doi: 10.4149/gpb_2024032

Sevoflurane regulates DICER1 expression by targeting miR-192-5p to protect cerebral ischemia-reperfusion injury in rats

Bo Yuan[1], Qian Zhao[1], Wei-Qiang Xiao[1], Jian-Jun Ouyang[1], Jun Huang[1] and Yu-Hong Fan[1]

[1] *Department of Anesthesia, The Fourth Hospital of Changsha, Changsha City, Hunan Province, China*

Abstract. Sevoflurane is considered an effective neuroprotector in cerebral ischemia/reperfusion injury (CIRI). Sevoflurane preconditioning in CIRI, however, remains unknown precisely by its molecular mechanism. The middle cerebral artery occlusion reperfusion (MCAO/R) rat model was established, and neurological function was evaluated by Zea-Longa score. Cerebral water content was determined to assess cerebral edema. Brain pathological condition was observed by hematoxylin and eosin staining, the intact changes of rat neurons were observed by Nissl staining, and neuronal apoptosis was measured by TUNEL staining. In addition, miR-192-5p and DICER1 levels were detected by RT-qPCR or Western blot, and the targeting relationship between miR-192-5p and DICER1 was verified by bioinformatics analysis and luciferase reporting experiment. miR-192-5p was up-regulated and DICER1 was down-regulated in MCAO/R rats. Sevoflurane preconditioning could decrease miR-192-5p and promote DICER1 expression. Sevoflurane preconditioning could alleviate brain tissue injury and neuronal apoptosis in MCAO/R rats. DICER1 expression was negatively regulated by targeting miR-192-5p. Elevating miR-192-5p or suppressing DICER1 rescued the protective effect of sevoflurane preconditioning on MCAO/R rats. Sevoflurane alleviates brain injury in MCAO/R rats *via* miR-192-5p/DICER1 axis.

Key words: Sevoflurane — miR-192-5p — DICER1 — Cerebral ischemia-reperfusion injury — Neuronal apoptosis

Introduction

By thrombolysis or mechanical recanalization, blood flow can be restored following an ischemic stroke. It is possible, however, that reperfusion may worsen the damage originally caused by ischemia, resulting in cerebral ischemia/reperfusion injury (CIRI) (Li et al. 2022). Several different pharmacological and mechanical strategies can be used to pretreat the brain to tolerate CIRI, such as ischemic preconditioning, ethanol-pharmacological preconditioning, and other preconditioning modalities (Yang H et al. 2022). Sevoflurane, as a commonly used inhalation anesthetic, has the characteristics of rapid onset, low blood gas coefficient, and little airway irritation (Huang et al. 2021). It is believed that sevoflurane can improve neurological function, reduce cerebral infarct volume and inflammatory cytokine levels, while protecting neurons against apoptosis and oxidant stress (Neag et al. 2020; Liang et al. 2021). In some animal studies, sevoflurane preconditioning is ameliorating in CIRI, which is potentially related to the regulation of certain miRNAs (Zhang et al. 2019; Jin and Bo 2021; Su et al. 2021).

Non-coding RNA transcripts, including miRNAs, involves in the pathophysiology of CIRI and have great potential as biomarkers to evaluate the degree of tissue damage (Ghafouri-Fard et al. 2020; Yang K et al. 2022). miR-192-5p has neurotoxicity in brain diseases, including but not limited to hypoxic-ischemic brain damage (Yan et al. 2022)

Correspondence to: Yu-Hong Fan, Department of Anesthesia, The Fourth Hospital of Changsha, No.70, Lushan Road, Yuelu District, Changsha City, Hunan Province, 410006, China
E-mail: fffanyuhong@hotmail.com

and Parkinson's disease (Kang et al. 2019). Moreover, high miR-192-5p expression has indication of poor outcomes in acute ischemic stroke patients administrated with thrombolysis (He et al. 2019). According to literature experience, downregulating miR-192-5p could alleviate neurobehavioral impairment and neuronal damage in hypoxic-ischemic brain damage (Yan et al. 2022). Protein-coding mRNAs are regulated by miRNAs by binding to the 3'UTR and triggering mRNA decay or movement (Correia de Sousa et al. 2019). In line with this theory, this research after bioinformatics screening and verification selected DICER1 as the interest mRNA of miR-192-5p.

Much study attention was paid to sevoflurane preconditioning in terms of attenuating CIRI in rat models of middle cerebral artery occlusion reperfusion (MCAO/R). This study found for the first time that sevoflurane preconditioning alleviates CIRI by modulating the miR-192-5p/DICER1 axis.

Materials and Methods

Laboratory animal

All animal experiments were approved by the Ethics Committee of The Fourth Hospital of Changsha and were in line with the Guidelines for the Care and Use of Experimental Animals. SPF SD rats (male; 10 weeks old; 260 ± 20 g) were provided with a standard food supply and free drinking water and were kept at 23 ± 2°C with a light/dark cycle of 12/12 h.

Construction of MCAO/R model

The rats were subjected to MCAO/R, as previously described (Wang et al. 2018). Briefly, the rats were anesthetized with pentobarbital sodium 3% intraperitoneally (50 mg/kg), then the left common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed, and a 3-0 nylon monofilament suture was inserted from the ECA into the ICA until reaching the middle cerebral artery (MCA). After 1.5 h of MCA occlusion, reperfusion was simulated by removing the filament. During the surgical procedure, the body temperature of all rats was maintained at 37.0°C. Sham-operated animals underwent the same anesthesia and surgical procedures except that the filament was not inserted into ICA.

Sevoflurane preconditioning

Sevoflurane (2.5%; Baxter International, USA) was preconditioned in a 33% O_2 and air mixture by inhalation delivery system for 30 min daily for 4 days prior to MCAO/R.

Experimental groups

Sixty-six rats were randomly divided into 11 groups with 6 rats in each group: (1) Sham group: only blood vessels were separated; (2) Model group: MCAO/R modeling; (3) Sevo group: sevoflurane preconditioning before MCAO/R; (4) Sevo+miR-192-5p antagomir group: rats pretreated with sevoflurane before MCAO/R were injected with miR-192-5p antagomir; (5) Sevo+antagomir NC group: rats pretreated with sevoflurane before MCAO/R were injected with antagomir negative control (NC); (6) Sevo+miR-192-5p agomir group: rats pretreated with sevoflurane before MCAO/R were injected with miR-192-5p agomir; (7) Sevo+agomir NC group: rats pretreated with sevoflurane before MCAO/R were injected with agomir NC; (8) Sevo+oe-DICER1 group: rats pretreated with Sevoflurane before MCAO/R were injected with DICER1 overexpression vector; (9) Sevo+oe-NC group: rats pretreated with sevoflurane before MCAO/R were injected with oe-NC; (10) Sevo+sh-DICER1 group: rats pretreated with sevoflurane before MCAO/R were injected with sh-DICER1; (11) Sevo+sh-NC group: rats pretreated with sevoflurane before MCAO/R were injected with sh-NC.

Right lateral ventricle injection: the anterior fontanelle was coordinated 0 point, 1.5 mm to the right, 1.2 mm backward, 4.5 mm deep. The corresponding plasmid (160 mg/l, 4 μl, Genepharma, Shanghai, China) was injected into the right lateral ventricle at 0.3 μl/min.

Neurological function assessment

Neurological function was evaluated by Zea-Longa score. No neurological defects = 0; left anterior extension disorder (mild neurological defect) = 1 point; crawling to the left (moderate neurological defect) = 2 points; turning to the hemiplegic side (severe neurological defect) during walking = 3 points; not able to walk (lost consciousness) = 4 points. The statistics were double-blind, statisticians were not involved in the modeling and did not know the groups (Sun et al. 2022).

Detection of brain water content (BWC)

Rat brains were weighed to record wet weight, and heated at 105°C for 24 h to measure dry weight. BWC was calculated: (wet weight − dry weight)/wet weight × 100% (Yang et al. 2021).

HE staining

Brain tissue was removed from CO_2-euthanized rats, fixed with 4% paraformaldehyde, and paraffin-embedded before preparation into slices of 5 μm. The slices were dewaxed with

xylene, washed with water, and stained with hematoxylin for 3 min and eosin for 1 min. After processing with gradient ethanol and xylene, the slices were sealed with neutral gum and examined under an optical microscope (Wang et al. 2020).

Nissl staining

Rat brain slices after dewaxing were treated with gradient ethanol and washed with distilled water. After that, the slices were dyed with cresyl violet solution (C9140, Solarbio) for 30 min, cleared with distilled water, and differentiated with 95% ethanol. The slices were observed under a microscope (Nikon, Japan) after dehydration by gradient ethanol and permeabilization by xylene (Luo et al. 2022).

TUNEL staining

Rat brain tissue was fixed with 4% paraformaldehyde, embedded in paraffin, and sliced. The sections were then stained according to the instructions provided by the TUNEL kit (Beyotime, China). Tissue was restained with DAPI, and positive-cells in each field were examined under a fluorescence microscope (Olympus) (Li et al. 2021).

RT-qPCR

Trizol (Invitrogen) was utilized to collect total RNA from rat brain tissue. The equivalent amount of RNA was reverse-transcribed using a reverse transcription kit (Takara, Japan), followed by RT-qPCR using SYBR-Green Supermix (Invitrogen) on the ABI PRISM 7000 Sequence Detection System (ABI/Perkin Elmer, USA). U6 was an internal reference for miRNA, and GAPDH was that for mRNA. The $2^{-\Delta\Delta Ct}$ method calculated expression levels (Table 1) (Chai et al. 2020).

Table 1. The primer sequences

Gene	Sequence
miR-192-5p	F: 5'-GACCTATGAATTGACAGCC-3'
	R: 5'-TGGTGTCGTGGAGTCG-3'
U6	F: 5'-CTCGCTTCGGCAGCACA-3'
	R: 5'-AACGCTTCACGAATTTGCGT-3'
DICER1	F: 5'-GAGCTGTCCTATCAGATCAGGG-3'
	R: 5'-ACTTGTTGAGCAACCTGGTTT-3'
GAPDH	F: 5'-GTCGGTGTGAACGGATTTG-3'
	R: 5'-TCCCATTCTCAGCCTTGAC-3'

F, forward; R, reverse; miR-192-5p, microRNA-192-5p; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Western blot

Total proteins were extracted from rat brain tissues using RIPA lysis buffer containing protease inhibitors (Beyotime). Following by protein concentration evaluation based on the BCA kit (Beyotime), the same amount of protein was isolated by 10% SDS/PAGE and transferred to the PVDF membrane, which was blocked with 10% milk powder solution for 1 h before overnight incubation with primary antibody DICER1 (ab14601, 1:1000, Abcam) and GAPDH (ab8245, 1:1000, Abcam) at 4°C. Next, the membrane was

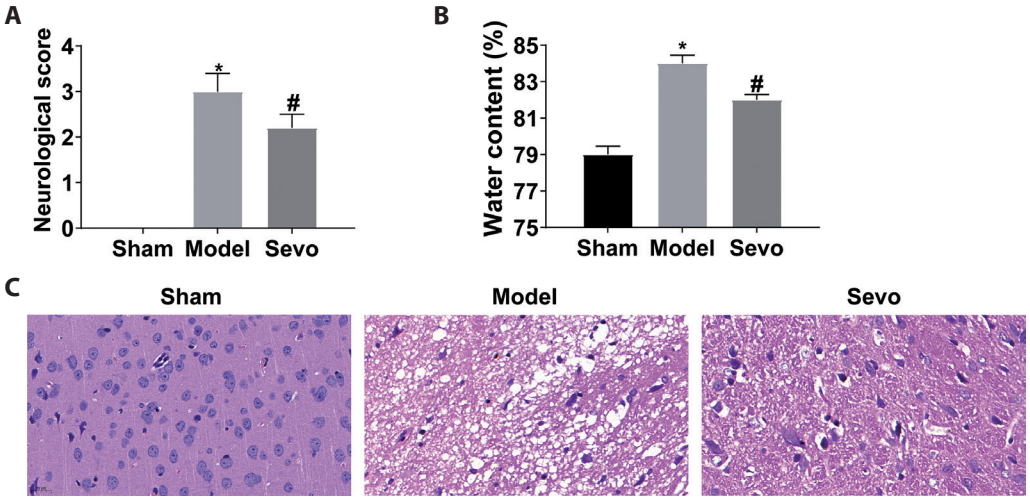

Figure 1. Sevoflurane preconditioning can improve neurological function and brain tissue damage in MCAO/R rats. **A.** Neurological deficit score of rats. **B.** Brain water content detection. **C.** HE staining. Measurement data were presented in the form of mean ± SD. * $p < 0.05$ *vs.* Sham; # $p < 0.05$ *vs.* Model.

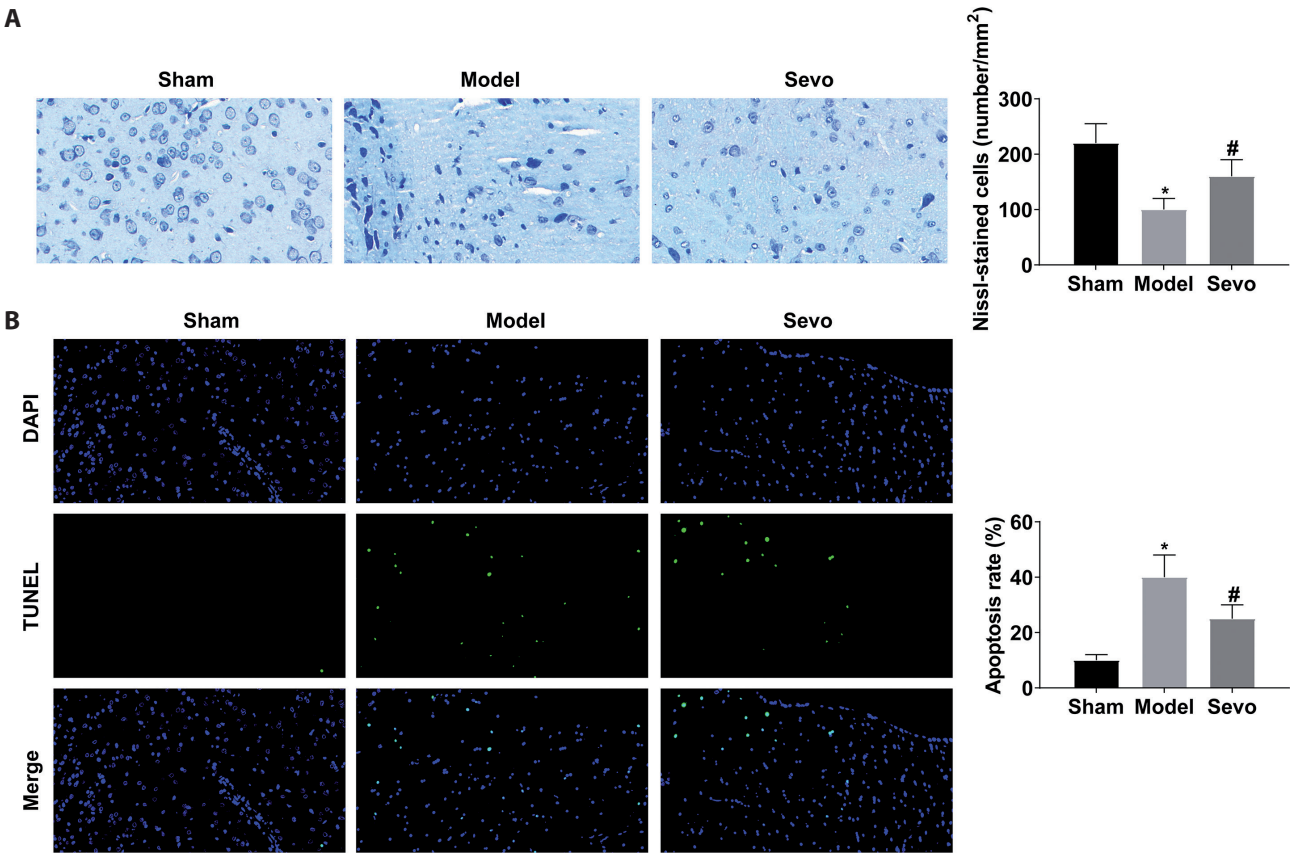

Figure 2. Sevoflurane preconditioning can inhibit neuronal loss and apoptosis MCAO/R rats. **A.** Nissl staining observed neuron loss. **B.** TUNEL staining analyzed neuronal apoptosis. Measurement data were presented in the form of mean ± SD. * $p < 0.05$ *vs.* Sham; # $p < 0.05$ *vs.* Model.

incubated with the secondary antibody for 1 h, developed with enhanced chemiluminescence, and analyzed by ImageJ software (Xiang et al. 2022).

Luciferase reporter assay

Bioinformatics software predicted the target genes of miR-192-5p. DICER1 wild type (WT) and mutant type (MUT) plasmids (Ambio) were constructed and generated. DICER1-WT, DICER1-MUT and miR-192-5p mimic and mimic NC were co-transfected into HEK293T cells. After 24 h, luciferase intensity was measured using a dual luciferase assay System kit (Promega, USA) (Sun et al. 2021).

Statistical methods

All data were statistically analyzed by SPSS 21.0. Measurement data were represented by mean ± standard deviation (SD). Comparison of measurement data subject to normal distribution was conducted by independent sample *t* test. $p < 0.05$ was considered statistically significant.

Resuslts

Sevoflurane preconditioning can alleviate brain tissue injury and neuronal apoptosis in MCAO/R rats

Neurological defect of MCAO/R rats was evaluated by Zea-Longa score. The neurological deficit score of MCAO/R rats was increased, while decreased in those preconditioned with sevoflurane (Fig. 1A). BWC detection revealed severe cerebral edema in MCAO/R rats, which could be improved by sevoflurane preconditioning (Fig. 1B). HE staining revealed significant structural brain damage in ischemic areas with dense, irregularly shaped consolidated nucleus areas in MCAO/R rats, while sevoflurane preconditioning alleviated brain damage (Fig. 1C). Neuronal loss was detected by Nissl staining, which showed increased neuronal loss in the MCAO/R rats, while sevoflurane preconditioning reduced neuronal loss (Fig. 2A). TUNEL staining was employed to detect apoptosis, and TUNEL-positive cells increased in MCAO/R rats, while sevoflurane preconditioning reduced apoptosis (Fig. 2B).

Deficiency of miR-192-5p can further inhibit neuronal apoptosis and alleviate brain tissue damage in MCAO/R rats

miR-192-5p levels were checked in MCAO/R rats by RT-qPCR. miR-192-5p was up-regulated in MCAO/R rats, while sevoflurane preconditioning could reduce miR-192-5p levels (Fig. 3A). Rats pretreated with sevoflurane were injected with miR-192-5p antagomir before MCAO/R to explore the effect of miR-192-5p on the neural function of MCAO/R rats, and the successful injection was verified by RT-qPCR (Fig. 3B). The results noted that MCAO/R rats injected with miR-192-5p antagomir had decreased neurological deficit scores and BWC (Fig. 3C,D), as well as improvements of brain damage (Fig. 3E), neuronal loss (Fig. 4A), and apoptosis (Fig. 4B).

miR-192-5p targets DICER1 negatively

DICER1 expression analysis in MCAO/R rats was performed by PCR and Western blot. DICER1 in MCAO/R rats was downregulated, while sevoflurane preconditioning could increase its expression (Fig. 5A). Biological information website starBase predicted the targeting binding sites of miR-192-5p and DICER1 (Fig. 5B). The results of dual luciferase activity experiment observed that the relative luciferase activity decreased after co-transfection of DICER1-WT and miR-192-5p mimic (Fig. 5C). Then, PCR and Western blot measured elevated DICER1 after down-regulating miR-192-5p (Fig. 6).

Forced expression of DICER1 can further inhibit neuronal apoptosis and relieve brain tissue damage in MCAO/R rats

Sevoflurane pretreated rats were injected with oe-DICER1 or oe-NC before MCAO/R, and the successful injection was verified by PCR and Western blot (Fig. 7A). Moreover, in MCAO/R rats injected with oe-DICER1, neurological deficit scores and BWC were reduced (Fig. 7B,C), brain damage (Fig. 7D), neuronal loss (Fig. 8A), and apoptosis were alleviated (Fig. 8B).

Elevating miR-192-5p or suppressing DICER1 weakens the protective effect of sevoflurane preconditioning on MCAO/R rats

To further determine the protective effect of sevoflurane on CIRI in rats by mediating miR-192-5p/DICER1 axis, rats

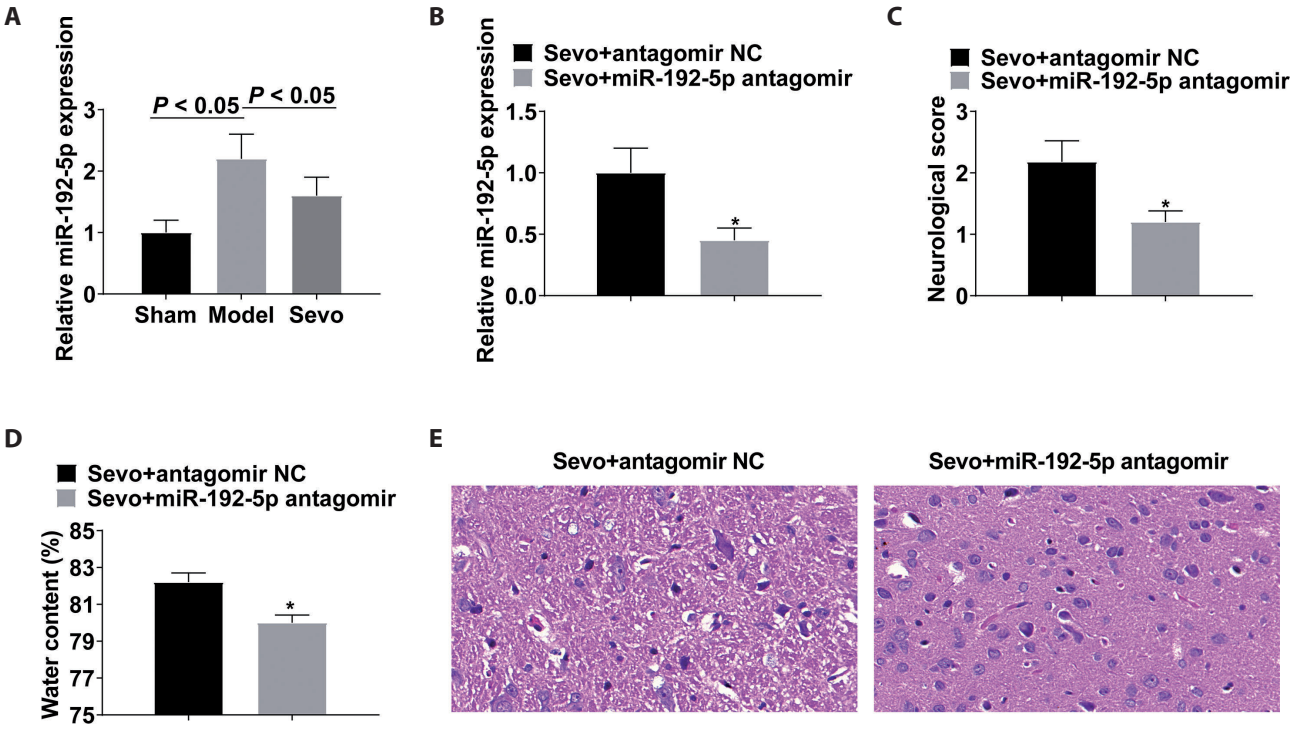

Figure 3. Deficiency of miR-192-5p can further improve neurological function and brain tissue damage in MCAO/R rats. **A.** RT-qPCR measured miR-192-5p in rats. **B.** RT-qPCR verified the successful upregulation of miR-192-5p. **C.** Neurological deficit score of rats. **D.** Brain water content detection. **E.** HE staining. Measurement data were presented in the form of mean ± SD. * $p < 0.05$ *vs.* Sevo+antagomir NC.

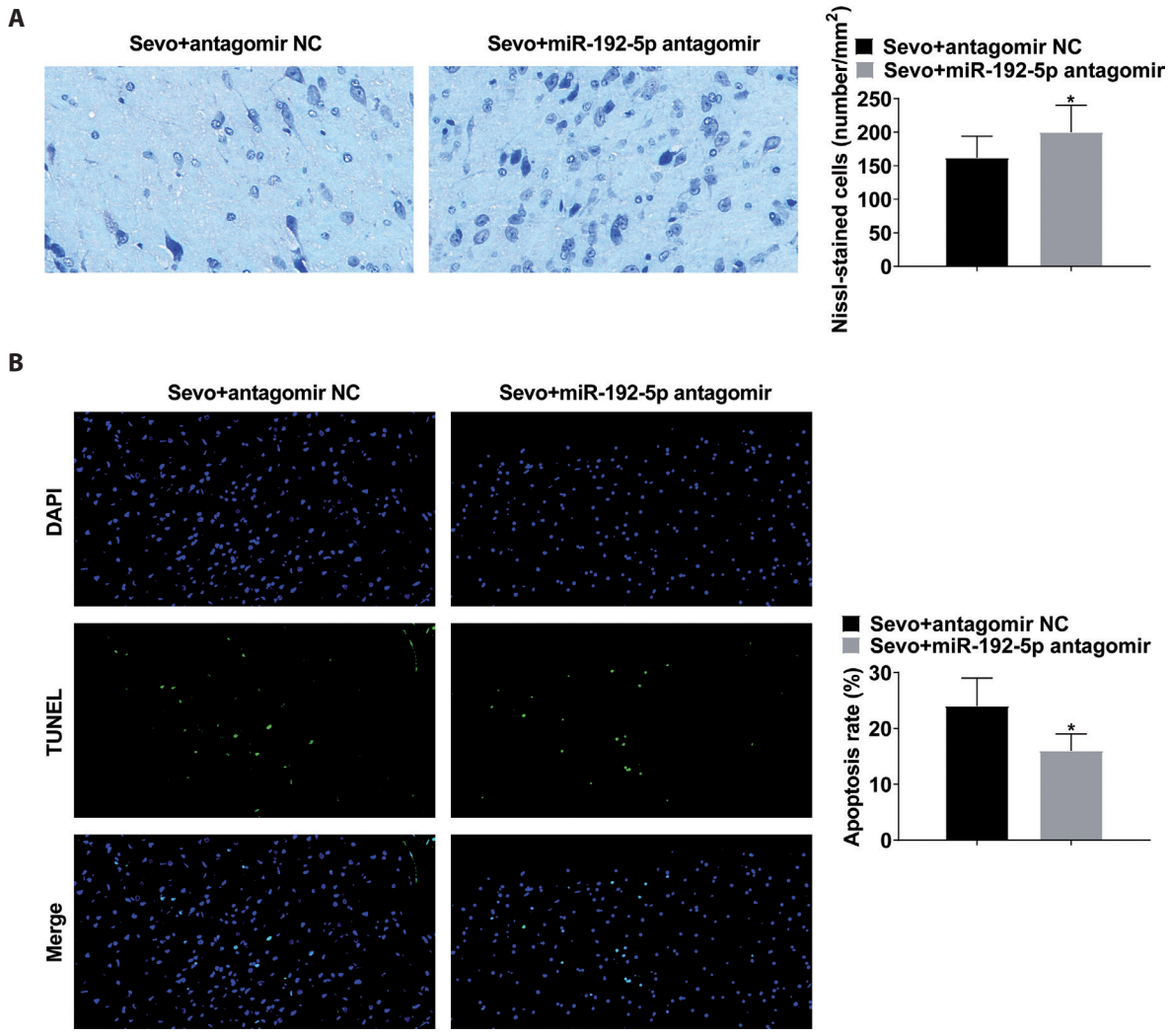

Figure 4. Deficiency of miR-192-5p can further inhibit neuronal loss and apoptosis in MCAO/R rats. **A.** Nissl staining observed neuron loss. **B.** TUNEL staining analyzed neuronal apoptosis. Measurement data were presented in the form of mean ± SD. * $p < 0.05$ *vs.* Sevo+antagomir NC.

pretreated with sevoflurane were injected with miR-192-5p agomir and sh-DICER1 before MCAO/R. RT-qPCR or Western blot verified the successful injection (Fig. 9A). After elevating miR-192-5p or silencing DICER1, neurological deficit scores and BWC were increased (Fig. 9B,C), and brain injury (Fig. 9D), neuronal loss (Fig. 10A), and apoptosis were aggravated (Fig. 10B).

Discussion

CIRI is a complex cascade process, which seriously hinders the rehabilitation of patients with acute ischemic stroke. For the disease, treatment options are currently limited to thrombolytic drugs and thrombus removal. At present, medical gases including volatile anesthetic gases, such as sevoflurane have neuroprotective indications in experimental studies on brain injuries (Wang YZ et al. 2019). The present work mainly explored the protective property of sevoflurane preconditioning in MCAO/R rats and subsequently analyzed its mechanistic action with the involvement of miR-192-5p/DICER1.

Till now, extensive experimental evidences have noted the significance of sevoflurane preconditioning in brain injuries. For instance, Lei and colleagues have noted that sevoflurane reduces neuronal injury in the hippocampal CA1 area of rats with CIRI (Jin and Bo 2021). Additionally, another research group has verified the protective effects of sevoflurane preconditioning on CIRI rats by reducing infarct volume, neurological deficit, and neuronal apoptosis (Zhang et al.

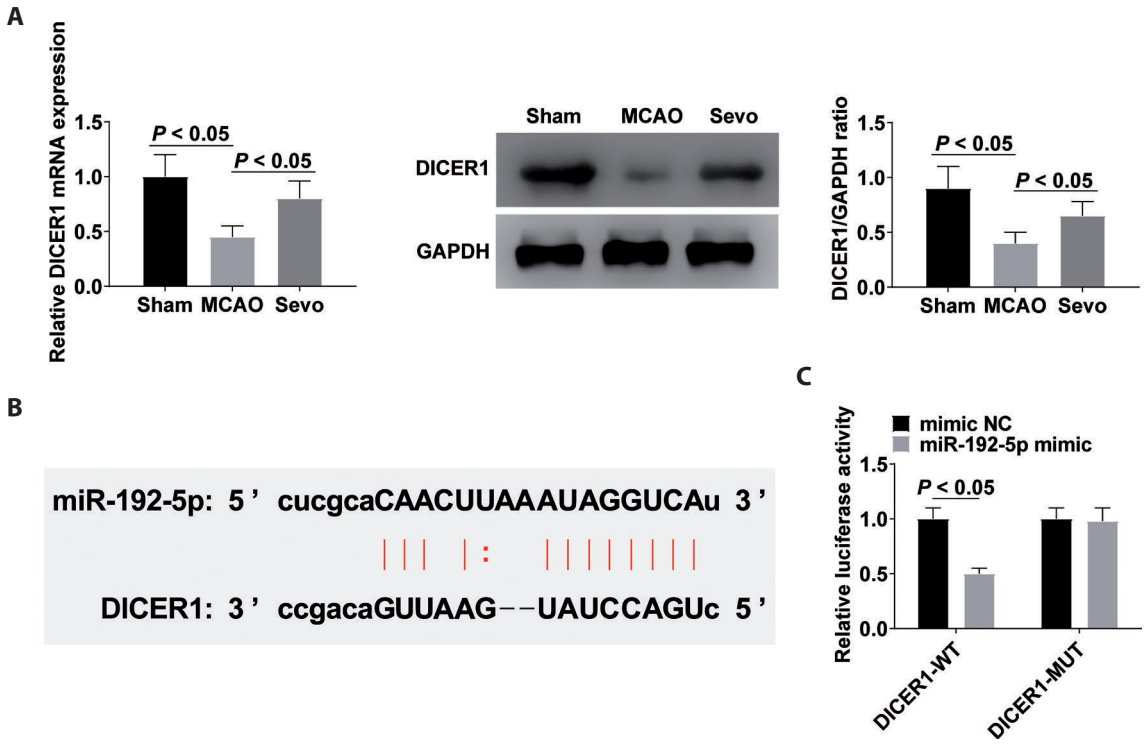

Figure 5. miR-192-5p targets DICER1. **A.** PCR and Western blot analyzed DICER1 in MCAO/R rats. **B.** Bioinformatics websites predicted the binding sites of miR-192-5p and DICER1. **C.** Luciferase reporter gene assay verified the targeting relationship between miR-192-5p and DICER1. Measurement data were presented in the form of mean ± SD.

2019). Also, a similar phenomenon exists in a current study revealing the attenuating effects of sevoflurane to reduce BWC, neurological deficits, and cell apoptosis (Hu et al. 2020). In this work, sevoflurane preconditioning could protect rats from MCAO/R-induced brain injury, as presented in the aspects of neurological deficits, BWC, as well as neuronal loss and apoptosis. Not only is sevoflurane preconditioning explored in CIRI treatment, but also sevoflurane postconditioning. For example, research has observed that neurological deficits, neuropathic damage, and inflammatory response in CIRI

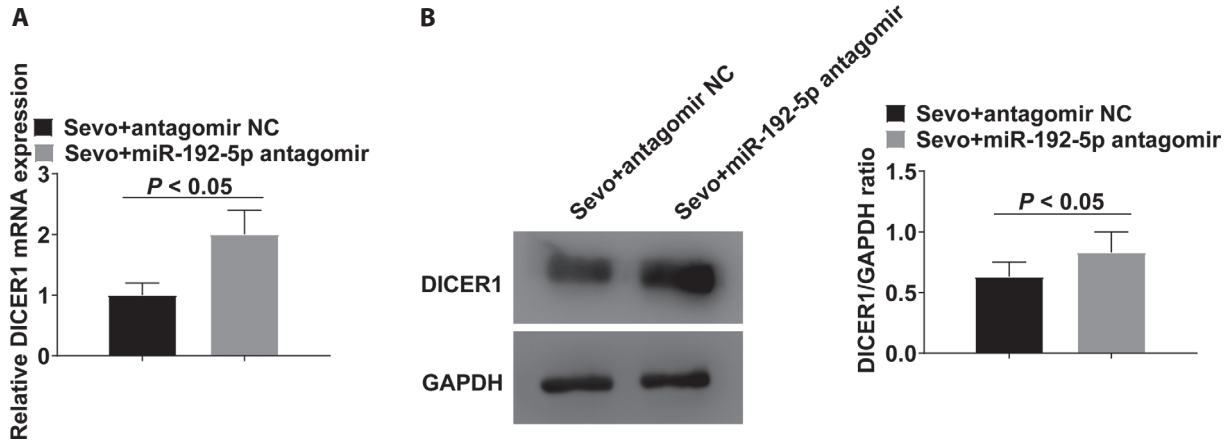

Figure 6. miR-192-5p negatively regulates DICER1 expression. PCR **(A)** and Western blot **(B)** analyzed DICER1. Measurement data were presented in the form of mean ± SD.

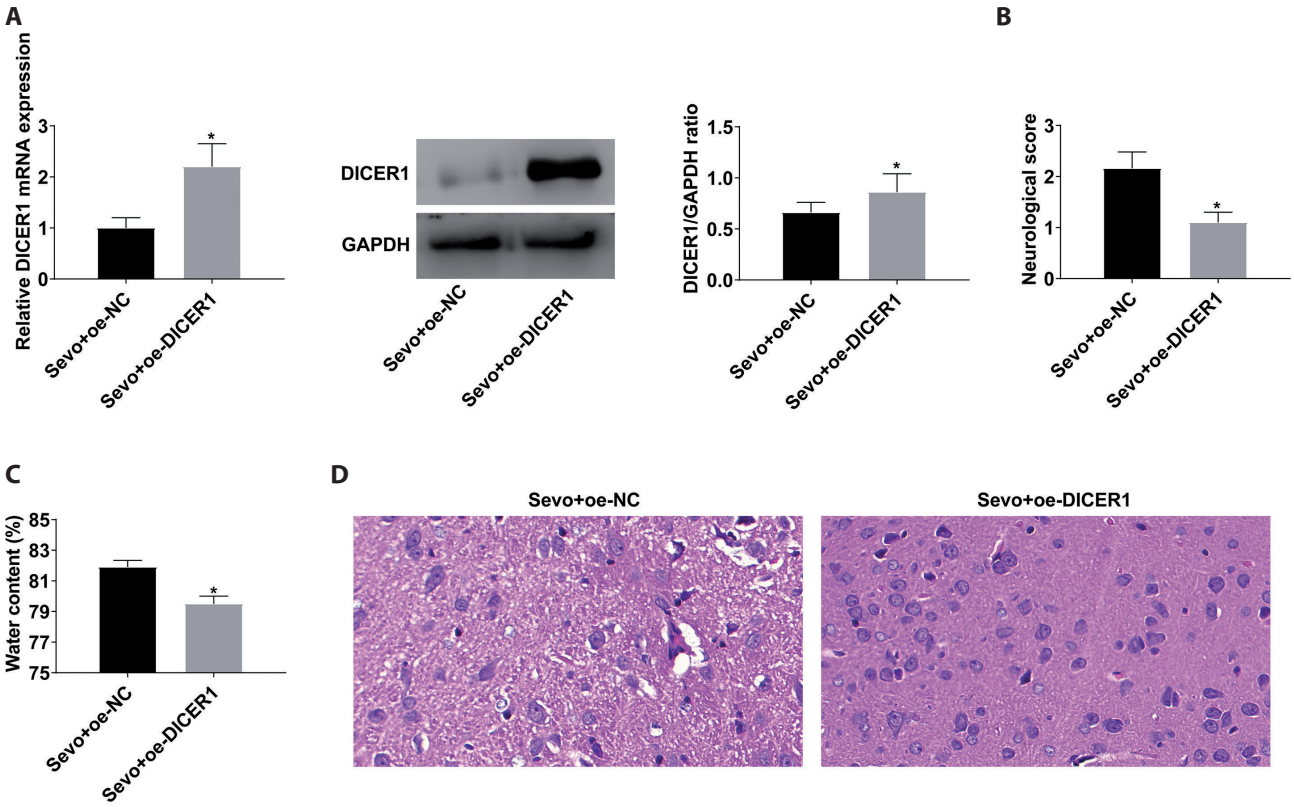

Figure 7. Forced expression of DICER1 can further improve neurological function and brain tissue damage in MCAO/R rats. **A.** PCR and Western blot verified the successful injection. **B.** Neurological deficit score of rats. **C.** Brain water content detection. **D.** HE staining. Measurement data were presented in the form of mean ± SD. * $p < 0.05$ *vs.* Sevo+oe-NC.

animals could be less severe after sevoflurane postconditioning (Zhao et al. 2022). Furthermore, sevoflurane postconditioning could attenuate learning and memory dysfunction following CIRI, narrow cerebral infarction area, and decrease neuronal apoptosis and autophagy in rats receiving sevoflurane administration (Shi et al. 2020).

Increasing studies have observed and ensured miRNA regulation during sevoflurane treating CIRI, such as miR-30c-5p (Su et al. 2021) and miR-203 (Zhong et al. 2020). This research clarified miR-192-5p overexpression in MCAO/R rats' brain tissues and further measured sevoflurane-mediated downregulation of miR-192-5p. As to functions, miR-192-5p deficiency befits the profound alleviation of brain injuries based on sevoflurane preconditioning. Consistently, miR-192-5p expression is elevated in renal IRI and is of possibility to be a diagnostic marker in the disease (Zou et al. 2017). Similarly, in the course of hepatic I/R, serum miR-192-5p expression is promoted in the course of hepatic IR, and silencing miR-192-5p reduces hepatic injury through mediating oxidative stress reaction (Roy et al. 2016). In a cardiomyocyte model of myocardial IRI, miR-192-5p downregulation suppresses cellular apoptosis under hypoxia/reoxygenation conditions (Zhang et al. 2017). Interestingly, miR-192-5p is upregulated in hypoxic-ischemic brain damage, and loss of miR-192-5p is conducive to neurobehavioral recovery and neuronal protection (Yan et al. 2022). Besides, it is clarified that inhibiting miR-192-5p induces sensory function recovery and reduces neuronal apoptosis in rats with left sciatic nerve injury (Expression of Concern: microRNA-192-5p is involved in nerve repair in rats with peripheral nerve injury by regulating XIAP 2023).

When it comes to the target of miR-192-5p, DICER1 was further analyzed and evaluated. It has been addressed that DICER1 expression drops in the ischemic boundary zone after permanent MCAO/R and it is mediated by miR-107 to modulate post-stroke angiogenesis (Li et al. 2015). However, no further reports have convinced DICER1's action in MCAO/R. In this research, DICER1 expression was suppressed in MCAO/R rats and sevoflurane could upregulate it in rats' brain tissues. Surprisingly, forced expression of DICER1 and sevoflurane preconditioning synergistically contributed to neuroprotection for MCAO/R rats.

To shortly summarize, sevoflurane preconditioning has protective indications in CIRI through silencing miR-192-5p and enhancing DICER1. The conclusion potentially supports the clinical value of sevoflurane in preventing brain injuries. On the whole, the research only investigated sevoflurane in animal experiments, and cell experiments are lacking. On the other hand, whether other miRNAs/mRNAs interacted in sevoflurane treatment of CIRI is waiting for many endeavors.

Conlict of interests. The authors have no conflicts of interest to declare.

Availability of data and materials. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate. All animal experiments were complied with the ARRIVE guidelines and performed in accordance with the National Institutes of Health Guide for

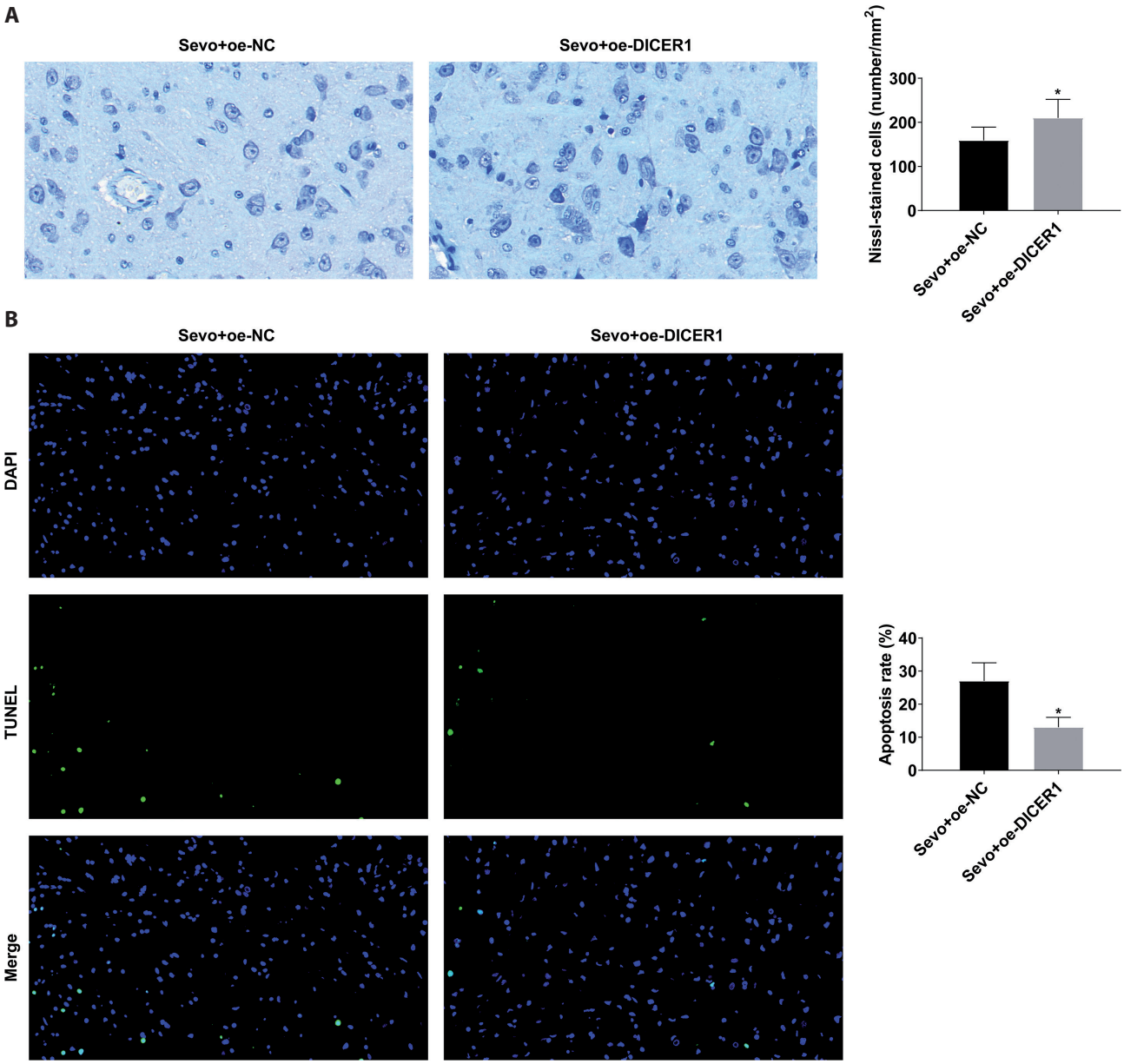

Figure 8. Forced expression of DICER1 can further inhibit neuronal loss and apoptosis in MCAO/R rats. **A.** Nissl staining observed neuron loss. **B.** TUNEL staining analyzed neuronal apoptosis. Measurement data were presented in the form of mean ± SD. * $p < 0.05$ *vs.* Sevo+oe-NC.

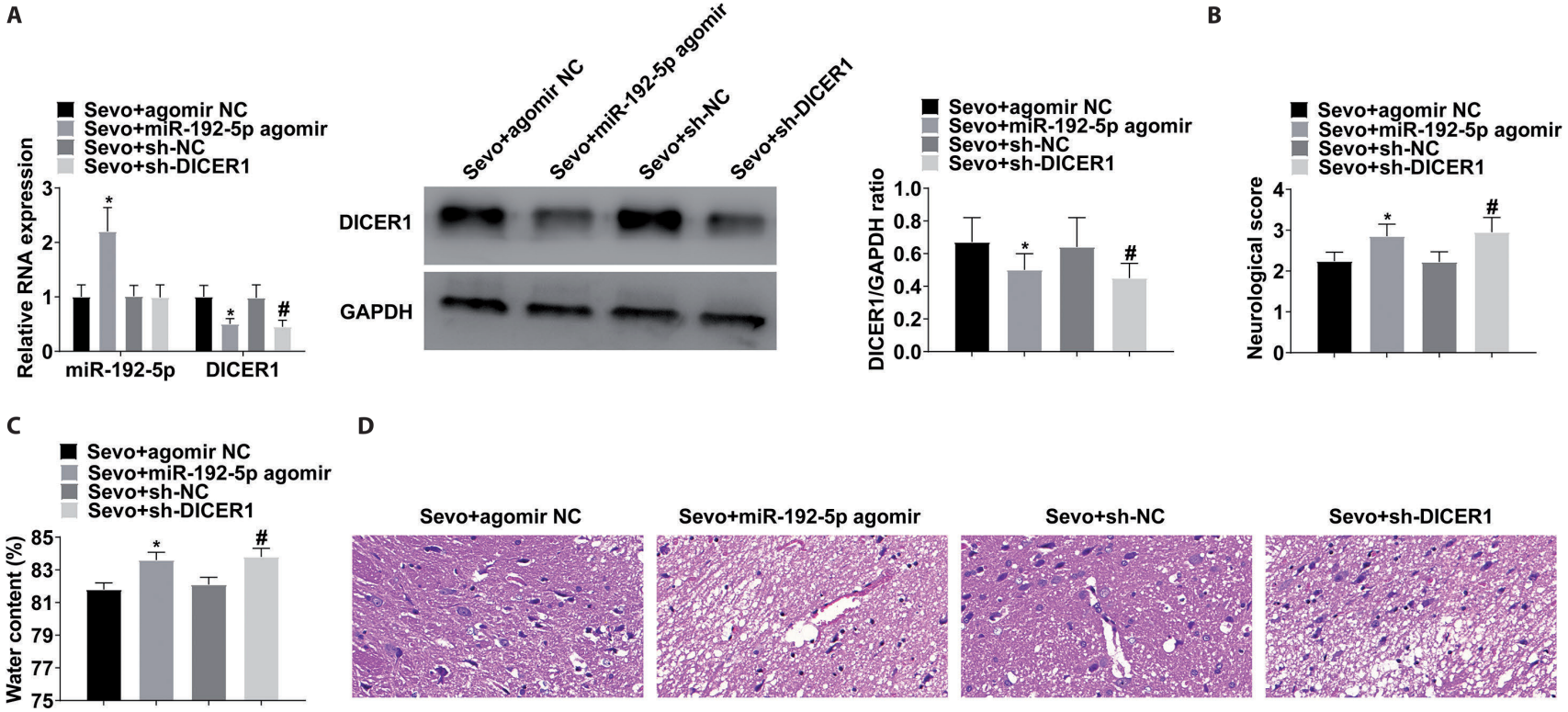

Figure 9. Elevating miR-192-5p or suppressing DICER1 weakens the protective effect of sevoflurane preconditioning on MCAO/R rats. **A.** PCR and Western blot verified the successful injection. **B.** Neurological deficit score of rats. **C.** Brain water content detection. **D.** HE staining. Measurement data were presented in the form of mean ± SD. * $p < 0.05$ *vs.* Sevo+agomir NC; # $p < 0.05$ *vs.* Sevo+sh-NC.

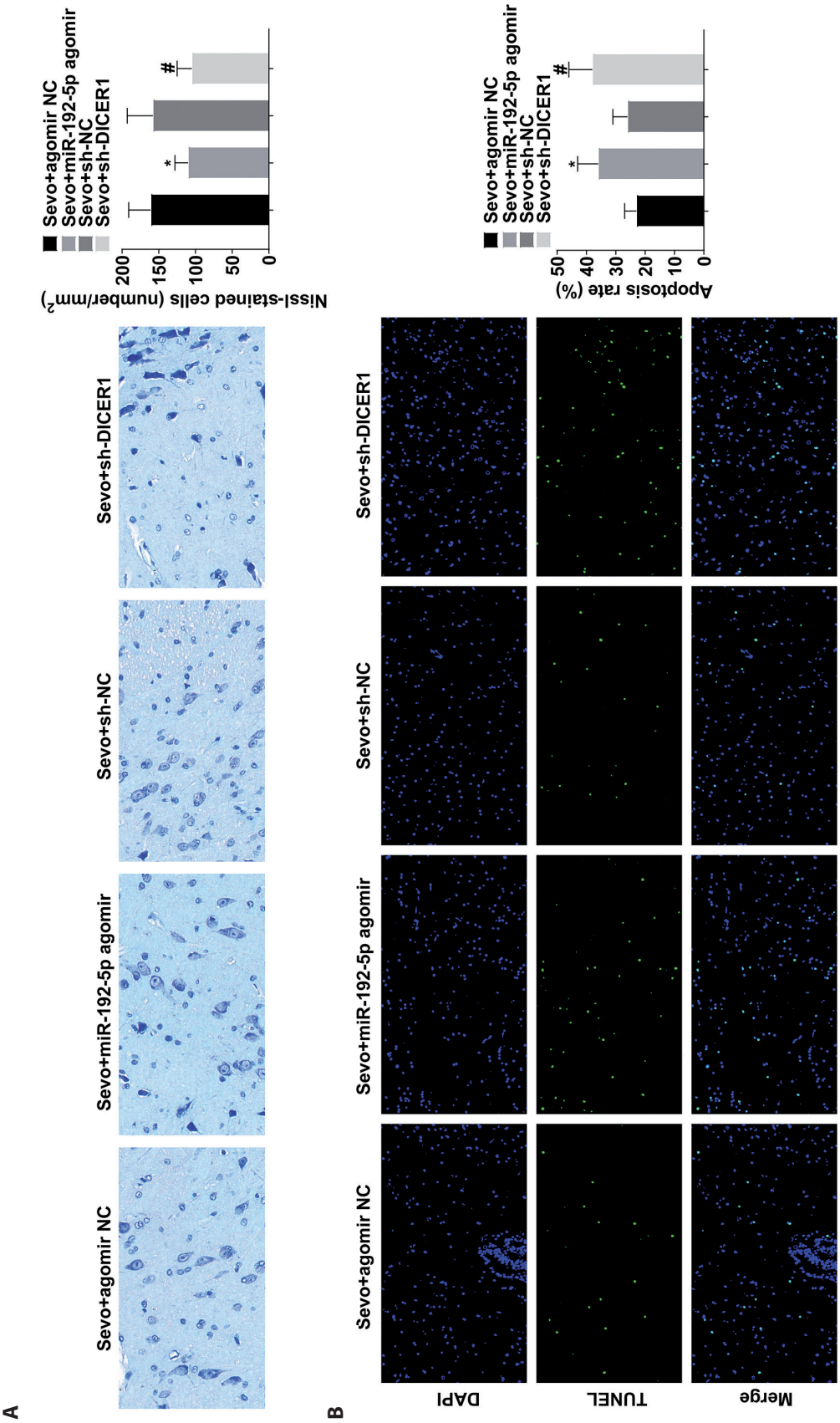

Figure 10. Elevating miR-192-5p or suppressing DICER1 weakens the protective effect of sevoflurane preconditioning on MCAO/R rats. **A.** Nissl staining observed neuron loss. **B.** TUNEL staining analyzed neuronal apoptosis. Measurement data were presented in the form of mean ± SD. * $p < 0.05$ *vs.* Sevo+agomir NC; $^{\#}$ $p < 0.05$ *vs.* Sevo+sh-NC.

the Care and Use of Laboratory Animals. The experiments were approved by the Institutional Animal Care and Use Committee of The Fourth Hospital of Changsha (No. 20190261LS).

Authors' contributions. BY designed the study and the experiments. BY and YF contributed to the conception of the study. QZ and WX were responsible for data collection. JO and JH analyzed the data. BY and YF drafted the manuscript. All authors read, critically revised, and approved the final manuscript.

References

Chai Z, Gong J, Zheng P, Zheng J (2020): Inhibition of miR-19a-3p decreases cerebral ischemia/reperfusion injury by targeting IGFBP3 in vivo and in vitro. Biol. Res. **53,** 1-11 https://doi.org/10.1186/s40659-020-00280-9

Correia de Sousa M, Gjorgjieva M, Dolicka D, Sobolewski C, Foti M (2019): Deciphering miRNAs' action through miRNA editing. Int. J. Mol. Sci. **20,** 6249 https://doi.org/10.3390/ijms20246249

Expression of Concern: microRNA-192-5p is involved in nerve repair in rats with peripheral nerve injury by regulating XIAP (2023): Cell Cycle **22,** 148 https://doi.org/10.1080/15384101.2022.2151240

Ghafouri-Fard S, Shoorei H, Taheri M (2020): Non-coding RNAs participate in the ischemia-reperfusion injury. Biomed. Pharmacother. **129,** 110419 https://doi.org/10.1016/j.biopha.2020.110419

He XW, Shi YH, Liu YS, Li GF, Zhao R, Hu Y, Lin CC, Zhuang MT, Su JJ, Liu JR (2019): Increased plasma levels of miR-124-3p, miR-125b-5p and miR-192-5p are associated with outcomes in acute ischaemic stroke patients receiving thrombolysis. Atherosclerosis **289,** 36-43 https://doi.org/10.1016/j.atherosclerosis.2019.08.002

Hu CY, Guo YQ, Hao YH, Zheng LN, Qi YH (2020): Research on mechanism of sevoflurane in alleviating cerebral ischemia-reperfusion injury in rats through JNK signaling pathway. Eur. Rev. Med. Pharmacol. Sci. **24,** 3907-3914

Huang X, Ying J, Yang D, Fang P, Wang X, Zhou B, Zhang L, Fang Y, Yu W, Liu X, et al. (2021): The mechanisms of sevoflurane-induced neuroinflammation. Front. Aging Neurosci. **13,** 717745 https://doi.org/10.3389/fnagi.2021.717745

Jin L, Bo XM (2021): Neuroprotection of sevoflurane against ischemia/reperfusion-induced brain injury through inhibiting GluN2A/GluN2B-PSD-95-MLK3 module. Exp. Brain Res. **239,** 2701-2709 https://doi.org/10.1007/s00221-021-06157-x

Kang C, Wang L, Kang M, Liu X, Fu Y, Gao J (2019): Baicalin alleviates 6-hydroxydopamine-induced neurotoxicity in PC12 cells by down-regulation of microRNA-192-5p. Brain Res. **1708,** 84-92 https://doi.org/10.1016/j.brainres.2018.12.015

Li J, Peng L, Bai W, Peng P, Chen W, Yang W, Shao J (2021): Biliverdin protects against cerebral ischemia/reperfusion injury by regulating the miR-27a-3p/Rgs1 axis. Neuropsychiatr. Dis. Treat. **17,** 1165-1181 https://doi.org/10.2147/NDT.S300773

Li M, Tang H, Li Z, Tang W (2022): Emerging treatment strategies for cerebral ischemia-reperfusion injury. Neuroscience **507,** 112-124 https://doi.org/10.1016/j.neuroscience.2022.10.020

Li Y, Mao L, Gao Y, Baral S, Zhou Y, Hu B (2015): MicroRNA-107 contributes to post-stroke angiogenesis by targeting Dicer-1. Sci. Rep. **5,** 13316 https://doi.org/10.1038/srep13316

Liang TY, Peng SY, Ma M, Li HY, Wang Z, Chen G (2021): Protective effects of sevoflurane in cerebral ischemia reperfusion injury: a narrative review. Med. Gas. Res. **11,** 152-154 https://doi.org/10.4103/2045-9912.318860

Luo L, Liu M, Fan Y, Zhang J, Liu L, Li Y, Zhang Q, Xie H, Jiang C, Wu J, et al. (2022): Intermittent theta-burst stimulation improves motor function by inhibiting neuronal pyroptosis and regulating microglial polarization via TLR4/NFkappaB/NLRP3 signaling pathway in cerebral ischemic mice. J. Neuroinflammation **19,** 141 https://doi.org/10.1186/s12974-022-02501-2

Neag MA, Mitre AO, Catinean A, Mitre CI (2020): An overview on the mechanisms of neuroprotection and neurotoxicity of isoflurane and sevoflurane in experimental studies. Brain Res. Bull. **165,** 281-289 https://doi.org/10.1016/j.brainresbull.2020.10.011

Roy S, Benz F, Alder J, Bantel H, Janssen J, Vucur M, Gautheron J, Schneider A, Schuller F, Loosen S, et al. (2016): Down-regulation of miR-192-5p protects from oxidative stress-induced acute liver injury. Clin. Sci. (Lond) **130,** 1197-1207 https://doi.org/10.1042/CS20160216

Shi CX, Jin J, Wang XQ, Song T, Li GH, Li KZ, Ma JH (2020): Sevoflurane attenuates brain damage through inhibiting autophagy and apoptosis in cerebral ischemia-reperfusion rats. Mol. Med. Rep. **21,** 123-130 https://doi.org/10.3892/mmr.2019.10832

Su G, Qu Y, Li G, Deng M (2021): Sevoflurane protects against cerebral ischemia/reperfusion injury via microrna-30c-5p modulating homeodomain-interacting protein kinase 1. Bioengineered **12,** 11858-11871 https://doi.org/10.1080/21655979.2021.1999551

Sun L, Ji D, Zhi F, Fang Y, Zhu Z, Ni T, Zhu Q, Bao J (2022): MiR-494-3p upregulation exacerbates cerebral ischemia injury by targeting Bhlhe40. Yonsei Med. J. **63,** 389-398 https://doi.org/10.3349/ymj.2022.63.4.389

Sun X, Wang L, Huang X, Zhou S, Jiang T (2021): Regulatory mechanism miR-302a-3p/E2F1/SNHG3 axis in nerve repair post cerebral ischemic stroke. Curr. Neurovasc. Res. **18,** 515-524 https://doi.org/10.2174/1567202618666211210155715

Wang F, Li R, Tu P, Chen J, Zeng K, Jiang Y (2020): Total glycosides of cistanche deserticola promote neurological function recovery by inducing neurovascular regeneration via Nrf-2/Keap-1 pathway in MCAO/R rats. Front. Pharmacol. **11,** 236 https://doi.org/10.3389/fphar.2020.00236

Wang FJ, Wang SX, Chai LJ, Zhang Y, Guo H, Hu LM (2018): Xueshuantong injection (lyophilized) combined with salvianolate lyophilized injection protects against focal cerebral ischemia/reperfusion injury in rats through attenuation of oxidative stress. Acta Pharmacol. Sin. **39,** 998-1011

https://doi.org/10.1038/aps.2017.128
Wang YZ, Li TT, Cao HL, Yang WC (2019): Recent advances in the neuroprotective effects of medical gases. Med. Gas. Res. **9,** 80-87
https://doi.org/10.4103/2045-9912.260649
Xiang P, Hu J, Wang H, Luo Y, Gu C, Tan X, Tu Y, Guo W, Chen L, Gao L, et al. (2022): miR-204-5p is sponged by TUG1 to aggravate neuron damage induced by focal cerebral ischemia and reperfusion injury through upregulating COX2. Cell Death Discov. **8,** 89
https://doi.org/10.1038/s41420-022-00885-x
Yan G, Tao Z, Xing X, Zhou Z, Wang X, Li X, Li F (2022): Down-regulated microRNA-192-5p protects against hypoxic-ischemic brain damage via regulation of YAP1-mediated hippo signaling pathway. Neurochem. Res. **47,** 1243-1254
https://doi.org/10.1007/s11064-021-03518-4
Yang D, Tan Y, Li H, Zhang X, Li X, Zhou F (2021): Upregulation of miR-20b protects against cerebral ischemic stroke by targeting thioredoxin interacting protein (TXNIP). Exp. Neurobiol. **30,** 170-182
https://doi.org/10.5607/en20046
Yang H, Qi C, Su F, Shan W, Guo A, Wu J, Wang Y, You H, Wang Q (2022): Cerebral ischemia/reperfusion injury and pharmacologic preconditioning as a means to reduce stroke-induced inflammation and damage. Neurochem. Res. **47,** 3598-3614
https://doi.org/10.1007/s11064-022-03789-5
Yang K, Zeng L, Ge A, Wang S, Zeng J, Yuan X, Mei Z, Wang G, Ge J (2022): A systematic review of the research progress of non-coding RNA in neuroinflammation and immune regulation in cerebral infarction/ischemia-reperfusion injury. Front. Immunol. **13,** 930171
https://doi.org/10.3389/fimmu.2022.930171
Zhang Y, Huang R, Zhou W, Zhao Q, Lu Z (2017): miR-192-5p mediates hypoxia/reoxygenation-induced apoptosis in H9c2 cardiomyocytes via targeting of FABP3. J. Biochem. Mol. Toxicol. **31,** 1-6
https://doi.org/10.1002/jbt.21873
Zhang Y, Shan Z, Zhao Y, Ai Y (2019): Sevoflurane prevents miR-181a-induced cerebral ischemia/reperfusion injury. Chem. Biol. Interact. **308,** 332-338
https://doi.org/10.1016/j.cbi.2019.06.008
Zhao Z, Li Y, Chi F, Ma L, Li Y, Hou Z, Wang Q (2022): Sevoflurane postconditioning ameliorates cerebral ischemia-reperfusion injury in rats via TLR4/MyD88/TRAF6 signaling pathway. Aging (Albany NY) **14,** 10153-10170
https://doi.org/10.18632/aging.204461
Zhong H, Chen H, Gu C (2020): Sevoflurane post-treatment up-regulated miR-203 expression to attenuate cerebral ischemia-reperfusion-induced neuroinflammation by targeting MyD88. Inflammation **43,** 651-663
https://doi.org/10.1007/s10753-019-01147-2
Zou YF, Wen D, Zhao Q, Shen PY, Shi H, Zhao Q, Chen YX, Zhang W (2017): Urinary microRNA-30c-5p and microRNA-192-5p as potential biomarkers of ischemia-reperfusion-induced kidney injury. Exp. Biol. Med. (Maywood) **242,** 657-667
https://doi.org/10.1177/1535370216685005

Received: November 13, 2023
Final version accepted: July 22, 2024