Wang Z, Guan W, Kang X, Tai X, *et al*. Silibinin declines blue light-stimulated apoptosis and inflammation through MEK/ERK/CREB of retinal ganglion cells. Artificial Cells, Nanomedicine, and Biotechnology. 2019; 47: 4059–4065.

[26] Dong Q, Jie Y, Ma J, Li C, Xin T, Yang D. Renal tubular cell death and inflammation response are regulated by the MAPK-ERK-CREB signaling pathway under hypoxia-reoxygenation injury. Journal of Receptors and Signal Transduction. 2019; 39: 383–391.

How to cite this article: Jingjing Hu, Yuanfang Li, Guoqiang Fei. Clematichinenoside AR improves isoflurane-stimulated cognitive dysfunction in aged mice by ameliorating neurotoxicity through ERK-CREB pathway. Signa Vitae. 2025; 21(4): 123-130. doi: 10.22514/sv.2025.059.