ORIGINAL RESEARCH

# Neuroprotective Effect of Dexmedetomidine on Cerebral Ischemia-Reperfusion Injury in Rats

Jianhong Lyu, MMed; Yang Zhou, BMed; Min Zhang, BMed; Lingling Tang, MMed; Xudan Bao, MMed; Zaijiao Sun, MMed; Li Huang, MMed; Liqin Zhao, MMed

## ABSTRACT

**Background** • The number of patients having ischemic stroke is increasing year on year. The anesthetic adjuvant dexmedetomidine is neuroprotective in rats and has potential for use in the treatment of ischemic stroke.

**Objective** • The neuroprotective mechanism of dexmedetomidine in cerebral ischemia-reperfusion injury was studied in relation to its regulation of the oxidative stress response, astrocyte response, microglia overactivation, and apoptosis-related protein expression.

**Methods** • We randomly and equally divided 25 male Sprague-Dawley rats into 5 groups: a sham-operation group, an ischemia-reperfusion injury group, and low-, medium-, and high-dose dexmedetomidine groups. A rat model of focal cerebral ischemia-reperfusion injury was established by embolization of the right middle cerebral artery for 60 minutes and reperfusion for 2 hours. The volume of cerebral infarction was calculated by triphenyl tetrazolium chloride staining. The protein expression levels of caspase-3, methionyl aminopeptidase 2 (MetAP2 or MAP2), glial fibrillary acidic protein, and allograft inflammatory factor 1 (AIF-1) in the cerebral cortex were determined by Western blot and immunohistochemistry.

**Results** • The volume of cerebral infarction in rats decreased with increasing dose of dexmedetomidine ($P = .039$, 95% CI = .027 to .044). The expression levels of caspase-3, glial fibrillary acidic protein, and allograft inflammatory factor 1 and the amount of 4-hydroxynonenal decreased with increasing doses of dexmedetomidine ($P = .033$, 95% CI = .021 to .037). Methionyl aminopeptidase 2 (MetAP2 or MAP2) expression increased with increasing doses of dexmedetomidine ($P = .023$, 95% CI = .011 to .028).

**Conclusion** • Dexmedetomidine has a dose-dependent protective effect on cerebral ischemic injury in rats. The neuroprotective effects of dexmedetomidine are achieved, in part, by reducing the oxidative stress response, inhibiting glial overactivation, and inhibiting expression levels of apoptosis-related proteins. (*Altern Ther Health Med.* 2023;29(6):164-169).

**Jianhong Lyu**, MMed; **Lingling Tang**, MMed; **Xudan Bao**, MMed; **Zaijiao Sun**, MMed; **Li Huang**, MMed; Department of Anesthesiology, The Third People's Hospital of Yunnan Province, Kunming, China; **Yang Zhou**, BMed; Department of ENT, The Third People's Hospital of Yunnan Province, Kunming, China; **Min Zhang**, BMed; Department of Anesthesiology, The Third People's Hospital of Yunnan Province, Kunming, China; **Liqin Zhao**, MMed; Department of Pharmacology, Yunnan University of Business Management, Kunming, China.

*Corresponding author: Liqin Zhao, MMed*
*E-mail: zhaoliqinkm@163.com*

## INTRODUCTION

The number of patients having stroke is increasing year on year.[1] China, with a population of 1.413 billion, has a growing number of stroke cases.[2] This disease not only causes damage to the patient's body but also brings heavy burden to the family and society.[3] The main intentions of treatment of ischemic stroke are to relieve ischemic obstruction as soon as possible and to restore or improve the blood supply to ischemic brain tissue.[4] Blockage of blood flow by ischemic stroke leads to poor blood supply to the surrounding tissue of brain, and the ischemic brain tissue produces pathological reactions. Cerebral ischemia-reperfusion injury is mainly related to oxidative stress response,[5-7] inflammatory response,[8,9] calcium overload,[10,11] brain edema, and apoptosis.[12] Most of the studies on the neuroprotective effects of neuroprotective drugs have focused on the mechanism of ischemia-reperfusion injury.

Dexmedetomidine is an α2 receptor agonist that can produce sedative, analgesic, diuretic, and antianxiety effects and has central antisympathetic effects.[13] It is widely used in the clinic and is a commonly used anesthetic adjuvant. Dexmedetomidine has a protective effect on brain,[14] heart,[15] kidney,[16] liver,[17] and digestive tract[18] organs.[19] Most of these

studies focused on a single cause of ischemic injury, so it is difficult to obtain a comprehensive understanding of the neuroprotective effects of dexmedetomidine.

In this study, the neuroprotective mechanism of dexmedetomidine in cerebral ischemia-reperfusion injury was studied from several aspects, including whether dexmedetomidine inhibited the oxidative stress response, astrocyte response, microglia overactivation, and apoptosis-related protein expression to better understand the neuroprotective effect of dexmedetomidine.

## METHODS

### Ethics approval

This study was approved by the Ethics Committee of the Third People's Hospital of Yunnan Province (approval number 2022-181-054). All animal experiments conducted for the study met the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### Rat grouping and treatment

We obtained 25 male Sprague-Dawley rats (220-300 g) from the Department of Experimental Animal Management, Kunming Medical University, China. The rats were maintained under a 12-hour light/dark cycle at 22 ± 2°C. After 1 week of adaptive feeding, the rats were randomly and equally divided into 5 groups: a sham-operation group (sham group), an ischemia-reperfusion group, a low-dose dexmedetomidine (L-Dex) group, a medium-dose dexmedetomidine (M-Dex) group, and a high-dose dexmedetomidine (H-Dex) group. The L-Dex, M-Dex, and H-Dex groups were treated with different doses of dexmedetomidine in accordance with expert consensus.[20] All other conditions of the rats remained consistent.

### Preparation of the cerebral ischemia-reperfusion injury rat model and treatment

All rats were fasted for 12 hours before surgery and were free to eat and drink after surgery. The rats were anesthetized by intraperitoneal injection of 0.3 mL/100 g 10% chloral hydrate (batch number 20200712; Qujing First People's Hospital, Yunnan Province, China). After anesthesia, the neck skin of the rats in the sham group was incised to expose the right common carotid artery, internal carotid artery, and external carotid artery, and the incision was closed after 0.5 hours. For the rats in the ischemia-reperfusion group, the neck skin was incised after anesthesia, and the distal end of the right common carotid artery was incised. A spherical-in-diameter middle cerebral artery occlusion nylon monofilament thread (0.34 ± 0.02 mm; Doccol Corporation) was inserted along the external carotid artery into the internal carotid artery to the beginning of the middle cerebral artery to interrupt the blood flow.[20] After 0.5 hours, the thread was removed, the middle cerebral artery reperfused, and the right common carotid artery and skin were sutured. The rats in the 3 dexmedetomidine groups received this same occlusion treatment, except that immediately after reperfusion, the rats in the dexmedetomidine groups were injected with dexmedetomidine via a femoral vein pump within 0.5 hours (batch number 20200610; Jiangsu Hengrui Pharmaceuticals Co., Ltd.). The doses of dexmedetomidine were 4 μg/kg (L-Dex), 7 μg/kg (M-Dex), or 12 μg/kg (H-Dex). Then, the maintenance dose of dexmedetomidine was injected via a femoral vein pump within 2 hours of the first dose of dexmedetomidine: 5 μg/kg (L-Dex), 7 μg/kg (M-Dex), or 17 μg/kg (H-Dex). The sham and ischemia-reperfusion groups were given the same volume of normal saline via a femoral vein pump as the L-Dex, M-Dex, and H-Dex groups received. All 25 rats were decapitated under chloral hydrate anesthesia 24 hours after reperfusion. The brain tissue was prepared as required for the following experiments.

### Cerebral infarction volume calculation

Triphenyl tetrazolium chloride (TTC) staining is one of the most direct methods of evaluating cerebral infarction. Rat brain tissue was frozen at -20°C for 15 minutes and then placed in a microtome. The brain tissue was cut into 10 slices along the coronal plane from front to back, with each slice being 2-mm wide. The brain tissue slices were placed in a freshly prepared 2% TTC solution and incubated for 30 minutes in a constant-temperature water bath at 37 °C in the dark. Then, the brain slices were fixed in 2% paraformaldehyde.

Each brain slice was photographed with a digital camera. ImageJ software (V 1.4.3.67, National Institutes of Health) was used to determine the infarct area in each image; the infarct volume of each slice was calculated as a cuboid of the infarct area, and the total infarct volume was calculated by adding the infarct volumes of all slices. To do this, the color thresholds of the images were adjusted to calculate the area of each brain slice. Next, the contour of the ischemic area was determined, and the TTC-labeled ischemic area was calculated using the following formula: ischemic area volume ratio = (sum of ischemic areas in each slice)/(sum of brain slice areas in each slice) × 100%.

### Western blot analysis

Half of the ischemic hemispheres of the rat brains were frozen with liquid nitrogen. These brain tissue samples were thawed, crushed, and centrifuged to yield tissue homogenates. The protein concentrations of the homogenates were determined using a BCA protein concentration kit. Homogenates were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using a 12% separation gel and then were transferred to an acetate membrane and sealed in a sealing solution for storage. The acetate membrane was washed before adding primary antibodies against allograft inflammatory factor 1 (Iba1; CST, 1:1000) or microtubule-associated protein 2 (MAP-2; CST, 1:1000); caspase-3 (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., 1:2000), glial fibrillary acidic protein (GFAP; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., 1:1000), or β-actin (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., 1:2000). The membrane with

primary antibody was shaken on a horizontal shaker for 1 hour at room temperature and then shaken overnight at 4 °C. Tris-buffered saline with Tween 20 (0.1%) was used to wash the acetate membrane after primary antibody incubation, and the secondary antibody (goat anti-rabbit or goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase, 1:2000; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) was added for 1 hour at room temperature. The chemiluminescence signal was imaged using a ChemiDoc XRS system (Bio-Rad Laboratories), and protein-band signals were quantified using ImageJ 1.4.3.67 software (National Institutes of Health). β-Actin was used as the internal reference.

### Immunohistochemistry

Ischemic hemispheres of the rat brain tissues were fixed in paraformaldehyde for 24 hours and then embedded in paraffin. The brain tissue was cut to 3 μm in thickness, and the sections on slides were added to 1% gelatin solution, removed, and dried. The slides were baked in a 65°C oven for 2 hours. After hydration in anhydrous ethanol and distilled water, the sections underwent antigen retrieval in a pressure cooker. A primary antibody (1:1000 anti-caspase-3, 1:500 anti-Iba1, 1:50 anti-MAP-2, 1:500 anti-GFAP, or 1:200 anti-4-HNE) was added, and the sections were incubated overnight at 4 °C. The sections were washed in phosphate-buffered saline with Tween 20 (0.1%) and then incubated in secondary antibody (goat anti-rabbit or goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase, 1:2000; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) for 40 minutes. The sections were stained with hematoxylin and eosin using a regressive staining method, dehydrated, and imaged using the color pathology report analysis system HPIAS (Wuhan Qianting Science and Technology Co.). The mean gray values of caspase-3, Iba1, MAP-2, GFAP, and 4-HNE antibody staining were determined by ImageJ 1.4.3.67 software. The mean gray level correlates with the protein expression level.

### Statistical analysis

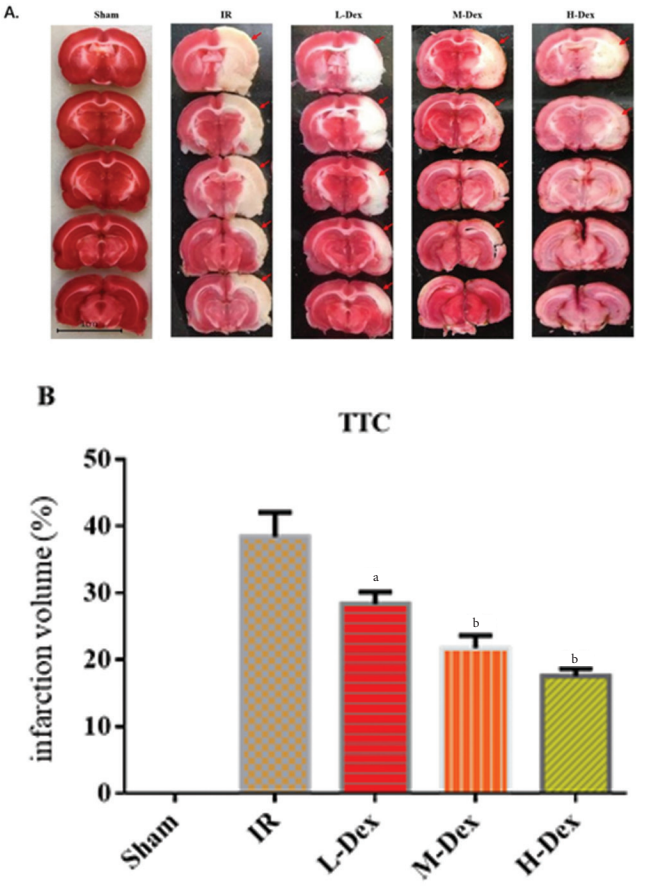
Data are expressed as mean ± SD. SPSS 18.0 (SPSS Inc.) was used for statistical analysis. The experimental data were analyzed by single-factor analysis of variance. $P < .05$ indicates a statistically significant difference.

## RESULTS

### Cerebral infarction volume assessment

TTC staining allowed the visualization of ischemic brain tissue (Figure 1A). Compared with the sham group, the ischemia-reperfusion, L-Dex, M-Dex, and H-Dex groups had obvious cerebral ischemia, and the differences were statistically significant ($P = .044$, 95% CI= .039 to .048). The cerebral infarction volume in the L-Dex, M-Dex, and H-Dex groups was smaller than in the ischemia-reperfusion group, and the differences were statistically significant ($P = .032$, 95% CI = .026 to .039). Among the 3 dexmedetomidine-treated groups, cerebral infarction volume decreased with increasing dexmedetomidine dose (Figure 1B).

**Figure 1.** Assessment of Cerebral Infarction Volume in 25 Rats. A, TTC staining of brain slices of the 5 groups of rats (n = 5 per group). Red tissue indicates TTC staining; white tissue and red arrows indicate ischemic tissue. B, The percentage of cerebral infarction volume in the rats.




[a]$P < .05$
[b]$P < .01$, vs. IR group

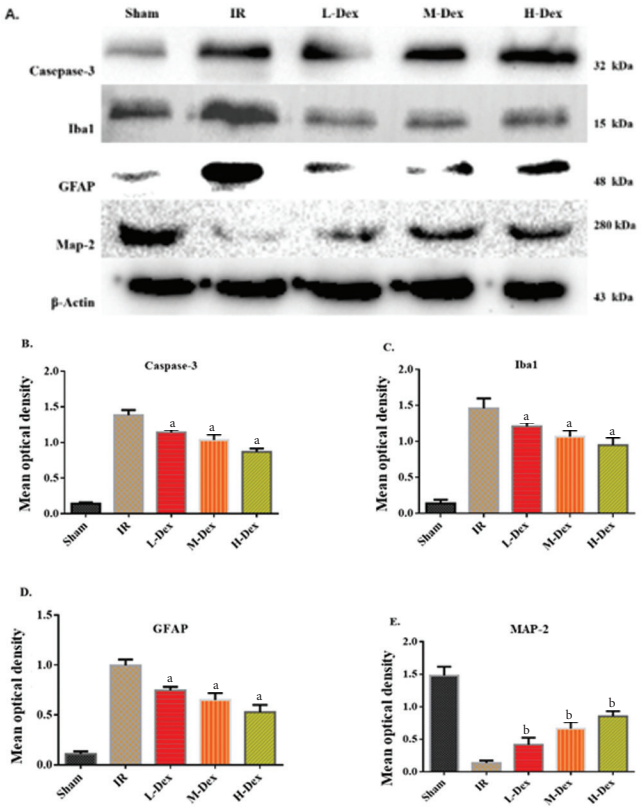
Note: Scale bar = 1 cm. Error bars are mean ± SD.

**Abbreviations**: H-Dex, high-dose dexmedetomidine; IR, ischemia-reperfusion; L-Dex, low-dose dexmedetomidine; M-Dex, medium-dose dexmedetomidine; TTC, triphenyl tetrazolium chloride.

### Western blotting results

The protein expression levels of caspase-3, Iba1, GFAP, and MAP-2 were quantified in the brain tissue of the 5 groups of rats (n = 5 per group) by analysis of Western blot results. The expression of MAP-2 was lower in the 3 dexmedetomidine-treated groups and the ischemia-reperfusion group than in the sham group ($P = .006$, 95% CI = .003 to .009); the expression of caspase-3, GFAP, and Iba1 was higher in the 3 dexmedetomidine-treated groups and the ischemia-reperfusion group than in the sham group ($P = .036$, 95% CI = .033 to .041); the expression of MAP-2 was higher in the 3 dexmedetomidine-treated groups than in the ischemia-

**Figure 2.** Western Blot Results of the Expression of Caspase-3, Iba1, GFAP, and MAP-2 in the Brain Tissues of Rats With Ischemia-Reperfusion Injury With Different Doses of Dexmedetomidine A, Expression of caspase-3, Iba1, GFAP, and MAP-2 by Western blotting. Quantification of the Western blotting results for (B) caspase-3, (C) Iba1, (D) GFAP, and (E) MAP-2.




[a]$P < .05$,
[b]$P < .01$, vs. IR group

Note: Error bars are mean ± SD.

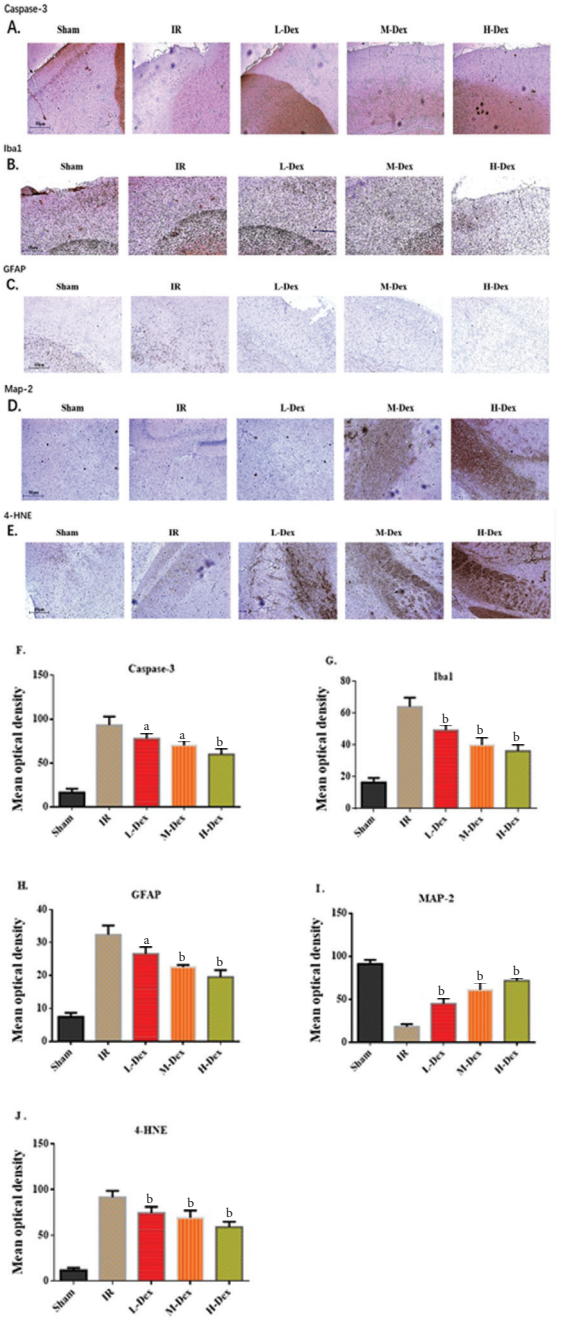
**Abbreviations**: H-Dex, high-dose dexmedetomidine; IR, ischemia-reperfusion; L-Dex, low-dose dexmedetomidine; M-Dex, medium-dose dexmedetomidine; TTC, triphenyl tetrazolium chloride.

reperfusion group; and the expression of caspase-3, GFAP, and Iba1 was lower in the 3 dexmedetomidine-treated groups than in the ischemia-reperfusion group ($P = .025$, 95% CI= .018 to .031) (Figure 2A-E).

### Immunohistochemistry results

The protein expression levels of caspase-3, Iba1, GFAP, and MAP-2 and the amount of 4-HNE in the brain tissues of the 5 groups of rats (n = 5 per group) were visualized by immunohistochemistry (Figure 3A-E) and quantified (Figure 3F-J). The expression of caspase-3, Iba1, and GFAP and the amount of 4-HNE was higher in the 3 dexmedetomidine-treated groups and the ischemia-reperfusion group than in

**Figure 3.** The Effect of Different Doses of Dexmedetomidine on the Expression Levels of Caspase-3, Iba1, GFAP, and MAP-2 and the Levels of 4-HNE in the Brain Tissue of Rats With Ischemia-Reperfusion Injury. Immunohistochemical staining of (A) caspase-3, (B) IbaI, (C) GFAP, (D) MAP-2, and the amount of (E) 4-HNE in rat brain tissue. The mean optical density of (F) caspase-3, (G) IbaI, (H) GFAP, (I) MAP-2, and (J) 4-HNE in rat brain tissue.




[a]$P < .05$,
[b]$P < .01$, vs. IR group

Note: n = 5 per groupScale bars = 50 μm. Error bars are mean ± SD.

the sham group, and the expression of MAP-2 was significantly lower in the 3 dexmedetomidine-treated groups and the ischemia-reperfusion group than in the sham group ($P = .036$, 95% CI = .023 to .044). The expression of caspase-3, Iba1, and GFAP and the amount of 4-HNE was lower in the 3 dexmedetomidine-treated groups than in the ischemia-reperfusion group, and the expression of MAP-2 was higher in the 3 dexmedetomidine-treated groups than in the ischemia-reperfusion group ($P = .041$, 95% CI = .038 to .045) (Figure 3F-J).

## DISCUSSION

The results show that dexmedetomidine reduced the volume of cerebral infarction in rats with ischemia-reperfusion injury. It also affected the expression levels of caspase-3, Iba1, GFAP, and MAP-2 and the amount of 4-HNE in damaged rat brain tissue.

Cerebral ischemia-reperfusion injury can affect the prognosis of patients' diseases and can even endanger their lives. The known pathogenesis of cerebral ischemia-reperfusion injury includes inflammatory reactions, excitatory amino acid toxicity, intracellular calcium overload, free radical damage, energy-metabolism disorders, and cell apoptosis, among which multiple complex mechanisms interact. At the cellular level, cerebral ischemia-reperfusion injury eventually leads to necrosis or apoptosis of neurons, microglia, astrocytes, and other brain tissue cells through a series of pathological changes that result in brain injury.[21] The mechanism of action of neuroprotective drugs is mainly studied to understand whether they affect the recovery from brain injury. Studies of the neuroprotective effects of dexmedetomidine are mostly based on experiments on laboratory animals and on in vitro studies, and most of the studies have confirmed the neuroprotective effects of dexmedetomidine.[22-24] However, there are few studies on the effects of dexmedetomidine on brain cells such as neurons, microglia, and astrocytes that have been damaged by brain injury. The results of this study show that dexmedetomidine significantly affected the protein expression levels of caspase-3, Iba1, GFAP, and MAP-2 and the amount of 4-HNE in brain tissues with ischemia-reperfusion injury. Changes in the expression levels of caspase-3, Iba1, GFAP, and MAP-2 and the amount of 4-HNE reflect the cell states of apoptosis, necrosis, or proliferation of neurons, microglia, and astrocytes in brain tissue.

Under normal conditions, aspartate-specific caspase-3 is present in normal cells as a dormant proenzyme. After the activation of an apoptosis signal, caspase-9 activates and cleaves caspase-3 into an active form by enzymatic catalysis.[25] Apoptosis is a cascade reaction process regulated by genes, among which the most important are the caspase cascade genes. As a key downstream factor of this cascade, caspase-3 performs the death process and is known as the "killer" protein. It promotes neuronal death in the brain and plays an important role in ischemic brain injury. Cell apoptosis is often quantified to reflect the state of tissue cells by detecting the cellular expression of caspase-3.[25] Enhanced expression of caspase-3 in tissue cells indicates tissue damage and cells that have entered the apoptotic process.[26]

Microglia activation is involved in neuronal repair and "shaping." Iba1 is a marker of macrophage and microglia activation. Microglia are the main immune cells in brain tissue and participate in the inflammatory immune response.[27] By detecting the expression of Iba1 in brain tissue, we can quantify the number of microglia activated in the stages of cerebral ischemia-reperfusion injury and injury repair. A high amount of Iba1 expression in brain tissue cells indicates that microglia are activated, and tissue repair is enhanced.[28] GFAP is a specific marker protein of mature astrocytes. Astrocytes are activated after brain injury.[29] The expression of GFAP in brain tissue reflects the activation of astrocytes in brain tissue and the repair state of brain tissue injury.[30] MAP-2 is a heat-stable phosphoprotein that is abundant in the mammalian brain and is the skeletal component of nerve cells. It is mainly expressed in the cell body, dendrites, and dendritic spines of neurons. It can regulate microtubule assembly and dynamics, which is often used as a marker of neurons. After cerebral ischemia-reperfusion injury, the functional state of the brain is directly related to the amount of neuron survival and regeneration. The neuronal state can be directly observed by detecting the expression of MAP-2.[31] When MAP-2 expression is enhanced in brain tissue, it indicates active neuron proliferation.[32] 4-HNE is the most commonly studied substance in the aldehyde group of products of lipid peroxidation. Excessive accumulation of 4-HNE in tissue cells is genotoxic and can inhibit the activity of enzyme systems and induce cell apoptosis. Excessive accumulation of 4-HNE in tissue cells is closely related to oxidation, inflammation, and apoptosis of tissue cells.[33]

The results of this study show that the expression levels of caspase-3, Iba1, and GFAP and the amount of 4-HNE in the brain tissue of rats with ischemia-reperfusion injury were all lower in the groups treated with dexmedetomidine, whereas the expression level of MAP-2 was higher in the groups treated with dexmedetomidine when compared with the ischemia-reperfusion group. This result is consistent with the smaller infarct volume after TTC staining in the dexmedetomidine-treated groups. Thus, the protective effect of dexmedetomidine on the brain may be partly related to the regulation of microglia and astrocyte proliferation, the promotion of neuron proliferation, the inhibition of oxidation and inflammation in brain tissue cells, and the inhibition of brain cell apoptosis. This study shows that the neuroprotective mechanisms of dexmedetomidine are multifaceted.

There are some limitations to this study. In recent years, research on dexmedetomidine has confirmed its neuroprotective effect in animal and *in vitro* experiments.[34] However, research on the neuroprotective effects of dexmedetomidine in patients with cerebral ischemic injury has not provided more positive results in terms of practical clinical value. There is even doubt about the clinical value of dexmedetomidine in brain protection.[35] Therefore,

researchers must study patients to draw clearer conclusions on the neuroprotective value of dexmedetomidine in patients. In fact, this study has preliminarily confirmed the neuroprotective value of dexmedetomidine in an animal model. However, owing to significant differences in physiological structures between animals and humans, future studies should include research on patients with cerebral ischemia-reperfusion injury to achieve the goal of serving patients from the laboratory to the clinic.

## CONCLUSIONS

The results of this study show that dexmedetomidine has a protective effect on the brain tissue of rats with ischemia-reperfusion injury. Part of the protective effect of dexmedetomidine is related to the promotion of neuronal regeneration, the regulation of microglia and astroglia proliferation, the inhibition of oxidative and inflammatory responses, and cell apoptosis. By studying the effects of dexmedetomidine on the expression levels of protein markers in injured brain tissue, we have provided ideas for the later study of the organ-protective effect of dexmedetomidine. However, clinical studies should be pursued so that the research ultimately serves patients.

### AUTHORS' CONTRIBUTIONS

Conception and design of the study: Lyu J, Bao X; acquisition of data: Zhao L; technical procedures: Tang L, Sun Z; statistical analysis: Zhou Y, Huang L; manuscript preparation: Zhang M; critical revision: Lyu J; final approval of the version to be published: Lyu J, Zhang M, Zhou Y; histological examinations: Zhao L. Lyu J, Zhou Y, Zhang M, Tang L, and Zhao L contributed equally to this work.

### ACKNOWLEDGEMENTS

The authors would like to express gratitude to the Third People's Hospital of Yunnan Province for its strong support for this study, as well as to the laboratory of the School of Pharmacy of Yunnan University of Economics and Management in China for providing the facilities for the experiments.

### CONFLICT OF INTEREST

Nothing to declare.

### DATA AVAILABILITY STATEMENT

Data will be available upon request.

### FUNDING

Not applicable.

### REFERENCES

1. Howard DPJ, Gaziano L, Rothwell PM; Oxford Vascular Study. Risk of stroke in relation to degree of asymptomatic carotid stenosis: a population-based cohort study, systematic review, and meta-analysis. *Lancet Neurol*. 2021;20(3):193-202. doi:10.1016/S1474-4422(20)30484-1
2. Wu S, Wu B, Liu M, et al; China Stroke Study Collaboration. Stroke in China: advances and challenges in epidemiology, prevention, and management. *Lancet Neurol*. 2019;18(4):394-405. doi:10.1016/S1474-4422(18)30500-3
3. Sewell K, Tse T, Harris E, et al. Pre-existing comorbidity burden and patient perceived stroke impact. *Int J Stroke*. 2021;16(3):273-279. doi:10.1177/1747493020920838
4. Ma H, Jiang Z, Xu J, Liu J, Guo ZN. Targeted nano-delivery strategies for facilitating thrombolysis treatment in ischemic stroke. *Drug Deliv*. 2021;28(1):357-371. doi:10.1080/10717544.2021.1879315
5. Wong CH, Crack PJ. Modulation of neuro-inflammation and vascular response by oxidative stress following cerebral ischemia-reperfusion injury. *Curr Med Chem*. 2008;15(1):1-14. doi:10.2174/092986708783330665
6. Zhao Y, Xu J. Sanggenon C ameliorates cerebral ischemia-reperfusion injury by inhibiting inflammation and oxidative stress through regulating RhoA-ROCK signaling. *Inflammation*. 2020;43(4):1476-1487. doi:10.1007/s10753-020-01225-w
7. Liu W, Guo Q, Zhao H. Oxidative stress-elicited YY1 potentiates antioxidative response via enhancement of NRF2-driven transcriptional activity: A potential neuronal defensive mechanism against ischemia/reperfusion cerebral injury. *Biomed Pharmacother*. 2018;108:698-706. doi:10.1016/j.biopha.2018.09.082
8. Wu MY, Yiang GT, Liao WT, et al. Current mechanistic concepts in ischemia and reperfusion injury. *Cell Physiol Biochem*. 2018;46(4):1650-1667. doi:10.1159/000489241
9. Franke M, Bieber M, Kraft P, Weber ANR, Stoll G, Schuhmann MK. The NLRP3 inflammasome drives inflammation in ischemia/reperfusion injury after transient middle cerebral artery occlusion in mice. *Brain Behav Immun*. 2021;92:223-233. doi:10.1016/j.bbi.2020.12.009
10. Zhang T, Wu C, Yang X, et al. Pseudoginsenoside-F11 protects against transient cerebral ischemia injury in rats involving repressing calcium overload. *Neuroscience*. 2019;411:86-104. doi:10.1016/j.neuroscience.2019.05.030
11. Halestrap AP. Calcium, mitochondria and reperfusion injury: a pore way to die. *Biochem Soc Trans*. 2006;34(Pt 2):232-237. doi:10.1042/BST0340232
12. Shi CX, Jin J, Wang XQ, et al. Sevoflurane attenuates brain damage through inhibiting autophagy and apoptosis in cerebral ischemiareperfusion rats. *Mol Med Rep*. 2020;21(1):123-130. doi:10.3892/mmr.2019.10832
13. Weerink MAS, Struys MMRF, Hannivoort LN, Barends CRM, Absalom AR, Colin P. Clinical pharmacokinetics and pharmacodynamics of dexmedetomidine. *Clin Pharmacokinet*. 2017;56(8):893-913. doi:10.1007/s40262-017-0507-7
14. Zheng D, Zhao S, Zhang N, Shi J. Brain protective effect and hemodynamics of dexmedetomidine hydrochloride in patients with intracranial aneurysm. *Saudi J Biol Sci*. 2020;27(7):1850-1855. doi:10.1016/j.sjbs.2020.03.027
15. Li H, Liu J, Shi H. Effect of dexmedetomidine on perioperative hemodynamics and myocardial protection in thoracoscopic-assisted thoracic surgery. *Med Sci Monit*. 2021;27:e929949. doi:10.12659/MSM.929949
16. Zhang QL, Wang L, Xu MJ, Wang TL. Protective effect of dexmedetomidine on kidney injury of parturients with preeclampsia undergoing cesarean section: a randomized controlled study. *Biosci Rep*. 2019;39(5):BSR20190352. doi:10.1042/BSR20190352
17. Lim H, Kim TY, Kim SY, et al. The protective effects of dexmedetomidine preconditioning on hepatic ischemia/reperfusion injury in rats. *Transplant Proc*. 2021;53(1):427-435. doi:10.1016/j.transproceed.2020.10.014
18. VanderBroek AR, Engiles JB, Kästner SBR, Kopp V, Verhaar N, Hopster K. Protective effects of dexmedetomidine on small intestinal ischaemia-reperfusion injury in horses. *Equine Vet J*. 2021;53(3):569-578. doi:10.1111/evj.13337
19. Bao N, Tang B. Organ-protective effects and the underlying mechanism of dexmedetomidine. *Mediators Inflamm*. 2020;2020:6136105. doi:10.1155/2020/6136105
20. Li JS, Liu JX, Yu HB, Wang D. [Establishment and evaluation of reproduction of middle cerebral artery occlusion to produce cerebral ischemia with autologous blood clot and nylon thread in rat]. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue*. 2006;18(5):272-274.
21. Huang Y, Chen S, Luo Y, Han Z. Crosstalk between inflammation and the BBB in stroke. *Curr Neuropharmacol*. 2020;18(12):1227-1236. doi:10.2174/1570159X18666200620230321
22. Jiang L, Hu M, Lu Y, Cao Y, Chang Y, Dai Z. The protective effects of dexmedetomidine on ischemic brain injury: A meta-analysis. *J Clin Anesth*. 2017;40:25-32. doi:10.1016/j.jclinane.2017.04.003
23. Zhao Q, Yu S, Ling Y, Hao S, Liu J. The protective effects of dexmedetomidine against hypoxia/reoxygenation-induced inflammatory injury and permeability in brain endothelial cells mediated by sigma-1 receptor. *ACS Chem Neurosci*. 2021;12(11):1940-1947. doi:10.1021/acschemneuro.1c00032
24. Andropoulos DB. Effect of anesthesia on the developing brain: infant and fetus. *Fetal Diagn Ther*. 2018;43(1):1-11. doi:10.1159/000475928
25. Lossi L, Castagna C, Merighi A. Caspase-3 mediated cell death in the normal development of the mammalian cerebellum. *Int J Mol Sci*. 2018;19(12):3999. doi:10.3390/ijms19123999
26. Shen X, Burguillos MA, Joseph B. Guilt by association, caspase-3 regulates microglia polarization. *Cell Cycle*. 2017;16(4):306-307. doi:10.1080/15384101.2016.1254979
27. Hendrickx DAE, van Eden CG, Schuurman KG, Hamann J, Huitinga I. Staining of HLA-DR, Iba1 and CD68 in human microglia reveals partially overlapping expression depending on cellular morphology and pathology. *J Neuroimmunol*. 2017;309:12-22. doi:10.1016/j.jneuroim.2017.04.007
28. Norden DM, Trojanowski PJ, Villanueva E, Navarro E, Godbout JP. Sequential activation of microglia and astrocyte cytokine expression precedes increased Iba-1 or GFAP immunoreactivity following systemic immune challenge. *Glia*. 2016;64(2):300-316. doi:10.1002/glia.22930
29. Shen XY, Gao ZK, Han Y, Yuan M, Guo YS, Bi X. Activation and role of astrocytes in ischemic stroke. *Front Cell Neurosci*. 2021;15:755955. doi:10.3389/fncel.2021.755955
30. Middeldorp J, Hol EM. GFAP in health and disease. *Prog Neurobiol*. 2011;93(3):421-443. doi:10.1016/j.pneurobio.2011.01.005
31. Ding K, Lai Z, Yang G, Zeng L. MiR-140-5p targets Prox1 to regulate the proliferation and differentiation of neural stem cells through the ERK/MAPK signaling pathway. *Ann Transl Med*. 2021;9(8):671. doi:10.21037/atm-21-597
32. Shafit-Zagardo B, Kalcheva N. Making sense of the multiple MAP-2 transcripts and their role in the neuron. *Mol Neurobiol*. 1998;16(2):149-162. doi:10.1007/BF02740642
33. Wang FX, Li HY, Li YQ, Kong LD. Can medicinal plants and bioactive compounds combat lipid peroxidation product 4-HNE-induced deleterious effects? *Biomolecules*. 2020;10(1):146. doi:10.3390/biom10010146
34. Bozorgi H, Zamani M, Motaghi E, Eslami M. Dexmedetomidine as an Analgesic Agent with Neuroprotective Properties: Experimental and Clinical Aspects. *J Pain Palliat Care Pharmacother*. 2021;35(3):215-225. doi:10.1080/15360288.2021.1914280
35. Hu Y, Zhou H, Zhang H, Sui Y, Zhang Z, Zou Y, Li K, Zhao Y, Xie J, Zhang L. The neuroprotective effect of dexmedetomidine and its mechanism. *Front Pharmacol*. 2022;20;13:965661. doi:10.3389/fphar.2022.965661.