The Effect of Dexmedetomidine on Oxidative Stress Response Following Cerebral Ischemia-Reperfusion in Rats and the Expression of Intracellular Adhesion Molecule-1 (ICAM-1) and S100B

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

ACE 1 **Yanwen Li**
BDFG 2 **Shikun Liu**

1 Department of Anesthesiology, Third Xiangya Hospital of Central South University, Changsha, Hunan, P.R. China
2 Department of Pharmacy, The Third Xiangya Hospital of the Central South University, Changsha, Hunan, P.R. China

Corresponding Author: Yanwen Li, e-mail: YanwenLi112@163.com
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Background: Ischemia-reperfusion injury of whole brain involves a complicated pathophysiology mechanism. Dexmedetomidine (Dex) has been shown to have neuro protective functions. This study observed the effect of Dex on serum S100B and cerebral intracellular adhesion molecule-1 (ICAM-1) in a rat model of cerebral ischemia-reperfusion.

Material/Methods: Healthy Sprague Dawley (SD) rats (males, 7 weeks old) were randomly divided into sham, model, and Dex groups (n=20 each). A cerebral ischemia-reperfusion model was prepared by clipping of the bilateral common carotid artery combined with hypotension. Dex (9 µg/kg) was infused intravenously immediately after reperfusion in the Dex group, while the other two groups received an equal volume of saline. Neural defect score (NDS) was measured at 6 hours, 24 hours, and 72 hours after surgery, with pathological observation of brain tissues. ELISA was then used to test serum S100B protein level. Malondialdehyde (MDA) and superoxide dismutase (SOD) were assayed by spectrometry. Nuclear factor-kappa B (NF-κB) and ICAM-1 levels were determined by real-time (RT)-PCR.

Results: Model rats had significant injury in the hippocampal CA1 region as shown by elevated NDS, S100B, and MDA levels, higher NF-κB and ICAM-1 mRNA expression, and lower SOD levels ($p<0.05$). Dex treatment improved pathological injury, decreased NDS, S100B, and MDA levels, decreased expression of mRNA of NF-κB and ICAM-1, and increased SOD levels.

Conclusions: Dex alleviated ischemia-reperfusion damage to rat brains, and inhibited NF-κB and ICAM-1 expression in brain tissues, possibly via inhibiting oxidative stress and inflammatory response.

MeSH Keywords: **Dexmedetomidine • Oxidative Stress • S100 Calcium Binding Protein beta Subunit**

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Background

Recently, cerebrovascular disease has increasing incidence, morbidity, and mortality [1,2]. Ischemia cerebrovascular disease accounts for increasingly more cases in clinics, as it is one potential complication in cardio surgery and neurosurgery. A frequent treatment for ischemia dysfunction is to regain blood perfusion, which, however, may cause tissues damage in cerebral regions [3,4].

The pathophysiological process of ischemia-reperfusion injury is complicated, with potent roles played by oxidative stress response and acute inflammatory response [5,6]. Inflammatory mediators, such as intracellular adhesion molecule-1 (ICAM-1) and tumor necrosis factor-α (TNF-α), can aggravate and block micro-vessels, further leading to the release of inflammatory mediators and cytokines by activating leukocytes, and exerting secondary neuron damage under the direction of oxygen free radicals [7,8]. Nuclear factor kappa B (NF-κB) has pluripotent modulatory roles, and can modulate gene expression levels of various inflammatory mediators, cytokines, and chemotactic factors such as interleukn-8 (IL-8), IL-6, and ICAM, thus playing an important role in cell inflammation and apoptosis. Some studies have shown the correlation between NF-κB and ischemia-reperfusion damage [9,10]. Calcium overload and oxidative stress can be induced by reperfusion after ischemia for further NF-κB activation. ICAM-1 is involved in the inflammatory pathological process, as its expression level can be increased by various inflammatory factors [11,12].

S100 protein is an acidic calcium binding protein specifically found in the central nervous system. Its concentration in serum is elevated after brain injury and the destruction of blood-brain barrier (BBB), and it is positively correlated with the severity of BBB and brain tissue damage [13,14].

Dexmedetomidine (Dex) is one selective α_2-adrenogladin receptor agonist that has been applied as an unique anesthetic and sedative reagent in severely ill patients. Some studies have shown that Dex could suppress inflammation via direct inhibition of inflammatory factors or activation of the cholinergic anti-inflammation pathway. It can protect neural tissues via activating α_2 receptors, decreasing free radicals, and suppressing the release of excitatory amino acids, although its detailed neuroprotective mechanism remains unclear [15,16].

ICAM-1 and S100B are involved in the inflammatory pathological process, which plays a vital role in the pathogenesis of brain ischemia-reperfusion. This study observed the effect of Dex on serum S100 protein levels and ICAM-1 expression in brain tissue during cerebral ischemia-reperfusion injury in rats, in order to investigate the function of Dex on brain ischemia-reperfusion injury and related mechanisms.

Material and Methods

Animals

Healthy male Sprague-Dawley (SD) rats (7 weeks old, body weight 220~250 g) were provided by the Laboratory Animal Center, Chinese Medicine Academy (Cert. No. SYXK-2013-0025) and were kept in a SPF-grade facility with food and water given *ad libitum*. Animals were randomly divided into sham, model, and Dex groups (n=20 each). 9 µg/kg Dex was given intravenously immediately after reperfusion t the Dex group. The other two groups received equal volumes of saline. Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Third Xiangya Hospital of Central South University.

Reagents and drugs

The following reagents and drugs were used: Dex (100 µg/mL, Hengrui Pharmaceutical, China); malondialdehyde (MDA) and superoxide dismutase (SOD) test kit (Jiancheng Bioengineering, China); S100 protein assay kit (Zhongshan Jinqiao, China); primers for NF-κB and ICAM-1 mRNA (Invitrogen, US); DNA polymerase (Toyobo, China); reverse transcription kit (Fermentas, USA); Trizol reagent (Invitrogen, USA); GAPDH antibody (Kangcheng Biotechnology, China).

Animal model

One hour before surgery, neural defect scores (NDSs) were examined to rule out those rats with congenital neural dysfunctions. A cerebral ischemia-reperfusion model was generated as previously reported [17]. In brief, the clipping of the bilateral common carotid artery combined with hypotension was applied. Rats were fasted for 8 hours before surgery. After anesthesia by 10% chloral hydrate, rats were placed in a supine position. The bilateral common carotid artery was exposed via mid-incision of the neck. The common carotid vein on the right side and the unilateral femoral artery were separated with intact nerves. A cannula was placed via femoral artery puncture to observe blood pressure. A second cannula was inserted into the carotid common artery toward the heart for blood collection and drug delivery. Venous blood was collected slowly via the femoral common vein (rats had blood volume at 30~50%) keeping average artery pressure at 35~45 mm Hg. The bilateral common carotid artery was closed by microclip. Electroencephalogram (EEG) was performed by insertion of electrodes on the bilateral temporal skull. The successful generation of a cerebral ischemia model was defined as the existence of static wave, pupil dilation, or the absence of cornea reflex. The bilateral common carotid artery was clipped for 15 minutes, followed by back-transfusion of venous blood via common carotid vein at 1.2 mL/minute. In the sham group,

Table 1. Primer sequence.

Primer	Size	Forward primer	Reverse primer
NF-kB	150bp	5'-ACGATCTGTTTCCCCTCATC-3'	5'-TGCTTCTCTCCCCAGGAATA-3'
ICAM-1	595bp	5'-AAGGTGTGATATCCGGTAGA-3'	5'-CCTTCTAAGTGGTTGGAACA-3'
β-actin	355bp	5'-TAAAGCCATGCCAAATGTCT-3'	5'-TAAAGACCTCTATGCCAACAC-3'

blood vessels were separated without ligation. Penicillin sodium was applied on the surgical site to prevent infection.

Drug delivery

Dex was diluted in saline and was infused into the rat veins at a total volume of 9 µg/kg body weight (9 µg/kg was based on our preliminary data and published pharmacokinetic studies of Dex). In the first hour, Dex was injected via a common carotid vein at 3 µg/kg, followed by 2 hour infusion at 3 µg/kg/hour. In the other two groups, equal volume of saline was applied.

NDS examination

At 6 hours, 24 hours, and 72 hours after reperfusion, NDS was performed, including vision, cognition, corena reflex, and motor and seizure episodes. A scale from 0 (normal) to 100 (brain death) was applied.

MDA and SOD assay

Six rats were sampled from each group at each time point. After sacrifice, the brain was extracted with the removal of the cerebellum and olfactory bulb. Cerebral cortex was quickly placed on ice, and was weighted after washing in cold saline and drying. Then a 10% homogenate was prepared for a 10 minute centrifugation. The supernatant was saved to determine protein content using Coomassie brilliant blue dye. MDA and SOD levels were quantified using spectrometry test kits.

HE staining for hippocampal CA1 morphology

Rat were fixed in 4% paraformaldehyde, and decapitated. The cerebellum, olfactory bulb, and lower brain stem were removed. Brain slices between 4 mm posterior of optic chiasm and cerebellum were immersed in paraformaldehyde. After paraffin embedding and sectioning, hematoxylin-eosin (HE) staining was performed for observation of tissue specimen under a light field microscope.

ELISA

Serum was extracted by centrifugation of rat blood samples. Serum S100B protein content was determined by ELISA following the manual instructions of the test kits.

RT-PCR

Hippocampal tissue posterior of the optic chiasm was extracted for preparing the tissue homogenate. The TransZol Up method was used to quantify total RNA. Using cDNA as the template, PCR amplification was performed under the following conditions: 94°C for 3 minutes (pre-denature), followed by 30 cycles each at 94°C for 30 seconds (denature), 56°C for 30 seconds (annealing), and 72°C for 45 seconds (elongation). Using β-actin as the internal reference, semi-quantitative analysis was performed by gel imaging analysis system to calculate relative absorption values. Primer sequences were shown in Table 1.

Statistical method

SPSS 20.0 software was applied for statistical analysis. Measurement data were used to test for normality. Data that fit the normal distribution are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare means across multiple groups. LSD test was used for between-group-comparison. A statistical significance was defined when $p < 0.05$.

Results

NDS of cerebral ischemia-reperfusion rats

Compared to the sham group, NDS was significantly increased in the model group at all study time points. NDS after reperfusion was lowest at 72 hours, followed by 24 hours and 6 hours ($p < 0.05$). The Dex group had significantly depressed NDS at all time points compared to the model group ($p < 0.05$, Figure 1).

Morphology of hippocampal CA 1 region

HE staining revealed regular arrangements of the CA1 region of the hippocampus in the sham group, with intact cell morphology, higher number of cells, normal structure, clear cytoplasm-nuclear boundary, and sharp nucleoid. However, irregular arrangements, incomplete morphology, fewer cell numbers with abnormal structure, blurred boundary of cytoplasm/nucleus, less cytoplasm, condensed nucleus, indistinguishable nucleoid, and occurrence of glial hyperplasia in the hippocampal
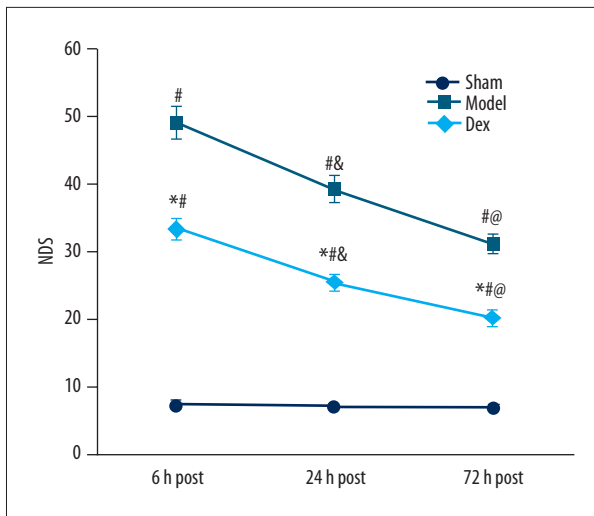
Figure 1. NDS of cerebral ischemia-reperfusion rats. # *p*<0.05 compared to control group; * *p*<0.05 compared to model group; & p<0.05 compared to 6 hour group; @ *p*<0.05 compared to 24 hour group.

CA1 zone were observed in the model group. The severity of hippocampal tissue injury was more at 72 hours than 24 hours. Dex treatment alleviated the injury in the hippocampal CA1 region at all time points (Figure 2).

MDA and SOD levels

Compared to the rats in the sham group, the model group rats had elevated MDA levels and decreased SOD levels. The MDA levels were lowest at 72 hours after reperfusion, followed by 24 hours and 6 hours. SOD levels, however, were highest at 72

hours, followed by 24 hours and 6 hours (*p*<0.05). Compared to the model group, the Dex group had lower MDA levels and elevated SOD levels at all time points (*p*<0.05, Figure 3).

Serum S100B protein concentration

Compared to the rats in the sham group, the model group rats had elevated serum S100B protein at all time points. S100B showed a peak value after 24 hours of reperfusion, and was then generally decreased. Compared to the model group, the Dex-treated group had lower protein content of S100B at all time points (*p*<0.05, Figure 4).

Expression of inflammatory factors in hippocampal tissues

Compared to the rats in the sham group, the model group rats had elevated NF-κB and ICAM-1 expression levels. Such values reached a peak after 24 hours of infusion, followed by decreased concentrations. Compared to the model group, the Dex group intervention lowered mRNA expression of NF-κB and ICAM-1 (*p*<0.05, Figure 5).

Discussion

Ischemia-reperfusion injury of brain is one common pathological alternation observed in clinics and that severely affects patient's quality of life. The alleviation of brain ischemia-reperfusion damage is thus of importance for decreasing morbidity and mortality. Dex exerts multiple functions, including sedative, anti-inflammation, and neuroprotection; although its neuroprotective mechanism has not been illustrated fully [15,16].
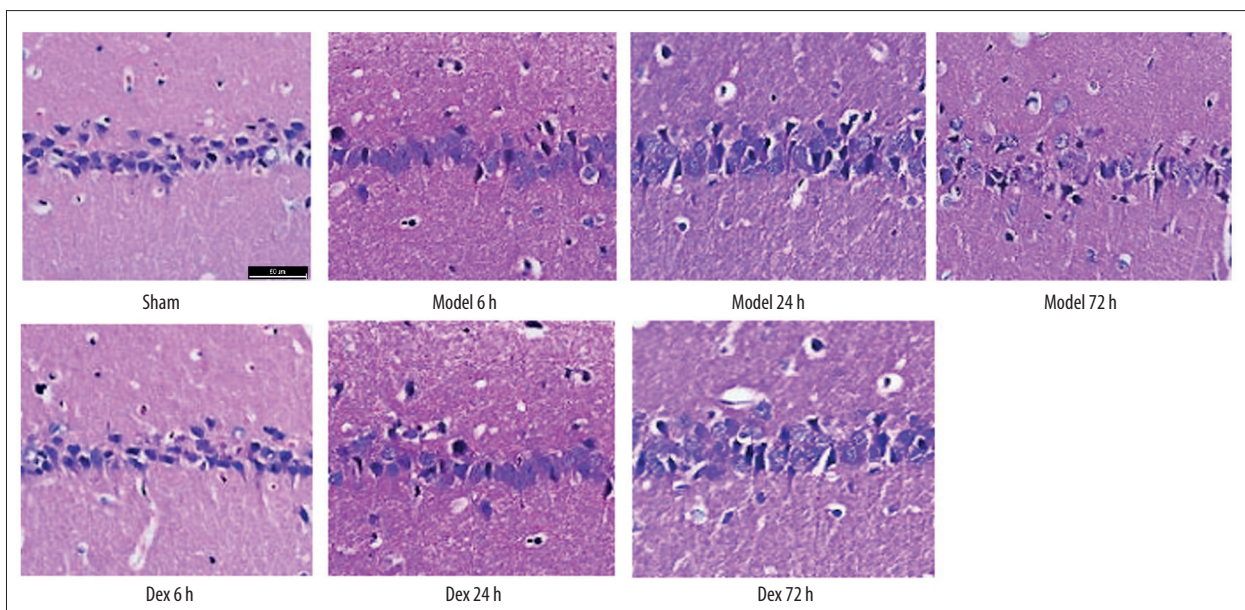

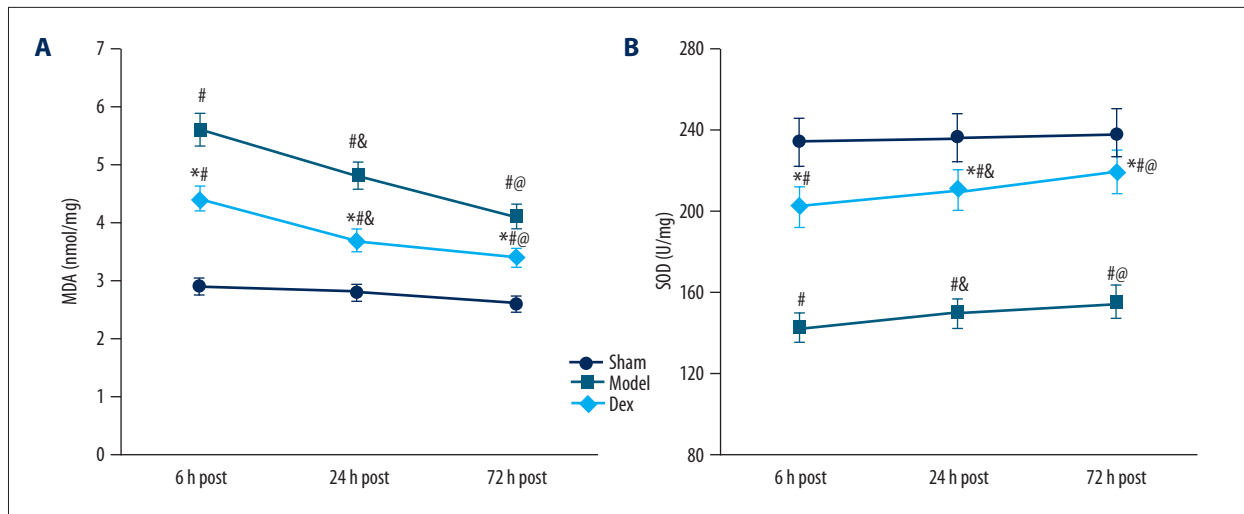**Figure 2.** Morphology of hippocampal CA1 zone (HE staining, ×400).

Figure 3. Cerebral levels of MDA (A) and SOD (B). [#] $p<0.05$ compared to control group; * $p<0.05$ compared to model group; [&] $p<0.05$ compared to 6 hour group; [@] $p<0.05$ compared to 24 hour group.

Figure 4. Serum S100 protein level. [#] $p<0.05$ compared to control group; * $p<0.05$ compared to model group; [&] $p<0.05$ compared to 6 hour group; [@] $p<0.05$ compared to 24 hour group.

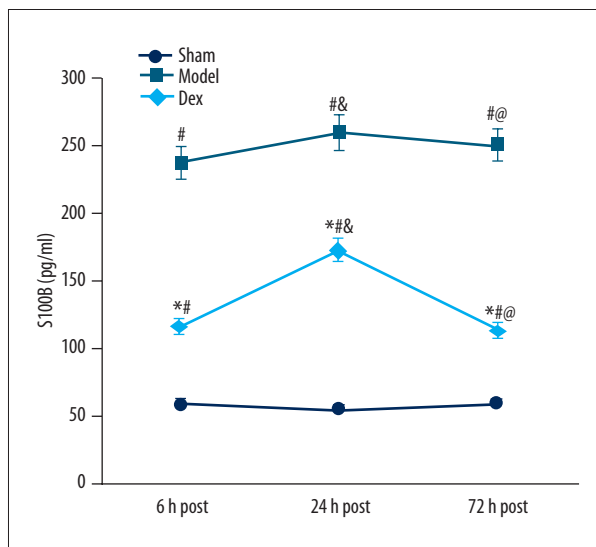During whole-brain ischemia-reperfusion injury, hippocampal CA1 and CA3 regions are most sensitive to ischemia as they show primary neuron apoptosis, followed by cell death three hours after reperfusion. Such cell apoptosis reaches a peak between 24 hours and 48 hours, followed by higher cell necrosis from 48 hours to 72 hours for the peak levels [18,19]. This study thus selected the three time points 6 hours, 24 hours, and 72 hours after reperfusion to investigate the neuroprotective function of Dex via both oxidative stress and inflammation. Pathology examination of the hippocampal CA1 region revealed irregular arrangement of cells after 6 hours of reperfusion, with incomplete tissue morphology, fewer cell numbers and minor tissue injury. After 24 hours or 72 hours of reperfusion, cell structure showed significant abnormalities, with blurred boundary between cytoplasm and nucleus, less cytoplasm, condensed nucleus, indistinguishable nucleoid, and glial hyperplasia. The degree of hippocampal damage at 72 hours was more severe than at 24 hours, as shown by abundant death of pyramidal cells. Treatment with Dex at all time points showed alleviation of neural injury. Under physiological condition, the body can maintain a homeostasis between oxidation and anti-oxidation functions, which are, however, disrupted under brain ischemia-reperfusion. As the body anti-oxidation is overwhelmed by oxidation, higher oxidative stress levels aggravate brain damage. MDA levels could reflect the lipid over-oxidation and cellular injury, while SOD could be the primary agent for clearing free radicals as it can block the cellular damage done by oxygen free radicals [18]. This study showed elevated MDA levels in the brains of model rats, accompanied by lower SOD levels and more severe hippocampal CA1 injury. Compared to the model group, the Dex group had lower MDA levels and higher SOD levels at all time points, resulting in alleviated CA1 injury. These results suggest that Dex could inhibit body oxidative stress levels, potentiate activity of anti-oxidation enzymes, and alleviate brain ischemia-reperfusion injuries in rats, possibly via mitochondrial ATP-sensitive potassium channel and mitochondrial membrane stability [19].

Under physiological condition, S100 protein could participate in the neural repair and regeneration process as one neurotrophic factor. With ischemia brain injury, serum S100B level is elevated. Thus, low concentration of S100B could antagonize glutamate neurotoxicity. Beyond certain thresholds, S100B could induce NOS activity, and activate NF-κB releasing factor for secondary inflammation and neural cell apoptosis. With the disruption of BBB, serum S100B level is elevated,
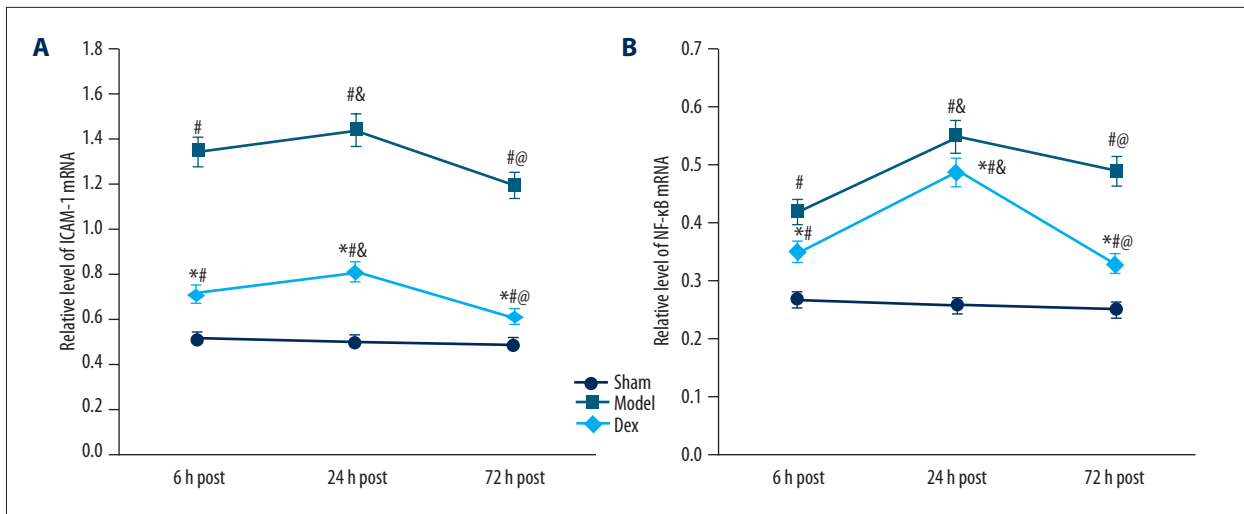
Figure 5. mRNA expression of NF-κB (A) and ICAM-1 (B). # *p*<0.05 compared to control group; * *p*<0.05 compared to model group; & *p*<0.05 compared to 6 hour group; @ *p*<0.05 compared to 24 hour group.

thus reflecting the severity of cerebral injury and integrity of BBB. This study showed elevation of S100B at all time points in the model group, with the peak level reached at 24 hours after reperfusion and maintained at a relatively high levels until 72 hours. Compared to the model group, the Dex group had lower serum S100B levels at all time points. At 24 hours after reperfusion, the severity of brain tissues and BBB permeability reached peak levels. After that, serum S100B levels began to decrease with the repair of BBB. A recent study showed the important role of inflammation during ischemia-reperfusion injury [20]. Due to the elevation of ICAM-1 mRNA expression, signal transduction will initiate via binding with neutrophil surface integrin LFA-1 to facilitate the adhesion between neutrophil and endothelial cells for inducing neutrophil infiltration. Multiple cellular pathways could regulate the release of inflammatory factors including IL-8 and IL-6. Among those pathways NF-κB is important, as it participates in cellular ischemia/hypoxia and acclimation of ischemia. Both *in vitro* and *in vivo* studies have shown involvement of NF-κB in ischemia-reperfusion process [21]. Normally, cytoplasmic NF-κB has no activity, as it binds with inhibitor protein IκB. After its activation, NF-κB facilitates the expression of IκBα, which regulates NF-κB expression via a feedback mechanism [21]. IL-1 and LPS signals initiate degradation of IκB. The activation of NF-κB following its release then facilitates the nuclear entry and binding with specific κB sequence, for facilitating transcription of inflammatory mediator or cytokine, thus leading to an inflammatory response. Secondary inflammatory responses after ischemia-reperfusion further aggravate the inflammation injury. During

the early stage of acute inflammation, one key step might be correlated with degradation of IκB following phosphorylation and translocation of NF-κB [21]. NF-κB binding sequence is widely distributed in the adhesion molecule ICAM-1 and the inflammatory mediator IL-8, whose expression level could be upregulated by NF-κB activation [20,21]. ICAM-1 is one ligand for integrin LFA-1 and can facilitate the adhesion between endothelial cells and leukocytes. This study showed elevation of mRNA of NF-κB and ICAM-1 expression in brain ischemia-reperfusion model rats, and decreased expression after Dex intervention, suggesting that Dex could inhibit the expression of inflammatory factors and NF-κB, thus decreasing the endothelial-leukocyte adhesion and consequent inflammation. Its neuroprotective role is related to the NF-κB signal pathway, as it can protect ischemia brain via suppressing inflammatory factors and potentiate vascular protective factors.

Conclusions

Dex could alleviate ischemia-reperfusion injury of the rat brain, and inhibit expression of NF-κB and ICAM-1 after cerebral injury, possibly via inhibiting oxidative stress and inflammatory response.

Disclosure of conflict of interest

The authors declare no competing financial or commercial interests in this manuscript.

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