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Parecoxib improves the cognitive function of POCD rats via attenuating COX-2

Y.-B. WANG[1], Z. CHEN[2], J. LI[1], J. SHI[1]

[1]Department of Anesthesiology, Ningbo Sixth Hospital Zhejiang, Ningbo, China
[2]Department of Anesthesiology, Affiliated Hospital of Qingdao University, Qingdao, China

Abstract. – OBJECTIVE: This study aims to explore the role of parecoxib in improving postoperative cognitive dysfunction (POCD) in rats, and to investigate the possible underlying mechanism.

MATERIALS AND METHODS: 60 Sprague Dawley (SD) rats were randomly divided into the control group (n=20), the model group (n=20), and the para group (n=20). The Morris water maze test was conducted to detect the postoperative cognitive ability of rats. The hematoxylin-eosin (HE) staining was applied to observe the neuronal density in the hippocampus of rats. The TdT-mediated dUTP Nick-End Labeling (TUNEL) was used to detect the hippocampal apoptosis in rats. Meanwhile, relative levels of inflammatory factors in rat hippocampus were detected by enzyme-linked immunosorbent assay (ELISA), including interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and prostaglandin E2 (PGE2). Furthermore, Western blot was conducted to detect the protein levels of cyclooxygenase-2 (COX-2), PGE2, EP1, and p-Akt in rat hippocampus.

RESULTS: Behavioral test results showed that the escape latency of rats in the para group was significantly shorter than that of the model group ($p<0.05$). Compared with the model group, rats in para group showed significantly longer time in target quadrant and more times across the platform, as well as higher Tn/Tn+Tf ($p<0.05$). In the control group, pathological changes of hippocampal neurons were slighter with rare apoptotic cells. Rats in model group showed great pathological lesions with abundant apoptotic neurons, which were markedly alleviated in the para group ($p<0.05$). Meanwhile, ELISA showed that the levels of IL-1β, IL-6, TNF-α, and PGE2 in the para group were remarkably lower than those of the model group ($p<0.05$). Expression levels of COX-2, PGE2, and EP1 in the para group were significantly lower than those of the model group ($p<0.05$). However, the expression level of p-Akt was significantly higher than the model group ($p<0.05$).

CONCLUSIONS: Parecoxib improves the cognitive function of POCD rats via inhibiting COX-2 overexpression in rat brain.

Key Words
Postoperative cognitive dysfunction (POCD), Cyclooxygenase-2 (COX-2), Parecoxib, Apoptosis.

Introduction

Postoperative cognitive dysfunction (POCD) is a common postoperative complication in the elderly. The clinical features of POCD are characterized by cognitive function decline (learning, memory, thinking, and attention) within several days to weeks after surgery. Some POCD patients may even develop into dementia. POCD prolongs the length of hospital stay and increases medical expenses, eventually posing a serious burden on their families and society[1,2]. Therefore, the prevention and treatment of POCD have been well explored in the field of neuroscience, exerting important clinical significances. The pathogenesis of POCD is a synergistic effect of various factors, involving the central nervous system, endocrine system, and immune system disorders. So far, the precise mechanism of POCD is still unclear[3-6]. Current researches[3-6] have indicated that age, surgery, anesthesia, postoperative pain, and infection may be associated with POCD. The central inflammatory response has been confirmed to be a key factor mediating the development of POCD. Surgical stress activates the peripheral immune system[7]. Meanwhile, elevated inflammatory factors affect the immune system response of the central nervous system through various signaling pathways, thus leading to cognitive dysfunction[8]. Studies have shown that cyclooxygenase-2 (COX-2) regulates the development of neurodegenerative diseases. COX-2 is associated with neuronal degeneration and apoptosis in the pathological process of various neurological diseases[9]. Cyclooxygenase is a key enzyme that catalyzes the conversion of substrate arachidonic acid (AA) to prostaglandins. It contains three subtypes, namely, COX-1, COX-2, and COX-3. Under normal physiological conditions, COX-2 is rarely expressed in the central nervous system. However, external stimulation greatly induces COX-2 upregulation in the cerebral cortex and hippocampus. Moreover, COX-2 overexpression

Corresponding Author: Yanbing Wang, MD; e-mail: lanyux@163.com

occurs in neurodegenerative diseases related to a central inflammatory reaction, such as Alzheimer's disease (AD), cerebral ischemia, hypoxia, and surgical trauma[10,11]. Parecoxib (para) is a selective COX-2 inhibitor, which exerts anti-inflammatory and antipyretic analgesic effects through inhibiting cyclooxygenase and PGE2 production[12]. Clinical studies have found the fat-solubility property of para. After intravenous injection of para for 15 minutes, it passes through the blood-brain barrier. Meanwhile, the blood concentration of para in cerebrospinal fluid is 1.57 ng/mL at 17 min. In addition to inhibition of peripheral inflammation, para simultaneously inhibits inflammation in the central nervous system, which is widely applied for analgesia[13,14]. COX-2 selective inhibitors have shown a neuroprotective role in brain injury diseases. Meloxicam, a COX-2 selective inhibitor, markedly improves memory and cognitive dysfunctions. Meanwhile, it reduces oxidative stress in the brain of AD mice induced by Amyloid β-protein (Aβ)[15,16]. Haile et al[17] have validated that meloxicam stimulates the activation of astrocytes, and decreases learning and memory function in mice after splenectomy. Moreover, it has also been reported[18] that therapeutic administration of para reduces cerebral oxidative stress and neuronal apoptosis in cerebral ischemia rats, thereby improving brain damage after cerebral ischemia. We aim to explore the role of parecoxib in improving POCD in rats, and to investigate the potential effects of para on POCD-induced apoptosis, oxidative stress, and inflammatory response.

Materials and Methods

Experimental Animals

Totally 60 adult male Sprague Dawley (SD) rats were housed in a quiet environment with 21-25°C of room temperature, 40%-60% of humidity, and 12-16/h of air exchange. All rats were maintained with a 12/12 h cycle of light. After one-week habituation, rats were randomly assigned into control group (no treatment), model group (abdominal surgery), and para group (abdominal surgery + para injection). Each group included 20 rats. Rats were intraperitoneally injected with 1 mL/kg para or saline 1 hour before abdominal surgery. At postoperative 6 h, 2 days and 3 days, 1 mL/kg para or saline was intraperitoneally injected. This study was approved by the Animal Ethics Committee of Ningbo Sixth Hospital Zhejiang Animal Center.

Behavioral Tests

Place navigation (acquisition phase): the rat head was placed toward the pool with a random initial position (four initial positions of east, west, south, and north). The time of the rat finding the underwater platform (the escape latency) was recorded. In the previous trainings, the rat was guided to the platform and stayed for 10 s if the escape latency exceeded 60 s. Each rat was trained 4 times per day (4 times in the east, west, south, and north quadrants). The interval between two trainings was 15-20 min, for a total of 5 days. Spatial probe test: on the second day after place navigation training, the platform was removed for space exploration training. The rat was placed in the water from the opposite side of the original platform quadrant. The time spent in the target quadrant where the platform was originally placed (time in target quadrant) and the times across the platform were recorded as indicators of spatial memory. Object recognition memory test: the time for exploring objects in rats was detected by novel and familiar object recognition test, for a total of 3 days. The exploring time of novel and familiar objects in rats were recorded as Tn and Tf, respectively. The time ratio of exploring novel object was calculated as Tn/Tn+Tf. Higher time ratio indicated stronger recognition memory of rats.

Sample Collection and Processing

After behavioral tests, 10 rats of each group were anesthetized for harvesting brain tissues. Fresh hippocampus tissues were separated on the ice and stored in a -80°C refrigerator. The remaining 10 rats in each group were anesthetized by intraperitoneal injection of 0.3% pentobarbital. After exposure of rat heart, intubation was performed through the left ventricular apex to the aorta. The right atrial appendage was cut, and 0.9% saline was quickly perfused until the rat lungs became pale with clear liquid flow from the auricula dextra. Subsequently, 300 mL of 4% paraformaldehyde was slowly infused for fixation. Brain tissue of rat was harvested and fixed again, followed by paraffin embed and serial coronal section preparation.

Hematoxylin-Eosin (HE) Staining

After 48 hours of fixation with 4% paraformaldehyde, brain tissues at 1-4 mm posterior to optic chiasma were cut into slices in the coronal direction. Brain slices were dehydrated with gradient alcohol, cleared in xylene, and embedded

in paraffin. Subsequently, brain tissues were cut into 5-μm slides for HE staining (Boster, Wuhan, China) and captured under a microscope.

Apoptosis Assay

The sections were routinely dewaxed, washed, hydrated, and fixed. Apoptosis determination was strictly performed according to the instructions of TdT-mediated dUTP Nick-End Labeling (TUNEL) Apoptosis Kit (Beyotime, Shanghai, China). Cells stained with a brown substance in the nucleus were considered as positive apoptotic cells. Five high-power fields in each sample were observed, with 100 cells counted in each field. The number of apoptotic cells was calculated as: AI = apoptotic cell number / total cell number × 100%.

Enzyme-Linked Immunosorbent Assay (ELISA)

Rat hippocampal tissue was lysed on ice with radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China) for 30 min, and the supernatant was collected for centrifugation. In this procedure, the serum levels of interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and prostaglandin E2 (PGE2) in rats were detected by ELISA using a microplate reader.

Western Blot

Total protein in hippocampus tissues was lysed with RIPA (radioimmunoprecipitation assay; Beyotime, Shanghai, China). The concentration of extracted protein was quantified using the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). Protein samples were electrophoresed on polyacrylamide gels and then transferred to polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Billerica, MA, USA). After blocking with 5% skimmed milk, the membranes were incubated with primary antibody of COX-2, PGE2, EP1, and p-Akt (Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. The membrane was incubated with the secondary antibody after rinsing with the Tris-Buffered Saline and Tween (TBST). Chemiluminescence was used to expose the protein bands on the membrane.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 (Chicago, IL, USA) was used for all statistical analysis. The experimental data were expressed as mean ± SD (Standard Deviation) ($\bar{x}\pm s$). The experimental results were analyzed with the standard *t*-test analysis. Categorical data were analyzed by the χ^2-test. $p<0.05$ was considered as statistically significant.

Results

Behavioral Test Results

The place navigation test indicated that rats in the model group showed prolonged escape latency compared with that of the control group, whereas para treatment markedly shortened escape latency ($p<0.05$, Figure 1A). Subsequently, the time spent in the target quadrant and the times across the platform in rats of the model group were significantly fewer than those of the control group ($p<0.05$). However, rats in the para group exhibited markedly longer time in target quadrant and more times across the platform than those of the model group ($p<0.05$, Figure 1B, 1C). The time ratio of exploring novel object was expressed as Tn/Tn+Tf. Results demonstrated that Tn/Tn+Tf in the model group was significantly lower than that of the control group. Meanwhile, the para group showed a remarkably higher level of Tn/Tn+Tf than the model group ($p<0.05$, Figure 1D). The behavioral test revealed that para administration could improve cognitive function in POCD rats.

Pathological Lesions in Hippocampal Neurons

In the control group, hippocampal neurons were arranged in a compact, continuous and uniform shape. The nucleoli were clear and basically located in the center of the nuclei. However, neurons in the model group were impaired with a disordered arrangement and unclear structure. Deeply stained nuclei and karyopyknosis were common, and the neuron density was reduced to a certain extent. Comparably, the para group showed significantly less neuronal destruction, and the arrangement was slightly sparse. Meanwhile, deeply stained nuclei and karyopyknosis were less observed than those of the model group (Figure 2A-2D).

Apoptosis of Hippocampal Neurons

In the control group, only several apoptotic cells were observed in the hippocampus. The model group showed much more apoptotic cells than the control group. Moreover, the number of apoptotic cells in the hippocampus of the para group was markedly lower than that of the model group (Figure 3A-3D).

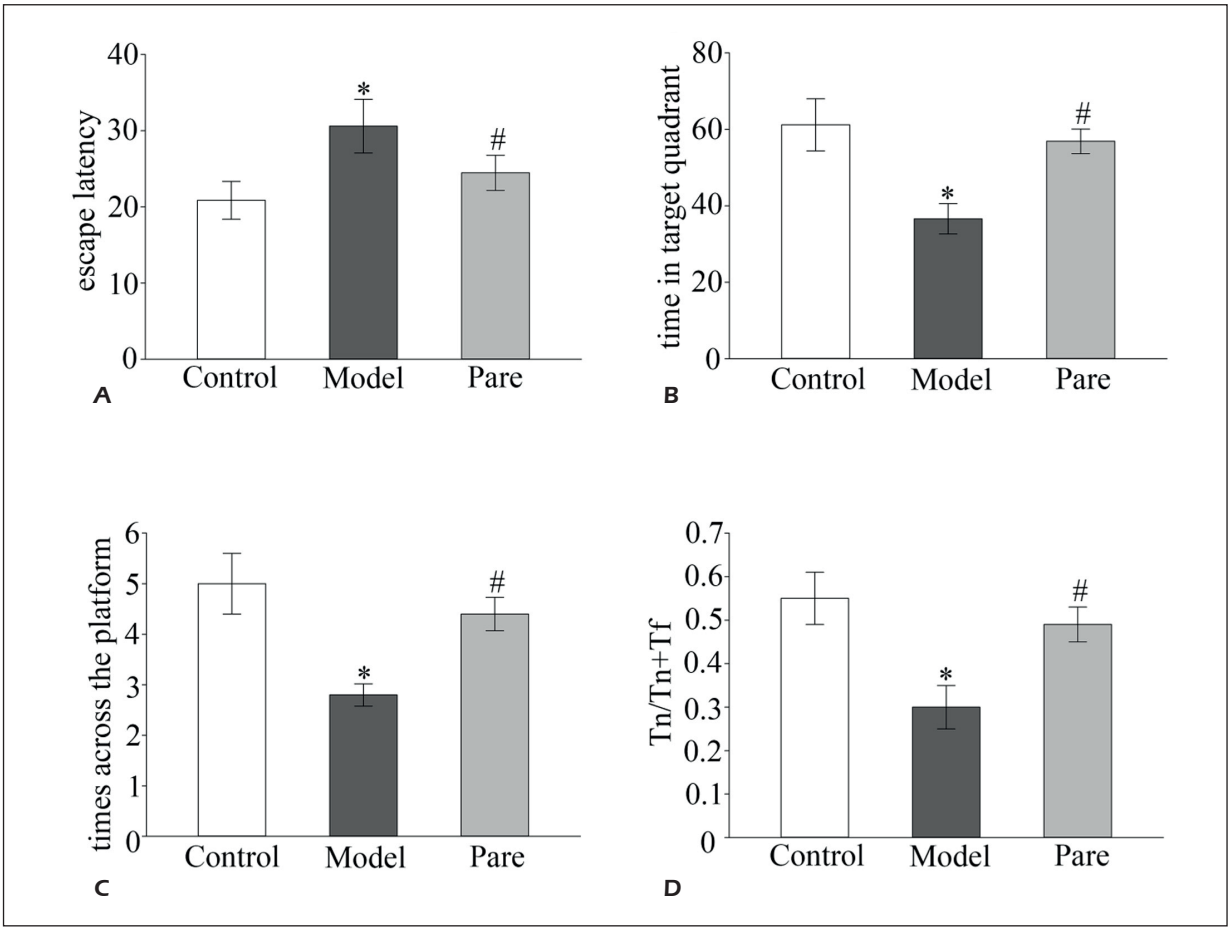

Figure 1. Behavioral test results. Rats were divided into the control group (n=20), the model group (n=20), and the para group (n=20). **A**, Comparison of escape latency among the three groups. **B**, Comparison of time in target quadrant among the three groups. **C**, Comparison of times across the platform among the three groups. **D**, Comparison of Tn/Tn+Tf among the three groups. *$p<0.05$, compared with the control group; #$p<0.05$, compared with the model group.

Levels of IL-1β, IL-6, TNF-α and PGE2 in Hippocampus

Results of ELISA showed that the levels of IL-1β, IL-6, TNF-α, and PGE2 in rats of the model group were significantly higher than those of the control group ($p<0.05$). However, remarkably decreased levels of IL-1β, IL-6, TNF-α, and PGE2 were found after para treatment ($p<0.05$, Figure 4A-4D).

Expression of COX-2 Relative Gene in Hippocampus

Western blot results showed that the protein expressions of COX-2, PGE2, and EP1 in rat hippocampus of the model group were significantly higher than those of the control group. However, the expressions of the above molecules were significantly downregulated after para treatment ($p<0.05$). On the contrary, the protein expression of p-Akt in the hippocampus of the model group was remarkably lower than that of the control group ($p<0.05$). Furthermore, the para group showed significantly higher expression of p-Akt than that of the model group ($p<0.05$, Figure 5A-5D).

Discussion

The incidence of POCD gradually increases, and its pathogenesis remains unclear. Current studies believed that high concentration of inflammatory factors resulted from surgical stress may interfere with normal hippocampal function and lead to neurotoxicity of hippocampal neurons. Impaired hippocampal neurons eventually

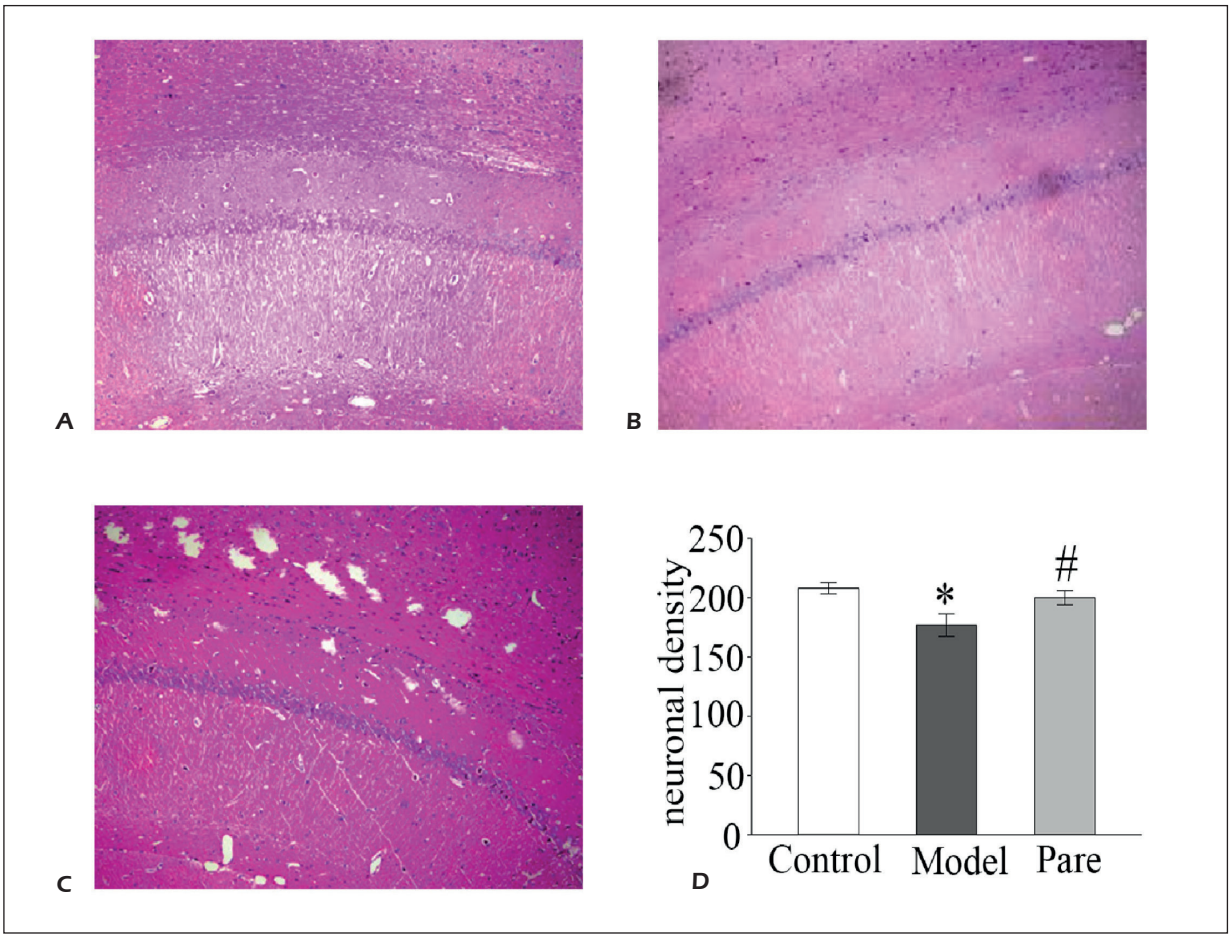

Figure 2. Pathological changes in hippocampal neurons. **A**, Pathological changes in hippocampal neurons in rats of the control group. **B**, Pathological changes in hippocampal neurons in rats of the model group. **C**, Pathological changes in hippocampal neurons in rats of the para group. **D**, Comparison of neuronal density among the control group, the model group, and the para group. *$p<0.05$, compared with the control group; #$p<0.05$, compared with the model group.

result in cognitive dysfunction[19,20]. COX-2 and its product PGE2 are found to be upregulated in multiple cognitive dysfunction-related diseases. Besides, EP1 receptor, the downstream factor of PGE2, mediates neurotoxicity in cerebral ischemia and hypoxia as well as AD models. Researches[21-23] have also shown that EP1 receptor is crucial in neurodegenerative diseases.

In normal or mammalian brain tissues, COX-2 is mainly distributed in the cerebral cortical neurons, hippocampal neurons, as well as dendrites and dendritic spines of other neurons. COX-2 is especially expressed in the postsynaptic membrane of hippocampal neurons[24]. COX-2 and its metabolites are necessary in normal physiological processes of the body, such as brain signal transduction, gene expression, neuronal synaptic plasticity formation, and synaptic remodeling. Meanwhile, COX-2 is also involved in basic brain functions, including neurotransmitter release, learning and memory abilities, and regulation of cerebral blood flow. Spinal cord pain response and fever reaction can be regulated by COX-2[25,26]. COX-2 triggers inflammatory cascades after trauma and infection, which is one of the important regulators of inflammation-related diseases[27-29]. Overexpression of COX-2 leads to the production of PGE2, which in turn stimulates glial cells to release cytotoxic factors and chemokines. Thereafter, this may eventually cause or aggravate the systemic inflammatory response[30,31]. A large amount of oxygen free radicals is produced during the synthesis of PGE2. Oxygen free radicals not only mediate excitatory neurotoxicity of glutamate, but also lead to neuronal damage by oxidative stress[32,33].

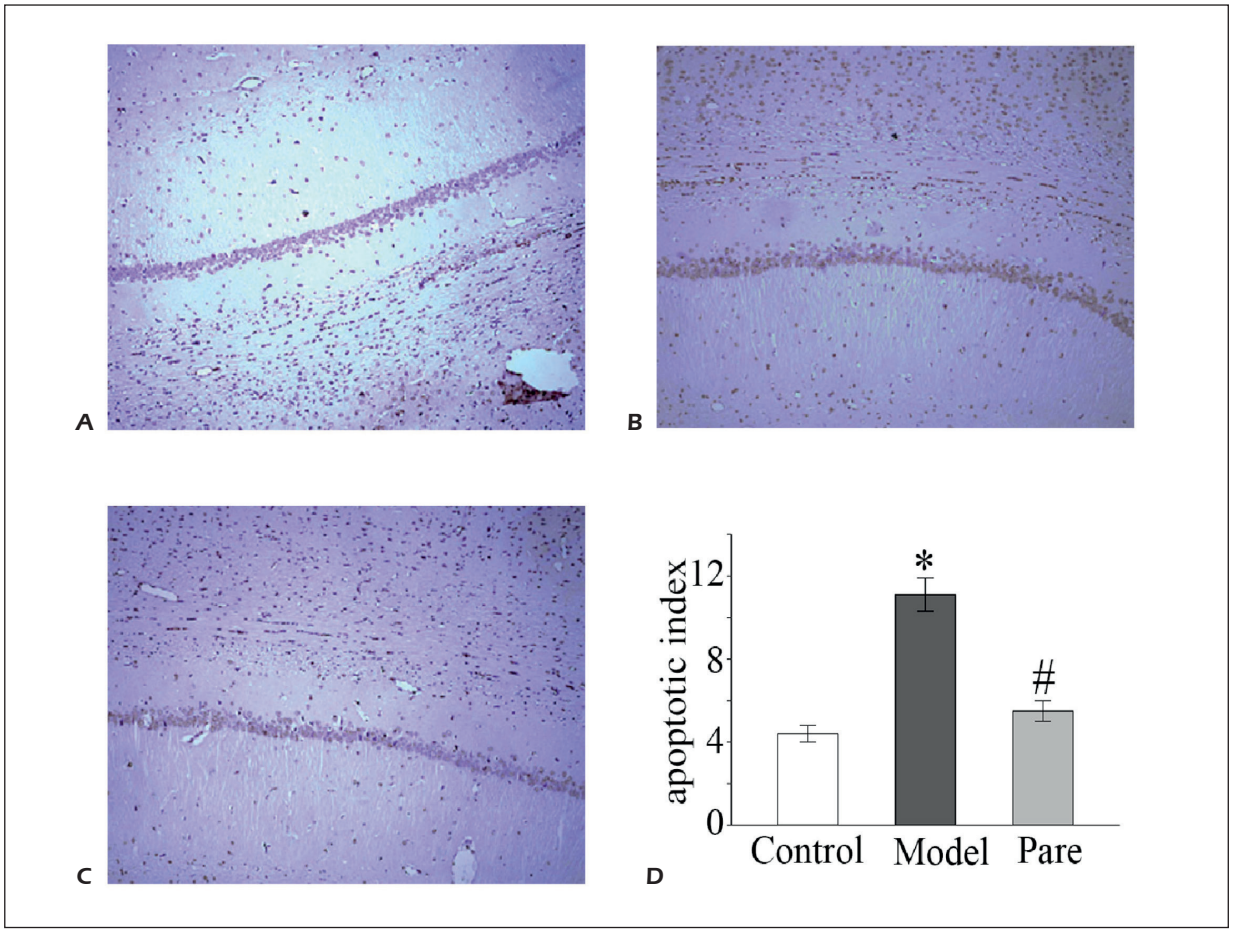

Figure 3. Apoptosis of hippocampal neurons. **A**, Apoptosis of hippocampal neurons in rats of the control group. **B**, Apoptosis of hippocampal neurons in rats of the model group. **C**, Apoptosis of hippocampal neurons in rats of the para group. **D**, Comparison of apoptotic index among the control group, the model group, and the para group. *$p<0.05$, compared with the control group; #$p<0.05$, compared with the model group.

In central nervous system diseases, COX-2 overexpression may lead to neuronal inflammation related neuronal damage, increased prostaglandin synthesis, prostaglandin receptor activation, oxidative stress, and apoptosis[34]. Our study showed that the levels of COX-2, PEG2, EP1, as well as cytokines of IL-1β, IL-6, and TNF-α in rat hippocampus were significantly upregulated in the model group. This indicated that COX-2 promoted free radicle production, oxidative stress, inflammation and apoptosis, ultimately leading to neuronal damage and cognitive dysfunction. Para administration could effectively reverse the levels of COX-2, PEG2, EP1, IL-1β, IL-6, and TNF-α in the brain. Prostaglandin production, neuronal inflammation and oxidative stress could be effectively controlled by para treatment. Finally, the release of neurotoxic free radicals was reduced, and the learning and memory functions were improved.

In this work, we also proposed a close relationship among surgery-induced neuro-inflammatory reaction, COX-2/PEG2/EP1 pathway and cognitive impairment, which was destructed by COX-2 inhibition after para treatment. Para exerts a strong lipophilicity and easily passes through the blood-brain barrier. Furthermore, it presents neuroprotective effects on improving the learning and memory ability of postoperative rats.

Conclusions

We showed that selective COX-2 inhibitor parecoxib suppressed COX-2 overexpression in postoperative rats. Moreover, para inhibited central inflammatory state, neuronal apoptosis, and oxidative stress, thus improving learning and memory functions in rats.

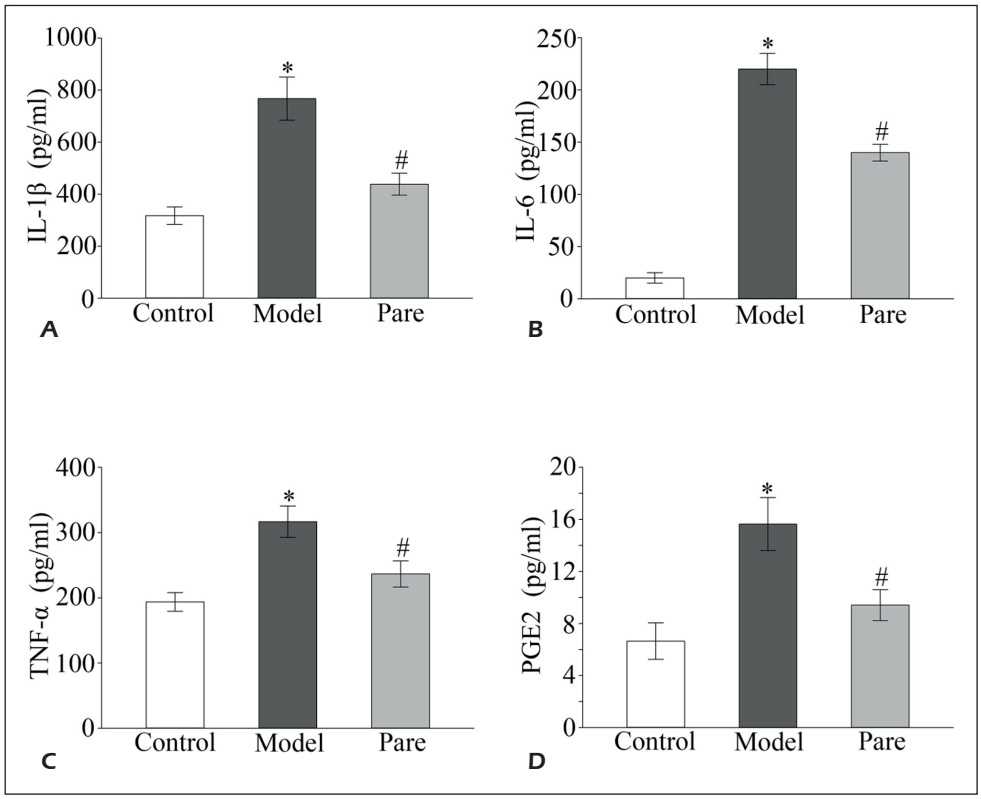

Figure 4. Levels of IL-1β, IL-6, TNF-α, and PGE2 in the hippocampus. **A**, IL-1β level in rat hippocampus of the control group, the model group, and the para group. **B**, IL-6 level in rat hippocampus of the control group, the model group, and the para group. **C**, TNF-α level in rat hippocampus of the control group, the model group, and the para group. **D**, PGE2 level in rat hippocampus of the control group, the model group, and the para group. *$p<0.05$, compared with the control group; $^{\#}p<0.05$, compared with the model group.

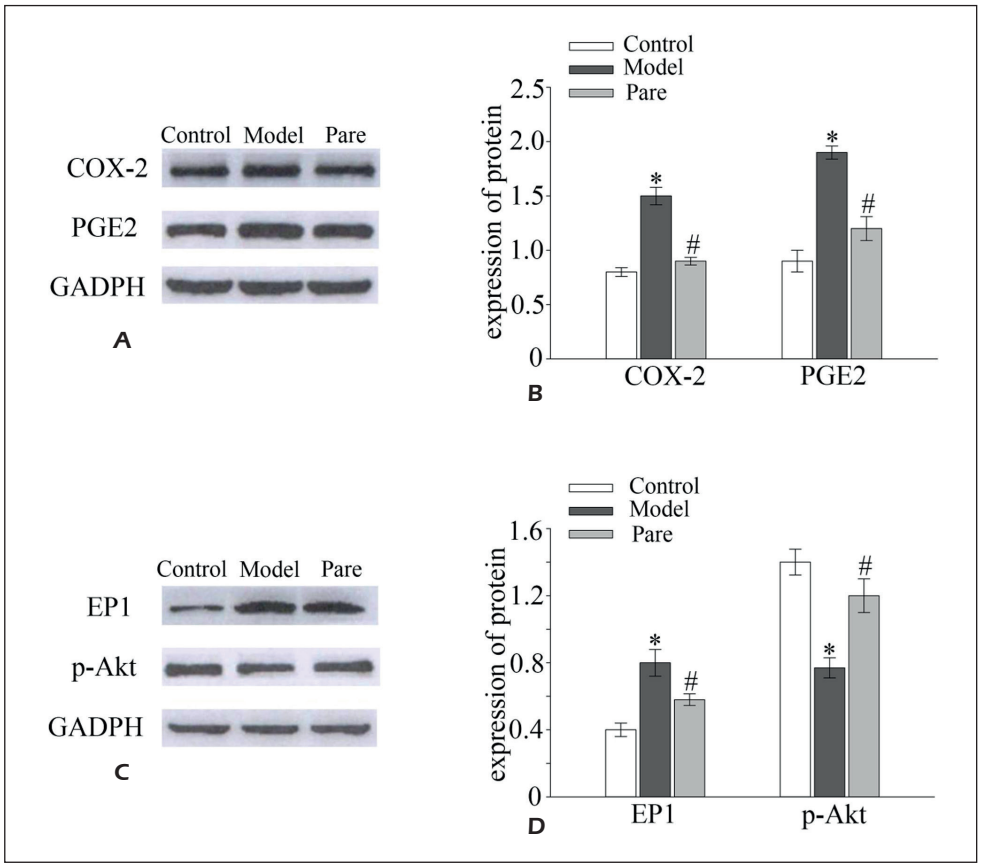

Figure 5. COX-2 relative gene expressions in the hippocampus. **A**, Western blot analyses of COX-2 and PEG2 in rat hippocampus of the control group, the model group, and the para group. **B**, Comparison of protein levels of COX-2 and PEG2 in rat hippocampus of the control group, the model group, and the para group. **C**, Western blot analyses of EP1 and p-Akt in rat hippocampus of the control group, the model group, and the para group. **D**, Comparison of protein levels of EP1 and p-Akt in rat hippocampus of the control group, the model group, and the para group. *$p<0.05$, compared with the control group; $^{\#}p<0.05$, compared with the model group.

Competing interests

The authors declare that they have no competing interests.

References

1) Gong GL, Liu B, Wu JX, Li JY, Shu BQ, You ZJ. Postoperative cognitive dysfunction induced by different surgical methods and its risk factors. Am Surg 2018; 84: 1531-1537.
2) Berger M, Nadler JW, Browndyke J, Terrando N, Ponnusamy V, Cohen HJ, Whitson HE, Mathew JP. Postoperative cognitive dysfunction: minding the gaps in our knowledge of a common post-operative complication in the elderly. Anesthesiol Clin 2015; 33: 517-550.
3) Wang W, Wang Y, Wu H, Lei L, Xu S, Shen X, Guo X, Shen R, Xia X, Liu Y, Wang F. Post-operative cognitive dysfunction: current developments in mechanism and prevention. Med Sci Monit 2014; 20: 1908-1912.
4) Uchino H, Nagashima F, Nishiyama R, Ishida Y, Saiki I, Yara M, Hara N. [Pathophysiology and mechanisms of postoperative cognitive dysfunction]. Masui 2014; 63: 1202-1210.
5) Liu SY, Wang HB, Wang HY, Wang GL. [Effect of sub-anesthetic doses of isoflurane and propofol on postoperative cognition and underlying mechanism in rats with mild cognitive impairment]. Zhonghua Yi Xue Za Zhi 2016; 96: 142-146.
6) Liu C, Han JG. Advances in the mechanisms and early warning indicators of the postoperative cognitive dysfunction after the extracorporeal circulation. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2015; 37: 101-107.
7) Rundshagen I. Postoperative cognitive dysfunction. Dtsch Arztebl Int 2014; 111: 119-125.
8) ISIK B. Postoperative cognitive dysfunction and Alzheimer disease. Turk J Med Sci 2015; 45: 1015-1019.
9) Bjork WD, Mirrasekhian E, Zajdel J, Kumar SA, Engblom D. Cyclooxygenase isoform exchange blocks brain-mediated inflammatory symptoms. PLoS One 2016; 11: e166153.
10) Maingret V, Barthet G, Deforges S, Jiang N, Mulle C, Amedee T. PGE2-EP3 signaling pathway impairs hippocampal presynaptic long-term plasticity in a mouse model of Alzheimer's disease. Neurobiol Aging 2017; 50: 13-24.
11) Bapna M, Chauhan LS. The ambidextrous cyclooxygenase: an enduring target. Inflamm Allergy Drug Targets 2015; 13: 387-392.
12) Lu J, Chen G, Zhou H, Zhou Q, Zhu Z, Wu C. Effect of parecoxib sodium pretreatment combined with dexmedetomidine on early postoperative cognitive dysfunction in elderly patients after shoulder ar-throscopy: a randomized double blinded controlled trial. J Clin Anesth 2017; 41: 30-34.
13) Vertiz-Hernandez AA, Martinez-Morales F, Valle-Aguilera R, Lopez-Sanchez P, Villalobos-Molina R, Perez-Urizar J. Parecoxib increases blood pressure through inhibition of cyclooxygenase-2 messenger RNA in an experimental model. Rev Invest Clin 2015; 67: 250-257.
14) Lv N, Kong Y, Mu L, Pan T, Xie Q, Zhao M. Effect of perioperative parecoxib sodium on post-operative pain control for transcatheter arterial chemoembolization for inoperable hepatocellular carcinoma: a prospective randomized trial. Eur Radiol 2016; 26: 3492-3499.
15) Ianiski FR, Alves CB, Ferreira CF, Rech VC, Savegnago L, Wilhelm EA, Luchese C. Meloxicam-loaded nanocapsules as an alternative to improve memory decline in an Alzheimer's disease model in mice: involvement of Na(+), K(+)-ATPase. Metab Brain Dis 2016; 31: 793-802.
16) Li H, Luo Y, Xu Y, Yang L, Hu C, Chen Q, Yang Y, Ma J, Zhang J, Xia H, Li Y, Yang J. Meloxicam improves cognitive impairment of diabetic rats through COX2-PGE2-EPs-cAMP/pPKA pathway. Mol Pharm 2018; 15: 4121-4131.
17) Haile M, Boutajangout A, Chung K, Chan J, Stolper T, Vincent N, Batchan M, D'Urso J, Lin Y, Kline R, Yaghmoor F, Jahfal S, Kamal R, Aljohani W, Blanck T, Bekker A, Wisniewski T. The Cox-2 inhibitor meloxicam ameliorates neuroinflammation and depressive behavior in adult mice after splenec-tomy. J Neurophysiol Neurol Disord 2016; 3: 101.
18) Li FT, Yao CH, Yao L, Huo ZF, Liu J. Study on the effects of parecoxib on hypothalamus orexin neuron of cerebral infarction rats. Eur Rev Med Pharmacol Sci 2018; 22: 1499-1505.
19) Peng L, Xu L, Ouyang W. Role of peripheral inflammatory markers in postoperative cognitive dysfunction (POCD): a meta-analysis. PLoS One 2013; 8: e79624.
20) Lin SY, Yin ZL, Gao J, Zhou LJ, Chen X. [Effect of acupuncture-anesthetic composite anesthesia on the incidence of POCD and TNF-alpha, IL-1beta, IL-6 in elderly patients]. Zhongguo Zhong Xi Yi Jie He Za Zhi 2014; 34: 795-799.
21) Evered L, Silbert B, Scott DA, Ames D, Maruff P, Blennow K. Cerebrospinal fluid biomarker for Alzheimer disease predicts postoperative cognitive dysfunction. Anesthesiology 2016; 124: 353-361.
22) Anglada-Huguet M, Vidal-Sancho L, Giralt A, Garcia-Diaz BG, Xifro X, Alberch J. Prostaglan-din E2 EP2 activation reduces memory decline in R6/1 mouse model of Huntington's disease by the in-duction of BDNF-dependent synaptic plasticity. Neurobiol Dis 2016; 95: 22-34.
23) Rojas A, Ganesh T, Manji Z, O'Neill T, Dingledine R. Inhibition of the prostaglandin E2 receptor EP2 prevents status epilepticus-induced deficits in the novel object recognition task in rats. Neuropharma-cology 2016; 110: 419-430.
24) Liu Y, Tang H, Liu X, Chen H, Feng N, Zhang J, Wang C, Qiu M, Yang J, Zhou X. Frontline Science: reprogramming COX-2, 5-LOX, and CYP4A-mediated arachidonic acid metabolism in macro-phages by salidroside alleviates gouty arthritis. J Leukoc Biol 2018:
25) Agundez JA, Blanca M, Cornejo-Garcia JA, Garcia-Martin E. Pharmacogenomics of cyclooxy-genases. Pharmacogenomics 2015; 16: 501-522.
26) Vosooghi M, Amini M. The discovery and development of cyclooxygenase-2 inhibitors as poten-tial anticancer therapies. Expert Opin Drug Discov 2014; 9: 255-267.

27) Yan W, Yun Y, Ku T, Li G, Sang N. NO2 inhalation promotes Alzheimer's disease-like progres-sion: cyclooxygenase-2-derived prostaglandin E2 modulation and monoacylglycerol lipase inhibition-targeted medication. Sci Rep 2016; 6: 22429.
28) Wang L, Jiang N, Lin QR, Qin CH, Hu YJ, Yu B. Cyclooxygenase-2 (COX-2) polymorphism rs689466 may contribute to the increased susceptibility to post-traumatic osteomyelitis in Chinese popula-tion. Infect Dis (Lond) 2017; 49: 817-823.
29) Tsai MH, Wu CH, Lin WN, Cheng CY, Chuang CC, Chang KT, Jiang RS, Hsu JF, Lee IT. In-fection with Staphylococcus aureus elicits COX-2/PGE2/IL-6/MMP-9-dependent aorta inflammation via the inhibition of intracellular ROS production. Biomed Pharmacother 2018; 107: 889-900.
30) Wang P, Guan PP, Wang T, Yu X, Guo JJ, Wang ZY. Aggravation of Alzheimer's disease due to the COX-2-mediated reciprocal regulation of IL-1beta and Abeta between glial and neuron cells. Aging Cell 2014; 13: 605-615.
31) Chu CH, Chen SH, Wang Q, Langenbach R, Li H, Zeldin D, Chen SL, Wang S, Gao H, Lu RB, Hong JS. PGE2 inhibits IL-10 production via EP2-mediated beta-arrestin signaling in neuroinflammatory condition. Mol Neurobiol 2015; 52: 587-600.
32) Luo D, Long Y, Chen GJ. Cyclooxygenase-2 gene polymorphisms and risk of Alzheimer's dis-ease: a meta-analysis. J Neurol Sci 2015; 359: 100-105.
33) Rojas A, Jiang J, Ganesh T, Yang MS, Lelutiu N, Gueorguieva P, Dingledine R. Cyclooxygen-ase-2 in epilepsy. Epilepsia 2014; 55: 17-25.
34) Guo Y, Lei W, Wang J, Hu X, Wei Y, Ji C, Yang J. Misoprostol reverse hippocampal neuron Cyclooxygenase-2 downstream signaling imbalance in aluminum-overload rats. Curr Alzheimer Res 2016; 13: 1006-1016.