Inflammation, Vol. 42, No. 6, December 2019 (© 2019)
DOI: 10.1007/s10753-019-01082-2

ORIGINAL ARTICLE

Role of JNK Signaling Pathway in Dexmedetomidine Post-Conditioning-Induced Reduction of the Inflammatory Response and Autophagy Effect of Focal Cerebral Ischemia Reperfusion Injury in Rats

Yulin Zhu,[1] Shihong Li,[2] Jingying Liu,[3] Qing Wen,[4] Jingui Yu,[5] Lingzhi Yu,[6] and Kun Xie[7,8]

Abstract— To investigate the effect of dexmedetomidine post-conditioning on the inflammatory response and autophagy effect of focal cerebral ischemia reperfusion injury in rats, and further to study its potential mechanisms. Water maze was conducted to evaluate spatial learning and memory ability of middle cerebral artery occlusion (MCAO) rats. TTC staining was used to observe the area of cerebral infarction. The expressions of inflammatory factors in serum were detected by ELISA. TUNEL assay, HE staining, and transmission electron microscopy were used to detect the apoptosis of neurons, neuro-cytopathic changes, and the formation of auto-phagosome in hippocampus CA1 region, respectively. The mRNA and protein expression of Beclin-1, Caspase-3, and light chain 3 (LC3) were detected by qRT-PCR and Western blot. Moreover, the activity of C-Jun N-terminal kinase (JNK) pathway was detected by Western blot. The escape latency (EL); cerebral infarction area ratio; positive apoptosis; neuron pathological changes; auto-phagosome numbers; inflammatory factor contents; mRNA and protein expressions of Beclin-1, Caspase-3 and LC3II/I; and the phosphorylation level of JNK were decreased, while the times across platform and the times stayed in the quadrant of the original platform were increased after dexmedetomidine treatment. However, the protective effect of dexmedetomidine

Yulin Zhu and Shihong Li contributed equally to this work.

[1] Department of Anesthesiology, Yantaishan Hospital, Yantai, 264000, China
[2] Department of Anesthesiology, Haiyang People's Hospital, Haiyang, 265100, China
[3] Department of Obstetrics, Yantaishan Hospital, Yantai, 264000, China
[4] Blood Purification Center, The Second Hospital of Shandong University, Jinan, 250000, China
[5] Department of Anesthesiology, Qilu Hospital of Shandong University, Jinan, 250000, China
[6] Departments of Pain, Jinan Central Hospital Affiliated to Shandong University, Jinan, 250000, China
[7] Department of Anesthesiology, The Second Hospital of Shandong University, No.247 Beiyuan Road, Tianqiao District, Jinan, 250000, China
[8] To whom correspondence should be addressed at Department of Anesthesiology, The Second Hospital of Shandong University, No.247 Beiyuan Road, Tianqiao District, Jinan, 250000, China. E-mail: xiekunxkk@sina.com

on brain injury in MCAO rats was reversed by JNK pathway activator. Dexmedetomidine post-conditioning could improve learning and memory dysfunction caused by MCAO in rats and reduce the inflammatory response and autophagy effect. The mechanism may be related to inhibition of JNK pathway activation.

KEY WORDS: Dexmedetomidine; Ischemia reperfusion injury; JNK signaling pathway; Autophagy; Inflammation.

INTRODUCTION

Ischemic cerebrovascular disease is the main type of cerebrovascular disease, accounting for 60–80% of the total number of patients with cerebrovascular disease [1, 2]. The main pathogenesis of ischemic cerebrovascular disease is irreversible injury and death of brain neurons, and the autophagy effect in this process has attracted increasing attention [3, 4]. Autophagy is a stress response of the body under the condition of ischemia and hypoxia [5]. Therefore, focal cerebral ischemia reperfusion can activate autophagy. Autophagy is the process by which cellular components are transported by vesicles to lysosomes for degradation [6]. In eukaryotic organisms, autophagy is involved not only in physiological processes such as growth and development, metabolism, and immunity but also in pathological processes such as cerebral ischemia, myocardial ischemia, and renal ischemia [7–9]. Several studies have showed that autophagy not only has a neuroprotective effect but also participates in the process of nerve cell death [10, 11]. Autophagy microtubule-associated protein light chain 3 antibody (LC3) is a marker protein for the formation of auto-phagosomes in mammals. After the occurrence of autophagy, the content of LC3 in cells and the transformation from LC3-I to LC3-II are significantly increased [12, 13]. Beclin-1 is a specific gene involved in autophagy in mammals and can be used as a marker of autophagy effect [14].

Inflammatory response is one of the mechanisms involved in the process of ischemia reperfusion injury [15]. Inhibiting inflammatory response can effectively alleviate ischemia reperfusion injury and reduce infarct area [16]. TNF-α, IL-6, and IL-1β are important inflammatory factors involved in the inflammatory response, which are released in large quantities during ischemia and reperfusion [17]. C-Jun N-terminal kinase (JNK) is a member of the mitogen-activated protein kinase superfamily. The JNK signaling pathway can be activated by cytokines, growth factors, stress, and other factors, and is involved in various physiological processes such as cell proliferation and differentiation, cell apoptosis, and stress response [18, 19]. In addition, JNK signaling pathway is closely related to the activation of autophagy and plays an important role in the occurrence and development of neurodegenerative diseases, ischemia reperfusion injury, and other diseases [20].

Dexmedetomidine (Dex), as a new type of highly selective 2 adrenoceptor agonist, has a good sedative effect and is a commonly used sedative in clinical practice [21]. Studies have found that dexmedetomidine can alleviate ischemia reperfusion injury in rats by reducing oxidative stress and reducing the release of inflammatory mediators [22–25]. However, there is no study that focuses on the mechanism of dexmedetomidine post-conditioning in inflammatory response and autophagy of focal cerebral ischemia reperfusion injury in rats. In the present study, a focal cerebral ischemia model was established in SD rats to explore the effect of dexmedetomidine post-conditioning on the spatial learning and memory ability, cerebral infarction area, the apoptosis, and pathological changes of nerve cells in the hippocampus CA1 region as well as its possible molecular mechanism involved in JNK signaling pathway.

MATERIALS AND METHODS

Animals

One hundred and eighty male healthy adult SD rats, 8–9 weeks of age, weighing 250–270 g, were provided by Jinan Pengyue Experimental Animal Breeding Co. LTD (License number SCXK 2014-0007). The rats were maintained in a SPF grade environment (22–24 °C, 55–60% humidity) on a 12-h light or 12-h dark cycle for a week, with free access to standard pellet diet and water. Animal experiments were conducted in accordance with NIH guidelines (NIH Pub. No. 85-23, revised 1996), and approved by the animal protection and use committee of Shandong University.

Modeling and Experimental Procedure

One hundred and eighty healthy adult rats were randomly divided into six groups (30 rats per treatment group)

for various treatments. There were sham-operated (Sham) group, ischemia reperfusion injury (I/R) group, dexmedetomidine post-conditioning (Dex) group, JNK inhibitor (SP600125) group, dexmedetomidine post-conditioning+ JNK activator (Dex + Anisomycin) group and positive drug nimodipine control (Nim) group.

The model of middle cerebral artery occlusion (MCAO) in rats was established by suture-occluded method. Rats fasted for 12 h and drank freely before surgery. After intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg) to anesthetize the rats, the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were selected as the infarction regions and the detailed steps that were used are the same as those described in previous studies [26, 27]. Sham group: The right ICA was free but not embolized or ligated. Dex group: 3 μg/kg dexmedetomidine was pumped with through the left femoral vein within 5 min during ischemia, the remaining dose was 6 g/(kg h) continuously pumped for 2 h. SP600125 group: The lateral ventricle was injected with 5 μL of SP600125 (6 mg/kg, HY-12041, MCE, USA) 30 min before ischemia. Dex + Anisomycin group: The lateral ventricle was injected with 5 μL of Anisomycin (10 mg/kg) 30 min before ischemia, and the same method was used to pump the same amount of dexmedetomidine in the ischemic state. Nim group: Nimodipine injection (0.2 mg/mL) was given at 1 mg/kg through the right common jugular vein or its branches in the ischemic state. The sham group and I/R group were given the same amount of normal saline.

Morris Water Maze Test

Twenty-four hours after cerebral ischemia reperfusion, the Morris water maze test was conducted to evaluate the spatial learning ability and memory ability of rats. Morris water maze (Huaibei Zhenghua Biological Instruments and Equipment Co., LTD.) is a circular pool, which is divided into four quadrants at four equidistant points on the pool wall, and the platform is placed in the middle of the third quadrant. Put each group of rats into water with their heads facing the wall of the pool, and randomly selected one of the four starting positions of east, west, south, and north. The rats entered the water and recorded the time from entering the water to finding the underwater platform(s) (escape incubation period, EL). If the platform position was not found within 90 s, the rats were guided to the platform position, and the time was recorded as 90 s. After the rats stayed on the platform for 10 s, they were removed from the pool and dried. Each rat was trained four times a day with an interval of 15–20 min. The mean value was taken as the result of the day, and the training lasted for 5 days. On the second day after the place navigation test, the platform was removed and the 90-s space exploration training was started. Put the rats in the water from the first quadrant. The times across platform and the times stayed in the quadrant of the original platform within 90 s were recorded to judge whether the rats had remembered the location of the original safety platform.

TTC Staining

Six rats in each group were randomly selected and intraperitoneally injected with pentobarbital sodium 40 mg/kg for deep anesthesia. The whole brain was cut off rapidly, and five coronal slices were sequentially cut from the frontal pole to the back with rat brain molds, 2 mm thick. Using 10 g/L of TTC solution (Batch no. 30187713, Shanghai Sinopharm Chemical Reagent Co., LTD., China) in 37 °C under dark stain for 15 min. After staining, PBS solution containing 4% paraformaldehyde was used for fixation and preservation. Image J 1.43 (National Institutes of Health, Bethesda, MD, USA) was used to calculate the infarct area of each layer after taking photos layer by layer. The normal brain tissue was stained red and the infarcted tissue was white.

TUNEL Assay

Six rats in each group were randomly selected and intraperitoneally injected with pentobarbital sodium 40 mg/kg for deep anesthesia. The isolated hippocampal tissue was fixed in 4% paraformaldehyde solution after cervical dislocation. The hippocampal tissue was embedded with paraffin and made coronal section (4 μm), and each specimen was cut into five pieces (biological tissue paraffin embedding machine, paraffin slicer, Leica, Germany). After conventional xylene dewax and gradient ethanol dehydration, apoptosis detection kit (Batch number: ZK-8005, Beijing Zhongshan Jinqiao Biotechnology Co., LTD., China) and TUNEL assay were used to quantitatively detect apoptotic nerve cells. Five fields were randomly selected under an optical microscope (BX50/Olympus, Japan) with 400 times (10 eyepieces, 40 objective lens). The normal nuclei were blue, and the positive apoptotic cells were brown-yellow, and the number of positive apoptotic cells was recorded.

Hematoxylin-Eosin (HE) Staining

Hippocampal tissue sections were processed as described in "TUNEL assay". Then the hippocampal tissue sections were stained with hematoxylin (Solarbio, Beijing, China) for 5 min and rinsed with running water. Hippocampal tissue sections were differentiated by hydrochloric acid ethanol for 30 s, soaked in tap water for 15 min, and then stained with eosin stain (Solarbio, Beijing, China) for 2 min. Routine dehydrated, transparency and tablet sealed. The pathological changes of neuron in the hippocampal CA1 region were observed under a 400-fold optical microscope (Olympus BX51, Olympus, Japan).

Enzyme-Linked Immunosorbent Assay (ELISA)

The contents of TNF-α (ABIN625216, RayBiotech), IL-6 (ab100767, abcam), and IL-1β (ab100772, abcam) inflammatory factors in serum were detected by ELISA assay, and the specific operation steps were strictly in accordance with the instructions of the kit.

Transmission Electron Microscopy

Six rats in each group were randomly selected and intraperitoneal injected with pentobarbital sodium 40 mg/kg for deep anesthesia. The isolated hippocampal tissue was fixed in 2.5% glutaraldehyde solution after cervical dislocation. After rinsing with PBS, the hippocampal tissue samples were fixed with 1% osmium acid. After gradient acetone dehydration, the samples were embedded by resin and 1-μm slices were prepared. Ultra-thin slices of 60 nm thickness were prepared after the light microscope was positioned. The ultra-thin sections were stained with lead citrate for 10 min, and then cleaned with CO_2-free double distilled water. The samples were stained with uranium acetate for 30 min, then washed with double distilled water and dried into slices. Transmission electron microscopy (JEM-1400/JEOL, Japan) was used to observe the ultrastructure of neurons in the hippocampal CA1 region and take pictures.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Six rats in each group were randomly selected and intraperitoneally injected with pentobarbital sodium 40 mg/kg for deep anesthesia. After putting into liquid nitrogen, the rat hippocampal tissues were saved to − 80 °C refrigerator. After the hippocampal tissue samples were homogenized, the total RNA was extracted by Trizol (15596018, Invitrogen, Carlsbad, CA, USA) method. When OD260/OD280 was between 1.8 and 2.0, the purity of RNA was qualified. RNA was reversely transcribed into cDNA using reverse transcription kits (Applied Biosystems, Waltham, MA, USA). qRT-PCR used Mastercycler® nexus X2 (Eppendorf, Hamburg, Germany) and the reaction conditions were 95 °C for 15 min, 95 °C for 10 s, and 59 °C for 30 s (40 cycles). The $2^{-\Delta\Delta Ct}$ method was used to process experimental data, and the mRNA of GAPDH was used as internal reference to calculate the relative expression levels of the genes.

Western Blot

The expressions of characteristic proteins in hippocampal tissues were measured by Western blot. Six rats in each group were randomly selected and intraperitoneally injected with pentobarbital sodium 40 mg/kg for deep anesthesia. After being put into liquid nitrogen, the rat hippocampal tissues were saved to − 80 °C refrigerator. After the hippocampal tissue samples were homogenized in RIPA buffer solution, the protein concentration was determined by BCA (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific, Waltham, USA) method. The sample size of SDS-PAGE electrophoresis (Mini-Protean-3, Bio-Rad, Hercules, CA, USA) was 40 g, and the 5% concentrated gel and 12% separation gel were used for the Western blotting analysis. All the primary antibodies were diluted with TBST solution containing 3% bovine serum protein, Beclin-1 (1:1000, ab62557, Abcam, UK), Caspase-3 (1:500, ab13847, Abcam, UK), LC3 (1:2000, ab192890, Abcam, UK), JNK1 (1:2500, ab199380, Abcam, UK), p-JNK1 (1:1000, ab47337, Abcam, UK), and β-actin (1:1000, ab8227, Abcam, UK) and were incubated with goat anti-rabbit IgG (1:2000, ab6721, Abcam, UK) for 1 h. The immune-reactive bands were detected using an enhanced chemiluminescence (ECL) kit. β-Actin was used as internal reference and Image J (NIH) software was used for grayscale scanning and quantification.

Statistical Analysis

SPSS19.0 statistical software was used to analyze the statistical data and the data were expressed as mean ± SD. One-way ANOVA was used for data analysis among multiple groups, and LSD test was used for subsequent analysis. $P < 0.05$ was considered statistically significant.

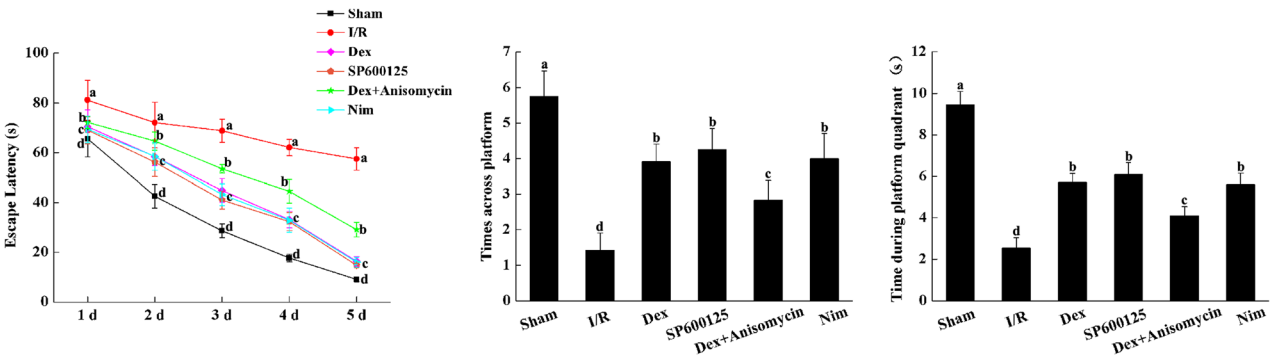

Fig. 1. Morris water maze test. **A** Escape latency (EL); **B** the times across platform; **C** the times stayed in the quadrant of the original platform. Note: The different letters mean significant difference ($P < 0.05$).

RESULTS

Behavioral Competence Test of Rats (Morris Water Maze Test)

Morris water maze experiment was used to evaluate the spatial learning and memory ability of rats. As shown in Fig. 1, compared to the Sham group, the escape latency (EL) were markedly prolonged, while the times across platform and the times stayed in the quadrant of the original platform were decreased in other groups ($P < 0.05$). Compared to the I/R group, the EL were markedly shorter, while the times across platform and during platform quadrant were significantly increased in Dex group, SP600125 group, Dex+Anisomycin group and Nim group ($P < 0.05$). There was no significant difference among Dex group, Nim group, and SP600125 group. Compared to the Dex group, the EL were markedly prolonged, while the times across platform and the during platform quadrant were significantly decreased in Dex + Anisomycin group. The results suggested that both the dexmedetomidine post-conditioning and the JNK pathway inhibitor treatment could improve the learning and memory dysfunction caused by focal cerebral ischemia reperfusion injury in rats.

Determination of the Infarct Area of Brain Tissue by TTC Staining

The infarct area of brain tissue was determined by TTC staining. As shown in Fig. 2, the infarcted areas were stained white and the non-infarcted areas were stained red. No white infarcts were observed in Sham group, while white infarcts of different sizes were observed in I/R group, Dex group, SP600125 group, Dex + Anisomycin group and Nim group. The infarct area of brain tissue in rats was expressed as the percentage of infarcted area to non-infarcted area. The infarct areas of brain tissue in the Dex group, SP600125 group, Dex + Anisomycin group and Nim group were significantly decreased ($P < 0.05$) compared with the I/R group. There was no significant difference in infarct area between the Nim group and the SP600125 group ($P > 0.05$) compared to the Dex group, while the white infarct increased in the Dex + Anisomycin group. The results showed that both the dexmedetomidine post-conditioning and the JNK pathway inhibitor treatment could reduce the infarct area of brain tissue caused by focal cerebral ischemia reperfusion injury in rats.

The Positive Apoptosis and Pathological Changes of Neurons in Hippocampal CA1 Region

TUNEL assay was used to detect the positive apoptosis of neurons in hippocampal CA1 region of rat brain. As shown in Fig. 3A, B, the number of apoptotic cells in the hippocampal CA1 region of Sham group was very small, and the cells were arranged neatly. The number of positive apoptosis of neurons were significantly increased in other groups ($P < 0.05$) compared with the Sham group. Compared to the I/R group, the number of positive apoptosis of neurons were significantly decreased to varying degrees, and the staining degree were decreased in Dex group, SP600125 group, Dex + Anisomycin group and Nim group ($P < 0.05$). Compared to the Dex group, there was no significant difference in apoptosis of neurons between the Nim group and the SP600125 group ($P > 0.05$), while the number of positive apoptosis of neurons was increased and the pathological staining was aggravated in the Dex + Anisomycin group.

HE staining was used to observe the pathological changes of neurons in the hippocampus CA1 region of

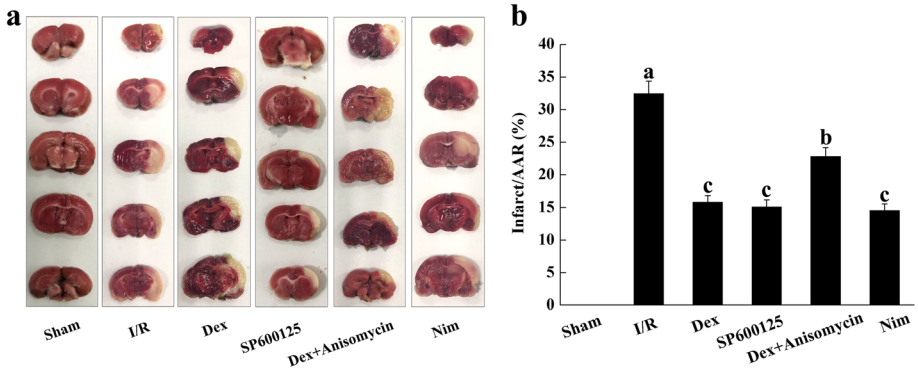

Fig. 2. Effect of dexmedetomidine post-conditioning on the infarct area of brain tissue. TTC staining of brain tissue in rat; **B** percentage of the infarct area of brain tissue. Note: The different letters mean significant difference ($P < 0.05$).

rats. As shown in Fig. 3C, neurons in the hippocampal CA1 region of Sham group were neatly arranged with complete morphology and clear hierarchy (layers 3–5). The morphological structure of neurons in other groups showed different degrees of abnormality compared with the Sham group. Especially in the I/R group, the heterogeneity was the most obvious in showing disordered arrangement of neuron cells, unclear hierarchy, significantly reduced number of neurons, partial nucleoplasmic shrinkage, nucleolus disappearance, and other phenomena. Compared to the I/R group, the degree of the pathological changes of neurons was reduced in the Dex group, SP600125 group, Dex + Anisomycin group, and Nim group. There were no significant differences among the Dex group, Nim group, and SP600125 group, while the degree of the pathological changes of neurons was slightly heavier in the Dex + Anisomycin group.

These results revealed that both the dexmedetomidine post-conditioning and the JNK pathway inhibitor treatment could reduce the positive apoptosis and pathological

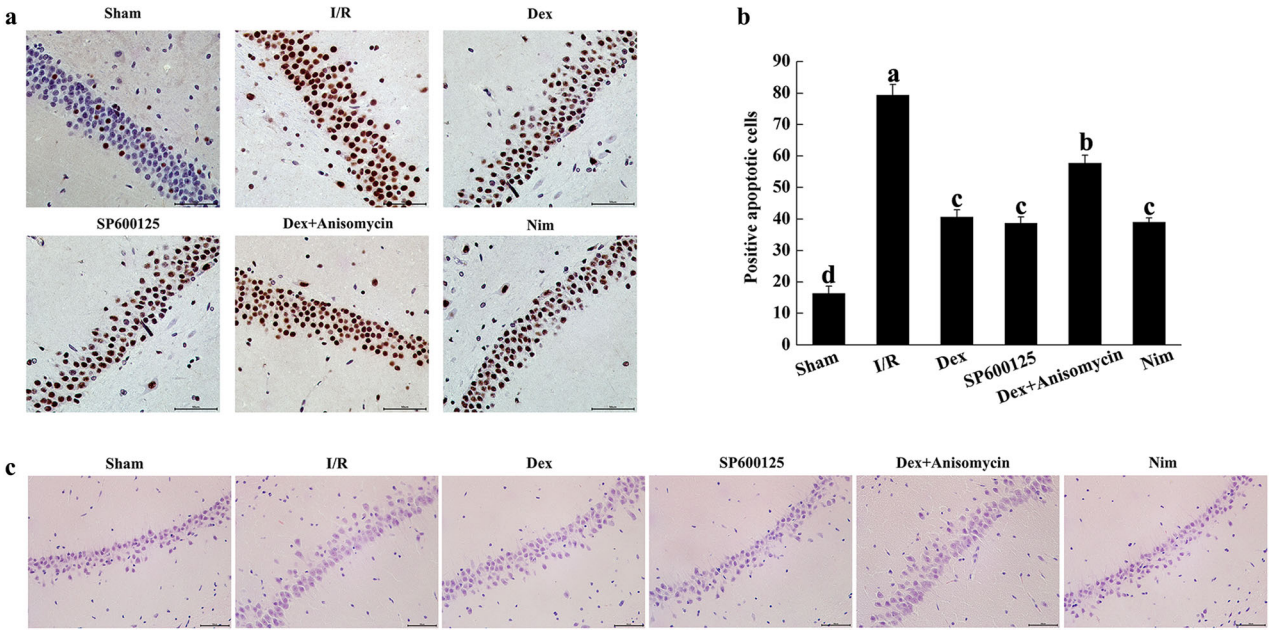

Fig. 3. Effect of dexmedetomidine post-conditioning on the positive apoptosis and pathological changes of neurons in hippocampal CA1 region. **A** TUNEL staining of neurons in hippocampal CA1 region of rats (400×); **B** comparison of the number of apoptotic nerve cells; **C** morphology of hippocampal CA1 in rats (HE, 400×). Note: The different letters mean significant difference ($P < 0.05$).

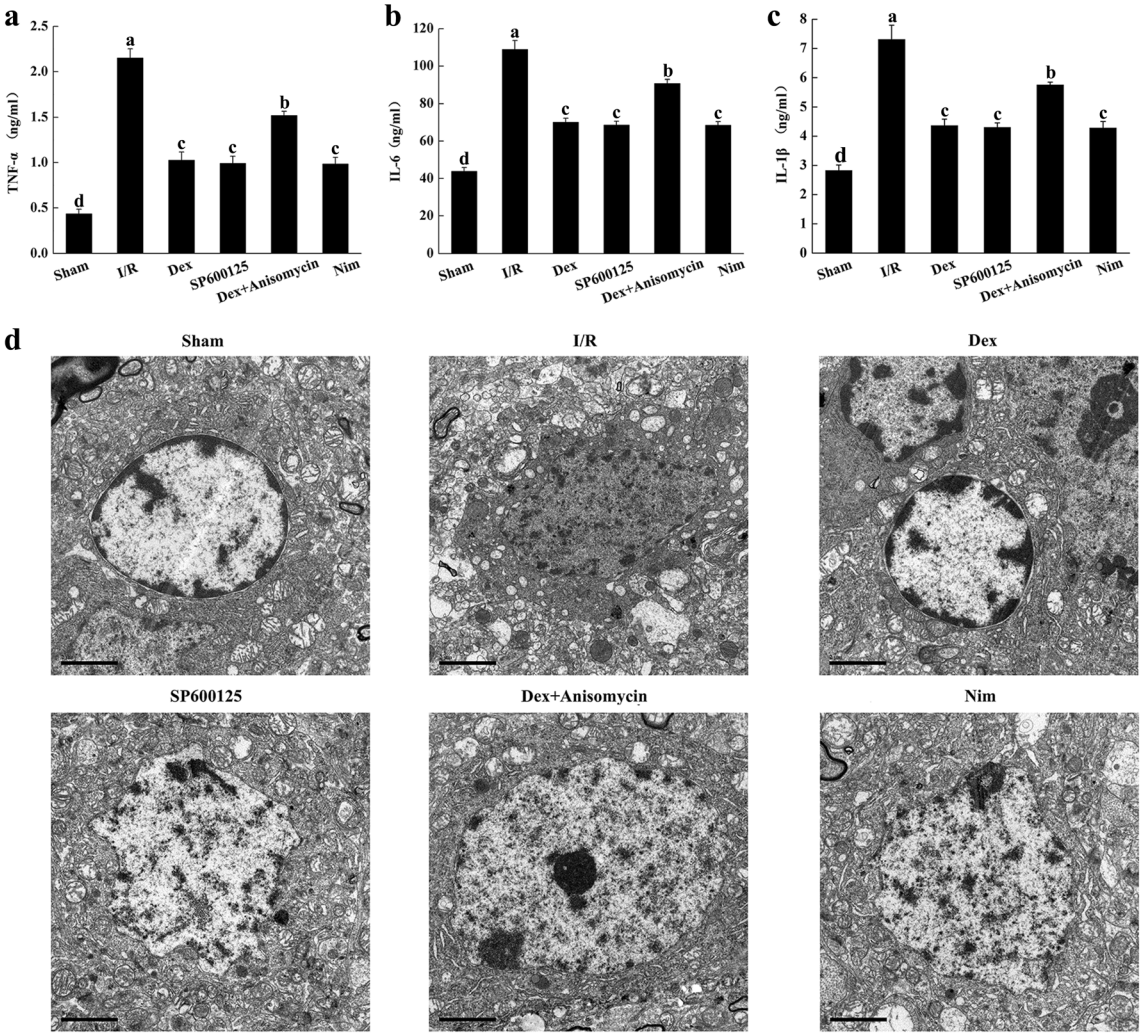

Fig. 4. Effect of dexmedetomidine post-conditioning on the inflammatory response and autophagy effect of MCAO in rats. **A**. The content of TNF-α; **B** the content of IL-6; **C** the content of IL-1β; **D** ultrastructure observation of autophagy of neurons in hippocampal CA1 region of rat brain. Note: The different letters mean significant difference ($P < 0.05$).

changes of neurons in hippocampal CA1 region caused by focal cerebral ischemia reperfusion injury in rats.

Effect of Dexmedetomidine Post-conditioning on the Inflammatory Response and Autophagy Effect of MCAO in Rats

The expression levels of inflammatory factors (TNF-α, IL-6, and IL-1β) in serum of rats were determined by ELISA. As shown in Fig. 4A–C, the contents of TNF-α, IL-6, and IL-1β in other groups were significantly increased ($P < 0.05$) compared to the Sham group. Compared to the I/R group, the contents of TNF-α, IL-6, and IL-1β were significantly decreased in the Dex group, SP600125 group, Dex + Anisomycin group, and Nim group, and the differences were statistically significant ($P < 0.05$). Compared to the Dex group, there was no significant difference in the levels of TNF-α, IL-6, and IL-1β between the Nim group and the SP600125 group ($P > 0.05$), while the contents of TNF-α, IL-6, and IL-1β in the Dex + Anisomycin group were increased. The results showed that both the dexmedetomidine post-conditioning and the JNK pathway inhibitor treatment could reduce the expressions of inflammatory factors caused by focal cerebral ischemia reperfusion injury in rats.

The autophagy in hippocampal CA1 region was observed by transmission electron microscopy. The auto-phagosomes or auto-phagocytes with bilayer or monolayer membrane in cells under electron microscopy is the morphological feature of autophagy. As shown in Fig. 4D, the cells in the hippocampal CA1 region of the Sham group were clearly structured, with normal intracytoplasmic organelles and clear mitochondrial cristae structure, but no lysosome or bilayer membrane structure. In the I/R group, there were obvious edema around the neurons in the hippocampal CA1 region, mitochondrial cristae fracture, and severe vacuolation. The auto-phagosomes of bilayer or monolayer membrane were observed, and the number of lysosomes was increased in the I/R group. Compared to the I/R group, the autophagy was reduced to varying degrees, with the degree of edema and cavitation reduced in the Dex group, the SP600125 group, the Dex + Anisomycin group and the Nim group. There were no significant differences among the Dex group, Nim group, and SP600125 group, while the autophagy was slightly heavier in the Dex + Anisomycin group. The results showed that both the dexmedetomidine post-conditioning and the JNK pathway inhibitor treatment could reduce autophagy effect of focal cerebral ischemia reperfusion injury in rats.

Effect of Dexmedetomidine Post-conditioning on the mRNA and Protein Expressions of Beclin-1, Caspase-3, and LC3 of Brain Tissues in Rats

The qRT-PCR results were shown in Fig. 5A–C, compared to the Sham group, the mRNA expressions of Beclin-1, Caspase-3, and LC3 were significantly increased in other groups ($P < 0.05$). The mRNA expressions of Beclin-1, Caspase-3, and LC3 in the Dex group, SP600125 group, Dex + Anisomycin group and Nim group were significantly decreased ($P < 0.05$) compared with the I/R group. In addition, there were no significant differences among the Dex group, Nim group, and SP600125 group, while the mRNA expressions of Beclin-1, Caspase-3, and LC3 were slightly higher in the Dex + Anisomycin group.

Western blot results were shown in Fig. 5D–G, compared to the Sham group, the protein expressions of Beclin-1, Caspase-3, and LC3II/I were significantly up-regulated in other groups ($P < 0.05$), and the overall change trend was consistent with the qRT-PCR experiment.

Detection of the Activity of JNK Signaling Pathway by Western Blot

Western blot results were shown in Fig. 6, the protein expressions of p-JNK1 in other groups were significantly up-regulated ($P < 0.05$) compared to the Sham group, and there were no significant changes in the total JNK1 content. Compared to the I/R group, the protein expressions of p-JNK1 were significantly decreased in the Dex group, SP600125 group, Dex + Anisomycin group, and Nim group ($P < 0.05$). Furthermore, compared to the Dex group, the protein expression of p-JNK1 was slightly up-regulated in the Dex + Anisomycin group.

DISCUSSION

Dexmedetomidine is a commonly used sedative and analgesic drug in clinical practice, which has the effect of improving ischemia reperfusion injury [28]. Dexmedetomidine post-conditioning could significantly reduce the content of malondialdehyde (MDA) and nitric oxide (NO) in the hippocampal tissue in the animal model of whole cerebral ischemia, which had a neuroprotective effect [29, 30]. In the present study, a model of focal cerebral ischemia reperfusion injury in SD rats was established to explore the protective effect of dexmedetomidine on focal cerebral ischemia reperfusion injury in rats and its possible mechanism. Morris water maze and TTC staining results showed that the dexmedetomidine post-conditioning could significantly shorten the EL compared to the I/R group, and could increase the times across platform and during platform quadrant, as well as reduce the proportion of cerebral infarction area. The results indicated that the dexmedetomidine post-conditioning could improve the spatial learning and memory ability of rats with focal cerebral ischemia reperfusion and reduce the cerebral infarction area.

Autophagy is widely found in neurodegenerative diseases, peripheral nerve injury, brain injury, and other neurological diseases, and is involved in the physiological and pathological processes of neurological diseases. Focal cerebral ischemia reperfusion injury in rats can activate autophagy and promote the formation of auto-phagosomes and lysosomal activation in ischemic penumbra [31, 32]. The auto-phagosomes or auto-phagocytes with bilayer or monolayer membrane in cells under electron microscopy is the morphological feature of autophagy [33]. Our study found that dexmedetomidine post-conditioning could reduce the apoptosis and pathological changes of nerve cells caused by focal cerebral ischemia reperfusion injury in rats, and reduce the

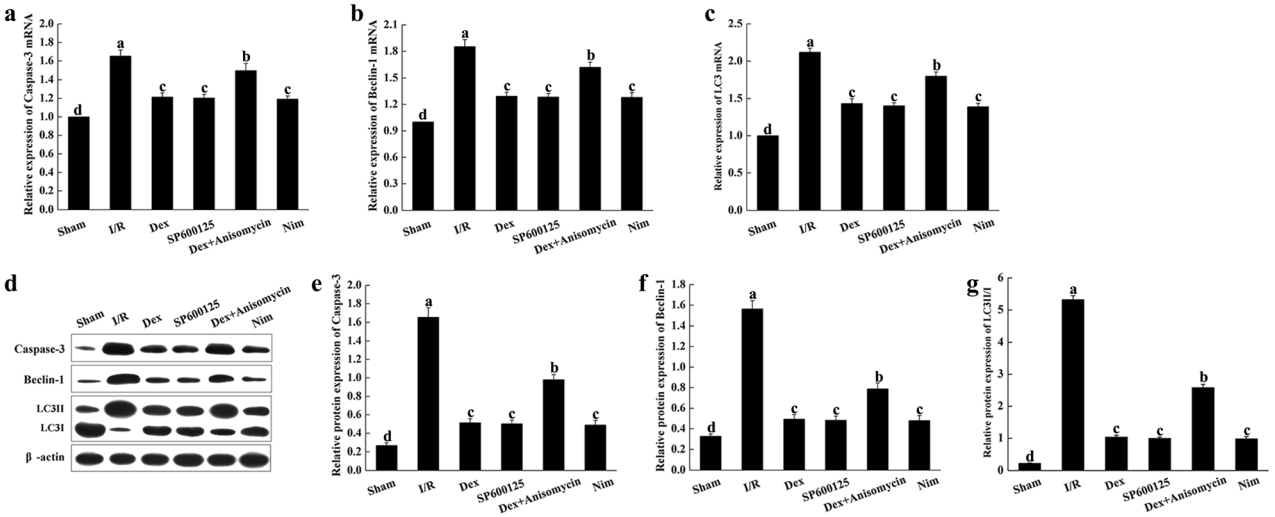

Fig. 5. The detection of mRNA and protein expressions of Beclin-1, Caspase-3, LC3 by qRT-PCR and Western blot. **A** the mRNA expression of Beclin-1; **B** the mRNA expression of Caspase-3; **C** the mRNA expression of LC3; **D** protein banding; **E** the protein expression of Beclin-1; **F** the protein expression of Caspase-3; **G** the protein expression of LC3II/I. Note: The different letters mean significant difference ($P < 0.05$).

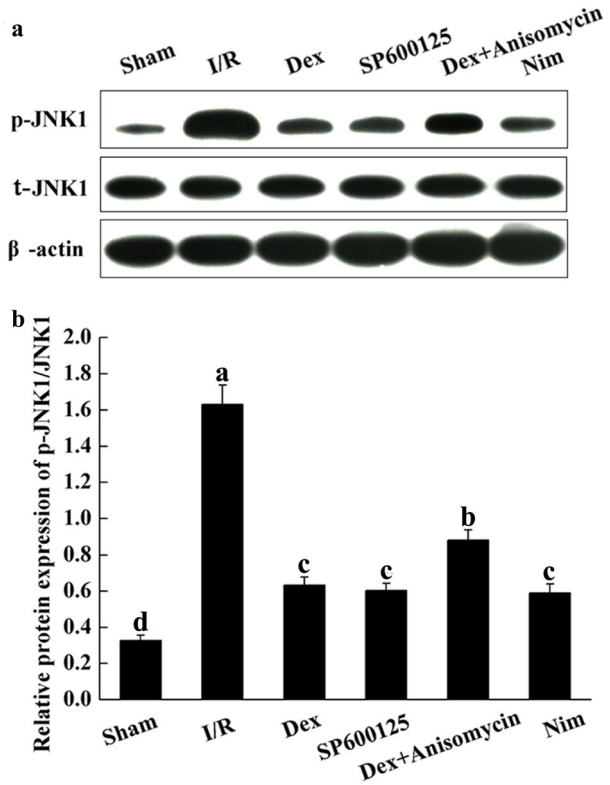

Fig. 6. The detection of protein expression of JNK1 by Western blot. **A** Protein banding; **B** the protein expression of p-JNK1 /JNK1. Note: The different letters mean significant difference ($P < 0.05$).

number of auto-phagosomes, which indicated that dexmedetomidine post-conditioning could reduce the autophagy effect activated by focal cerebral ischemia reperfusion injury in rats. In addition, the formation of autophagy is related to autophagy-related genes and microtubule-related protein 3 (LC3). LC3 is mainly involved in the modification of autophagy vesicles, and its protein expression level is an important indicator of autophagy detection. Microtubule associated protein LC3-I and autophagy membrane on the surface of phosphatidyl ethanolamine form LC3-II in the process of autophagy. Because of the difference in molecular weight, two different bands can be formed in Western blot. Therefore, the LC3-II and LC3-I ratio can reflect the strength of the autophagy. Beclin-1 is a specific gene involved in autophagy in mammals, and the expression level of Beclin-1 can be detected to dynamically monitor the autophagy activity of cells [34]. Our results showed that dexmedetomidine post-conditioning could significantly down-regulate the mRNA and protein expressions of Beclin-1, Caspase-3, and LC3II/I, which indicated that dexmedetomidine could reduce the autophagy effect activated by focal cerebral ischemia reperfusion injury in rats by inhibiting the expressions of autophagy-related genes and proteins.

Inflammatory response is one of the mechanisms involved in the process of ischemia reperfusion injury. TNF-α, IL-6, and IL-1β are important inflammatory factors involved

in the inflammatory response, which are released in large quantities during ischemia and reperfusion. Wang et al. found that isoquercetin played a neuroprotective role by inhibiting the phosphorylation of ERK1/2, JNK1/2, and p38 mitogen-activated protein kinase (MAPK) and the release of TNF-α, IL-1β, and IL-6 [35]. Beatriz et al. found that Allopurinol protected ischemic kidneys through a mechanism associated with downregulation of TNF-α, IL-1 β, and IL-6 [17]. In the present study, we found that the concentrations of TNF-α, IL-6, and IL-1β in the I/R group were higher than those in the Sham group, which indicated that focal cerebral ischemia reperfusion in rats might promote the activation of inflammatory cells. Also, the serum concentrations of TNF-α, IL-6, and IL-1β in the Dex group were lower than those in the I/R group, which suggested that dexmedetomidine could reduce the inflammatory response induced by focal cerebral ischemia reperfusion.

JNK signaling pathway was found to be related to the activation of autophagy [36, 37]. The results shown that the protein expression of p-JNK was significantly down-regulated after dexmedetomidine post-conditioning, which indicated that dexmedetomidine might play a certain inhibitory effect on the JNK signaling pathway. Also, we found that JNK pathway inhibitor treatment (SP600125 group) could effectively improve the brain injury caused by focal cerebral ischemia in rats, and the results were not significantly different from those in the Dex group ($P > 0.05$). Activation of JNK signaling pathway can induce the autophagy and apoptosis of nerve cells [38], dexmedetomidine could inhibit the formation of inflammatory mediators *in vivo*, and could inhibit the activation of JNK signaling pathway, as well as down-regulate the level of autophagy, which might involve in alleviating the degree of focal cerebral ischemia reperfusion injury in rats. We speculated that this might be the neuroprotective mechanism of dexmedetomidine.

CONCLUSIONS

In conclusion, the present study assessed the effect of dexmedetomidine post-conditioning on the inflammatory response and autophagy effect of myocardial ischemia-reperfusion injury in rats. The results showed that the dexmedetomidine post-conditioning could improve the learning and memory dysfunction caused by focal cerebral ischemia reperfusion injury in rats, and reduce the inflammatory response and autophagy effect. The mechanism of the effect might be related to the inhibition of JNK pathway activation, and to affect the expressions of inflammatory factors and autophagy-related proteins.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

REFERENCES

1. Chen, F.Y., X.Y. Fang, and H. Zhang. 2018. Effect of polydatin on expression of p53 and Notch1 in brain tissue of ischemic cerebrovascular disease. *Journal of Biological Regulators and Homeostatic Agents* 32 (1): 133.
2. Hartley, A., D.C. Marshall, J.D. Salciccioli, M.B. Sikkel, M. Maruthappu, and J. Shalhoub. 2016. Trends in mortality from ischemic heart disease and cerebrovascular disease in EuropeClinical PERSPECTIVE: 1980 to 2009. *Circulation* 133 (20): 1916–1926.
3. Balestrino, M., M. Sarocchi, E. Adriano, and P. Spallarossa. 2016. Potential of creatine or phosphocreatine supplementation in cerebrovascular disease and in ischemic heart disease. *Amino Acids* 48 (8): 1955–1967.
4. Wang, M., J. Wang, Z. Liu, X. Guo, N. Wang, N. Jia, Y. Zhang, and J. Yuan. 2017. Effects of intermedin on autophagy in cerebral ischemia/reperfusion injury. *Neuropeptides* 68: 15-21.
5. Morselli, E., M.C. Maiuri, M. Markaki, E. Megalou, A. Pasparaki, K. Palikaras, A. Criollo, L. Galluzzi, S.A. Malik, and I. Vitale. 2010. The life span-prolonging effect of Sirtuin-1 is mediated by autophagy. *Autophagy* 6 (1): 186–188.
6. Bao, H., A. Benavides, Y. Shi, P. Frost, and A. Lichtenstein. 2009. Effect of autophagy on multiple myeloma cell viability. *Molecular Cancer Therapeutics* 8 (7): 1974.
7. Dosenko, V.E., V.S. Nagibin, L.V. Tumanovska, and A.A. Moibenko. 2006. Protective effect of autophagy in anoxia-reoxygenation of isolated cardiomyocyte? *Autophagy* 2 (4): 305–306.
8. Gu, Y., C. Wang, and A. Cohen. 2004. Effect of IGF-1 on the balance between autophagy of dysfunctional mitochondria and apoptosis. *FEBS Letters* 577 (3): 357–360.
9. Zhang, X., Y. Dong, X. Zeng, X. Liang, X. Li, W. Tao, H. Chen, Y. Jiang, L. Mei, and S.S. Feng. 2014. The effect of autophagy inhibitors on drug delivery using biodegradable polymer nanoparticles in cancer treatment. *Biomaterials* 35 (6): 1932–1943.
10. Lu, N., B. Wang, X. Deng, H. Zhao, Y. Wang, and D. Li. 2014. Autophagy occurs within an hour of adenosine triphosphate treatment after nerve cell damage:the neuroprotective effects of adenosine triphosphate against apoptosis. *Neural Regeneration Research* 9 (17): 1599–1605.
11. Wang, L., P. Wang, H. Dong, S. Wang, H. Chu, W. Yan, and X. Zhang. 2018. Ulk1/FUNDC1 prevents nerve cells from hypoxia-induced apoptosis by promoting cell autophagy. *Neurochemical Research* 43 (8): 1539–1548.
12. Cherra, S.J., S.M. Kulich, G. Uechi, M. Balasubramani, J. Mountzouris, B.W. Day, and C.T. Chu. 2010. Regulation of the autophagy protein LC3 by phosphorylation. *The Journal of Cell Biology* 190 (4): 533–539.

13. Tanida, I., N. Minematsu-Ikeguchi, T. Ueno, and E. Kominami. 2005. Lysosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. *Autophagy* 1 (2): 84–91.
14. Ashkenazi, A., C.F. Bento, T. Ricketts, M. Vicinanza, F. Siddiqi, M. Pavel, F. Squitieri, M.C. Hardenberg, S. Imarisio, and F.M. Menzies. 2017. Polyglutamine tracts regulate beclin 1-dependent autophagy. *Nature* 545 (7652): 108–111.
15. Kawamura, N., A.M. Schmeichel, Y. Wang, J. Schmelzer, and P. Low. 2006. Multiple effects of hypothermia on inflammatory response following ischemia-reperfusion injury in experimental ischemic neuropathy. *Experimental Neurology* 202 (2): 487–496.
16. Shin, I.W., I.S. Jang, S.M. Lee, K.E. Park, S.H. Ok, J.T. Sohn, H.K. Lee, and Y.K. Chung. 2011. Myocardial protective effect by ulinastatin via an anti-inflammatory response after regional ischemia/reperfusion injury in an in vivo rat heart model. *Korean Journal of Anesthesiology* 61 (6): 499–505.
17. Prieto-Moure, B., J.M. Lloris-Carsí, M. Belda-Antolí, L.H. Toledo-Pereyra, and D. Cejalvo-Lapeña. 2016. Allopurinol protective effect of renal ischemia by downregulating TNF-α, IL-1β, and IL-6 response. *Journal of Investigative Surgery* 30 (3): 143–151.
18. Xiang, Y., W. Ye, C. Huang, B. Lou, J. Zhang, D. Yu, X. Huang, B. Chen, and M. Zhou. 2017. Brusatol inhibits growth and induces apoptosis in pancreatic cancer cells via JNK/p38 MAPK/NF-κb/Stat3/Bcl-2 signaling pathway. *Biochemical and Biophysical Research Communications* 487 (4): 820–826.
19. Zhong, L., Z.L. Zhang, X. Li, C. Liao, P. Mou, T. Wang, Z. Wang, Z. Wang, M. Wei, and H. Xu. 2017. TREM2/DAP12 complex regulates inflammatory responses in microglia via the JNK signaling pathway. *Frontiers in Aging Neuroscience* 9: 204.
20. Ge, X.H., G.J. Zhu, D.Q. Geng, H.Z. Zhang, J.M. He, A.Z. Guo, L.L. Ma, and D.H. Yu. 2017. Metformin protects the brain against ischemia/reperfusion injury through PI3K/Akt1/JNK3 signaling pathways in rats. *Physiology & Behavior* 170: 115–123.
21. Hoy, S.M., and G.M. Keating. 2011. Dexmedetomidine: a review of its use for sedation in mechanically ventilated patients in an intensive care setting and for procedural sedation. *Drugs* 71 (11): 1481–1501.
22. Gu, J., P. Sun, H. Zhao, H.R. Watts, R.D. Sanders, N. Terrando, P. Xia, M. Maze, and D. Ma. 2011. Dexmedetomidine provides renoprotection against ischemia-reperfusion injury in mice. *Critical Care* 15 (3): R153.
23. Ibacache, M., G. Sanchez, Z. Pedrozo, F. Galvez, C. Humeres, G. Echevarria, J. Duaso, M. Hassi, L. Garcia, and G. Díazaraya. 2012. Dexmedetomidine preconditioning activates pro-survival kinases and attenuates regional ischemia/reperfusion injury in rat heart. *Biochimica et Biophysica Acta* 1822 (4): 537–545.
24. Si, Y., H. Bao, L. Han, H. Shi, Y. Zhang, L. Xu, C. Liu, J. Wang, X. Yang, and A. Vohra. 2013. Dexmedetomidine protects against renal ischemia and reperfusion injury by inhibiting the JAK/STAT signaling activation. *Journal of Translational Medicine* 11: 141.
25. Yeda, X., L. Shaoqing, H. Yayi, Z. Bo, W. Huaxin, C. Hong, X. Zhongyuan, X. Yeda, L. Shaoqing, and H. Yayi. 2017. Dexmedetomidine protects against renal ischemia and reperfusion injury by inhibiting the P38-MAPK/TXNIP signaling activation in streptozotocin induced diabetic rats. *Acta Cirúrgica Brasileira* 32 (6): 429–439.
26. Huang, J., X. Ye, Y. You, W. Liu, Y. Gao, S. Yang, J. Peng, Z. Hong, J. Tao, and L. Chen. 2014. Electroacupuncture promotes neural cell proliferation in vivo through activation of the ERK1/2 signaling pathway. *International Journal of Molecular Medicine* 33 (6): 1547–1553.
27. Xie, G., S. Yang, A. Chen, L. Lan, Z. Lin, Y. Gao, J. Huang, J. Lin, J. Peng, and J. Tao. 2013. Electroacupuncture at Quchi and Zusanli treats cerebral ischemia-reperfusion injury through activation of ERK signaling. *Experimental and Therapeutic Medicine* 5 (6): 1593–1597.
28. Cheng, J., P. Zhu, H. Qin, X. Li, H. Yu, H. Yu, and X. Peng. 2018. Dexmedetomidine attenuates cerebral ischemia/reperfusion injury in neonatal rats by inhibiting TLR4 signaling. *The Journal of International Medical Research* 46 (7): 2925–2932.
29. Dong, J., X. Guo, S. Yang, and L. Li. 2017. The effects of dexmedetomidine preconditioning on aged rat heart of ischaemia reperfusion injury. *Research in Veterinary Science* 114: 489–492.
30. Riquelme, J.A., F. Westermeier, A.R. Hall, J.M. Vicencio, Z. Pedrozo, M. Ibacache, B. Fuenzalida, L. Sobrevia, S.M. Davidson, and D.M. Yellon. 2016. Dexmedetomidine protects the heart against ischemia-reperfusion injury by an endothelial eNOS/NO dependent mechanism. *Pharmacological Research* 103: 318–327.
31. Li, W.L., S.P. Yu, D. Chen, S.S. Yu, Y.J. Jiang, T. Genetta, and L. Wei. 2013. The regulatory role of NF-κB in autophagy-like cell death after focal cerebral ischemia in mice. *Neuroscience* 244: 16–30.
32. Zheng, Y., J. Hou, J. Liu, M. Yao, L. Li, B. Zhang, H. Zhu, and Z. Wang. 2014. Inhibition of autophagy contributes to melatonin-mediated neuroprotection against transient focal cerebral ischemia in rats. *Journal of Pharmacological Sciences* 124 (3): 354–364.
33. Eskelinen, E.L., F. Reggiori, M. Baba, A.L. Kovács, and P.O. Seglen. 2011. Seeing is beieving: the impact of electron microscopy on autophagy research. *Autophagy* 7 (9): 935–956.
34. Kang, R., H.J. Zeh, M.T. Lotze, and D. Tang. 2011. The Beclin 1 network regulates autophagy and apoptosis. *Cell Death and Differentiation* 18 (4): 571–580.
35. Wang, C.P., Y.W. Shi, M. Tang, X.C. Zhang, Y. Gu, X.M. Liang, Z.W. Wang, and F. Ding. 2017. Isoquercetin ameliorates cerebral impairment in focal ischemia through anti-oxidative, anti-inflammatory, and anti-apoptotic effects in primary culture of rat hippocampal neurons and hippocampal CA1 region of rats. *Molecular Neurobiology* 54 (3): 2126–2142.
36. Haberzettl, P., and B.G. Hill. 2013. Oxidized lipids activate autophagy in a JNK-dependent manner by stimulating the endoplasmic reticulum stress response. *Redox Biology* 1: 56–64.
37. Zhou, Y.Y., Y. Li, W.Q. Jiang, and L.F. Zhou. 2015. MAPK/JNK signalling: a potential autophagy regulation pathway. *Bioscience Reports* 35: 3.
38. Gomez-Sanchez, J.A., L. Carty, M. Iruarrizaga-Lejarreta, M. Palomo-Irigoyen, M. Varela-Rey, M. Griffith, J. Hantke, N. Macias-Camara, M. Azkargorta, and I. Aurrekoetxea. 2015. Schwann cell autophagy, myelinophagy, initiates myelin clearance from injured nerves. *The Journal of Cell Biology* 210 (1): 153–168.