



The role of Wnt/ β -catenin pathway in the protection process by dexmedetomidine against cerebral ischemia/reperfusion injury in rats

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ABSTRACT

Aims: To assess the role of glycogen synthase kinase-3 β (GSK3 β) and β -catenin in the protection of ischemic injury by dexmedetomidine (Dex).

Main methods: Adult male Sprague-Dawley rats were subjected to (middle cerebral artery occlusion, MCAO) for 2 h followed by reperfusion and Dex was administered 30min before MCAO. The neurological deficit score, cerebral infarct size and neuron survival were evaluated at 24 h after reperfusion. The expression of pAKT, pGSK3 β and β -catenin in the ischemic penumbra was assayed by Western blot at 2 h after reperfusion.

Key findings: We found that the Dex-induced increment of neuron survival in the ischemic penumbra was diminished by the PI3K inhibitor LY294002 and the β -catenin inhibitor XAV939, respectively. The increasing expression of pAKT, pGSK3 β and β -catenin induced by Dex was markedly inhibited by LY294002. And the increasing expression of β -catenin in nuclei induced by Dex was markedly inhibited by XAV939. At the same time, the GSK3 β inhibitor SB216763 also caused an increment of neuron survival and an increasing expression of pGSK3 β and β -catenin in the ischemic penumbra.

Significance: Our data suggested that treatment with Dex reduced cerebral injury in rats exposed to cerebral ischemia-reperfusion (I/R) by the activation of the PI3K/AKT/GSK3 β pathways as well the activation of downstream Wnt/ β -catenin pathway. And the Wnt/ β -catenin pathway may play an important role in the protection against cerebral ischemia/reperfusion injury in rats.

1. Introduction

Ischemic stroke is a leading cause of death or long-term disability. Once it occurs, the goal of clinical treatment is to restore the blood supply as soon as possible, allowing timely supply of oxygen to ischemic brain tissue, and minimize the consequent ischemia-reperfusion (I/R) injury. In previous studies [1], researchers have found that during cerebral ischemia, the infarction focus can be divided into ischemic penumbra and ischemic core based on histopathology and pharmacological characteristics. Ischemic penumbra is the transition zone between the ischemic core and non-ischemic areas, which still has a small amount of collateral arterial blood supply after ischemia, with lighter ischemic extent than the ischemic core area. The function of ischemic penumbra cells could possibly be restored after ischemia, so researchers pay more attention to penumbra in the process of ischemia-reperfusion, hoping to rescue more neurons [2]. However, due to surgery, anesthesia and other reasons, perioperative ischemic stroke is often difficult to detect in time and result in poor prognosis.

Dexmedetomidine (Dex) is a highly selective α 2-adrenoceptor agonist with central anti-sympathetic effects that can produce sedative, analgesic, diuretic and anti-anxiety effects. It is widely used during perioperative period. In recent years, dexmedetomidine has been demonstrated to provide protective effect on ischemia-reperfusion injury in brain, heart [3], kidney [4], liver [5], gastrointestinal tract and other organs and tissues [6,7]. The mechanisms underlying this protection have always been the focus of research. A large number of studies have shown that activation of the phosphatidylinositol-3kinase/protein kinase B (PI3K/AKT) signaling pathway has protective effects in a variety of ischemic tissues [8–10]. A previous study has shown that during cerebral ischemia and reperfusion, dexmedetomidine can promote the phosphorylation of downstream glycogen synthase kinase-3 β (GSK3 β) protein by activating the PI3K/AKT signaling pathway, thus providing brain protection in the process of ischemia and reperfusion [11]. GSK3 β is involved in multiple signaling pathways and the Wnt signaling is one of them [12]. Wnt/ β -catenin is the classic pathway, in which β -catenin functions as a downstream effector molecule for GSK3 β . GSK3 β can

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phosphorylate β -catenin and degrade it in the cytoplasm [13,14]. The enzymatic activity of GSK3 β can be attenuated by phosphorylating Ser-9. Inhibition of GSK3 β activity therefore leads to stabilization and accumulation of β -catenin in the cytosol, which is shuttled into the nucleus where it binds to the transcription factor TCF/LEF to form transcription complexes, activating or inhibiting important target genes. For the past few years, researchers have found that activation of the Wnt/ β -catenin signaling pathway in the process of ischemia and reperfusion produces corresponding organ protective effects in kidney [15,16], liver [17,18], myocardia and brain [19]; However, other researchers have stimulated organ protection by inhibiting Wnt/ β -catenin signaling through drug intervention [20]. Because there is not much relevant research, the role of the Wnt/ β -catenin signaling pathway in the process of organ ischemia and reperfusion remains controversial.

So here we used (middle cerebral artery occlusion, MCAO) induced cerebral I/R rat model to observe the activation of PI3K/AKT/GSK3 β and Wnt/ β -catenin pathways in ischemia penumbra and explore the role of these two pathways in the protection by dexmedetomidine against cerebral ischemia-reperfusion injury.

2. Materials and methods

2.1. Animals

The study was approved by the Ethical Committee on Animal Experiments of China Medical University. All experiments were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Healthy male Sprague-Dawley (SD) rats (7 weeks old, body weight 220–300 g) were purchased from Beijing Huafukang Biotechnology Co., Ltd. (China).

2.2. Reagents and drugs

Dexmedetomidine (Dex) was provided by Hengrui Pharmaceutical Co., Ltd. (Jiangsu, China). LY294002 (PI3K inhibitor), SB216763 (GSK-3 inhibitor) and XAV939 (inhibitor of Wnt/ β -catenin signaling) were purchased from Sigma-Aldrich Co., LLC. (St. Louis, MO, USA).

2.3. Animal model

The cerebral I/R model was produced by MCAO as described in a previous study [21]. Briefly, the MCAO device consisted of two pieces: an occluder filament (made from a fishing line about 6 cm in length and 0.26 mm in diameter with a rounded tip) and a guide sheath (made from a polyethylene catheter). After the rat was anesthetized with 1% pentobarbital (50 mg/kg, i.p), the left common carotid artery (CCA), the external carotid artery (ECA) and the internal carotid artery (ICA) were carefully isolated under sterile conditions. The CCA and ECA were ligated, and the ICA was clamped. Then a fishing line was carefully inserted from the left CCA into the internal carotid to occlude the origin of the left middle cerebral artery through a guide sheath. And the fishing line was fixed by ligation. After 120 min of occlusion [2], the fishing line was withdrawn to allow reperfusion. The rat was maintained under effective anesthesia with additional doses of pentobarbital (15 mg/kg, i.p) and rectal temperature was maintained at 37 °C throughout the procedure. Rats in the sham-operated group were subjected of the same surgical procedure, but without occlusion of the middle cerebral artery.

2.4. Drug delivery

Dex was infused through the tail vein for 30 min (a bolus of 6 μ g/kg) before the onset of ischemia, then continuously infused for another 2 h (9 μ g/kg) during and after cerebral ischemia-reperfusion injury done. Rats in the sham-operated group were intravenously infused with the solvent of Dex (0.9% NaCl) at the same volume as that in Dex-treated

rats. A burr-hole was drilled in the skull (1 mm posterior and 1.5 mm lateral to bregma) for intracerebroventricular (i.c.v.) injection of 10 μ l PI3K inhibitor LY294002 (2 mM), 10 μ l β -catenin inhibitor XAV939 (0.5 mM), 10 μ l GSK3 β inhibitor SB216763 (1 mM), or the solvent (0.9% NaCl with 10% DMSO) 30 min before ischemia using a microinjector. All these inhibitors were dissolved in 10% dimethylsulfoxide (DMSO).

2.5. Neurologic deficit scoring in rats

Evaluation of the neurologic deficit in rats at 24 h of reperfusion was based on the method of Longa et al. as follows [22]. 0 points: normal, no neurologic deficit; 1 point: contralateral forepaw could not be fully extended, a mild focal neurologic deficit; 2 points: when walking, rats circled to the contralateral (paralyzed) side, a moderate focal neurologic deficit; 3 points: when walking, rats fell to the contralateral (paralyzed) side, a severe focal neurologic deficit; 4 points: did not walk spontaneously and had a depressed level of consciousness.

2.6. Cerebral infarct size assay

After neurologic assessment, rats under anesthesia were decapitated, and the brain was removed quickly. Each brain was quickly placed in a –20 °C freezer after removal of the olfactory bulb and cerebellum. Frozen rat brains were sectioned into 5 coronal slices. Each slice was about 2 mm thick, and were weighed and recorded individually. The brain slices were placed in 2,3,5-triphenyltetrazolium chloride (TTC) staining solution (Nanjing Jiancheng Research Institute of Bioengineering, Nanjing, China) and photographed after incubation at 37 °C for 15 min. Active brain tissue contains dehydrogenase, which reduces TTC to red, showing red dye. Dehydrogenase activity in infarcted brain tissue disappeared, so infarcted tissue remained unstained. Therefore, slices showed two colors after staining: normal brain tissue was red, while the infarct area was white. Images were processed with ImageJ software, and the degree of cerebral infarction was represented by the ratio between the infarct area and the entire brain area.

2.7. Nissl stain and neuron counts

After 24 h of reperfusion, two rats in each group were randomly selected and perfused intracardially with 0.1 mol/L PBS and 4% paraformaldehyde. Brain slices were collected and processed into paraffin slices. We prepared 2-mm coronal slices posterior to the chiasma opticum for Nissl staining. Morphological observation of brain tissue was performed under high-power light microscopy and the number of morphologically normal (surviving) neurons in the ischemic penumbra was counted using ImageJ by an investigator who was blind to the experimental condition.

2.8. Western blotting

After 2 h of reperfusion, the rats were anesthetized and decapitated, with brains collected on ice. Brain tissue sections 4-mm thick were cut at 3 mm and 7 mm from the front end of the frontal coronary. The middle part 2 mm from the sagittal suture on both sides was removed (blood supply to this part relies on the anterior cerebral artery). For the remaining left and right sides of the brain block, sections were cut 2 mm from the sagittal side and 30° oblique to the sagittal section. The lateral area was the ischemic core area (core), while the medial area was the penumbra [1], which were quickly placed at –80 °C for storage.

Total protein was isolated from the brains by homogenization in sample buffer (RIPA/PMSF/phosphatase inhibitors = 98/1/1) and centrifuged at 12,000 g for 30 min at 4 °C. Nuclear and cytoplasmic protein was extracted respectively by Nuclear and cytoplasmic protein extraction kit (Sangon Biotech Co., Ltd., Shanghai, China). Protein concentrations were calculated by BCA protein assay kit (Beyotime

Biotechnology Co., Jiangsu, China). The proteins (50 μ g, nuclear protein 25 μ g) were denatured at 100 °C for 5 min and separated by 12% SDS-PAGE, in a Tris-glycine-SDS buffer. Separated proteins were transferred to PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% skim milk for 1h at room temperature, and then incubated with a 1:1000 dilution of the primary antibodies. The following primary antibodies were used: Akt (pan) (C67E7) Rabbit mAb (Cell Signaling Technology, Beverly, MA., USA), Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (Cell Signaling Technology), GSK-3 β (D5C5Z) XP® Rabbit mAb (Cell Signaling Technology), Phospho-GSK-3 β (Ser9) (D85E12) XP® Rabbit mAb (Cell Signaling Technology), β -Catenin (D10A8) XP® Rabbit mAb (Cell Signaling Technology), GAPDH (D16H11) XP® Rabbit mAb (Cell Signaling Technology) and Lamin B Rabbit Ab (Wanlei Biotechnology Co., Shenyang, China). The antibodies were diluted in 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBST), and applied overnight at 4 °C. Membranes were washed with TBST three times and incubated with HRP-conjugated secondary antibodies (goat anti-rabbit IgG-HRP Secondary Antibody, Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China). Protein bands were detected using the ECL luminous liquid (Beyotime).

2.9. Protocol

As shown in Fig. 1, All Rats were randomly divided into seven groups (n = 10 in each group): (i) sham-operated: rats were subjected to the same surgical procedures except for MCAO and drug administration; (ii) I/R: rats were subjected to MCAO for 2 h (intravenously infused with the same volume of 0.9% NaCl) and reperfusion; (iii) Dex: rats were subjected to MCAO for 2 h (intravenously infused with Dex) and reperfusion; (iv) Dex + LY: rats were given LY (2 mM, 10 μ l, i.c.v.) before MCAO, Dex and reperfusion; (v) I/R + XAV: rats were given XAV (0.5 mM, 10 μ l, i.c.v.) before MCAO, 0.9% NaCl and reperfusion; (vi) Dex + XAV: rats were given XAV (0.5 mM, 10 μ l, i.c.v.) before MCAO, Dex and reperfusion; and (vii) I/R + SB: rats were given SB (1 mM, 10 μ l, i.c.v.) before MCAO, 0.9% NaCl and reperfusion.

2.10. Statistical analysis

All data are representative of 6 independent experiments and are as MEAN \pm SD. SPSS 22.0 statistical software (IBM, Armonk, NY, USA) was used for analysis. Univariate ANOVA analysis was employed, with $p < 0.05$ considered statistically significant.

3. Results

3.1. Effect of Dex on neurological deficit score (Longa score), and cerebral infarct size in rats subjected to focal cerebral I/R

The Longa score and cerebral infarct area size were all increased in the I/R group compared with the sham group (I/R: 2.00 ± 0.63 vs. Sham: 0.00 ± 0.00 , one-way ANOVA, $p < 0.01$, Fig. 2a). Treatment with Dex or SB21673 markedly diminished the increment of Longa score (Dex: 0.67 ± 0.52 vs. I/R: 2.00 ± 0.63 , one-way ANOVA, $p < 0.05$; I/R + SB: 0.67 ± 0.82 vs. I/R: 2.00 ± 0.63 , one-way ANOVA, $p < 0.05$, Fig. 2a) and cerebral infarct size induced by I/R (Dex: 4.41 ± 3.50 vs. I/R: 19.83 ± 5.42 , one-way ANOVA, $p < 0.05$; I/R + SB: 3.23 ± 0.85 vs. I/R: 19.83 ± 5.42 , one-way ANOVA, $p < 0.05$, Fig. 2b and c), both of which were still higher than in the sham group (Dex: 0.67 ± 0.52 vs. Sham: 0.00 ± 0.00 , one-way ANOVA, $p < 0.05$; I/R + SB: 0.67 ± 0.82 vs. Sham: 0.00 ± 0.00 , one-way ANOVA, $p < 0.05$, Fig. 2a; Dex: 4.41 ± 3.50 vs. Sham: 0.00 ± 0.00 , one-way ANOVA, $p < 0.05$; I/R + SB: 3.23 ± 0.85 vs. Sham: 0.00 ± 0.00 , one-way ANOVA, $p < 0.05$, Fig. 2b and c). And the Dex + LY, I/R + XAV and Dex + XAV groups showed no significant difference in Longa score and cerebral infarct size compared with the I/R group.

3.2. Effect of Dex on neuron survival in rats subjected to focal cerebral I/R

As shown in Fig. 3, in the sham group, the number of nerve cells was greater, with large cell bodies and clear margins, as well as clearly visible Nissl bodies, nuclei and nucleoli, and with glial cells scattered

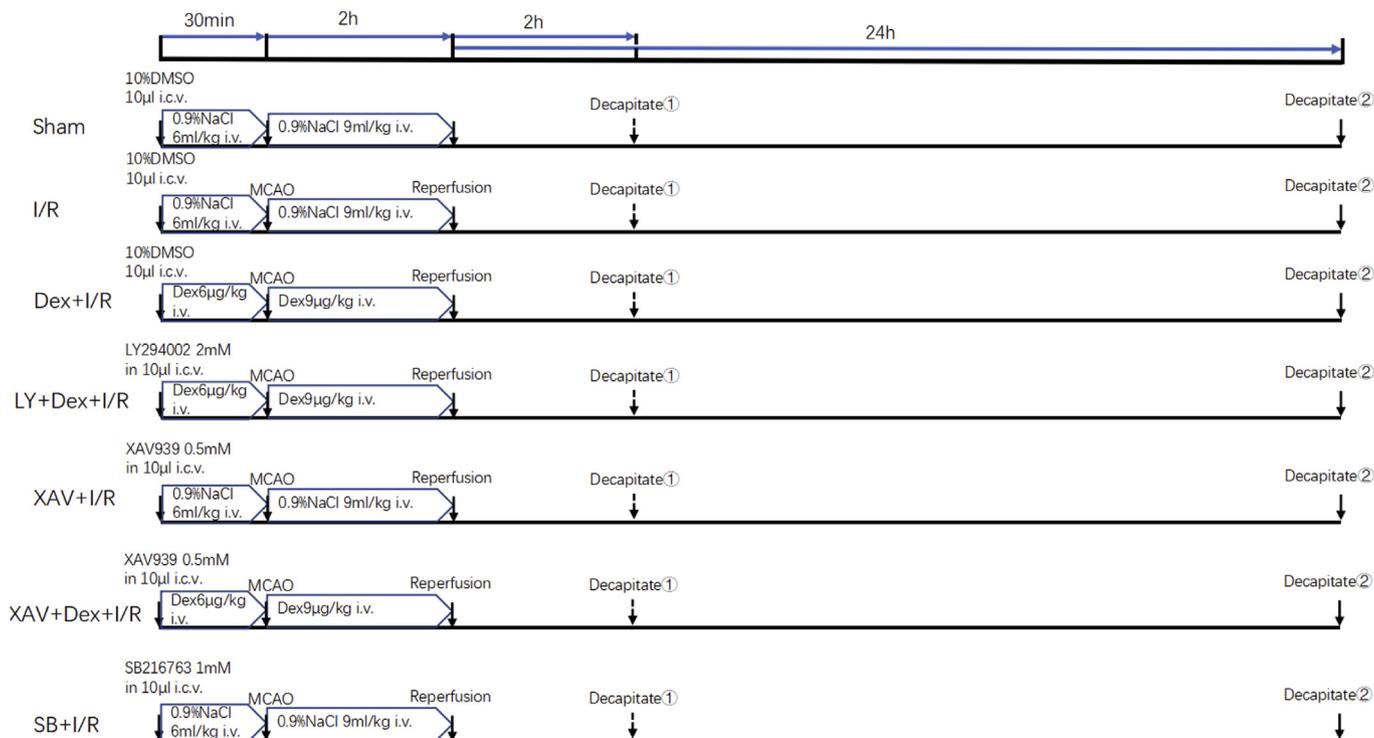


Fig. 1. Experimental groups and protocols for the cerebral protection by Dex in rats subjected to middle cerebral artery occlusion (MCAO) induced ischemia-reperfusion (I/R).

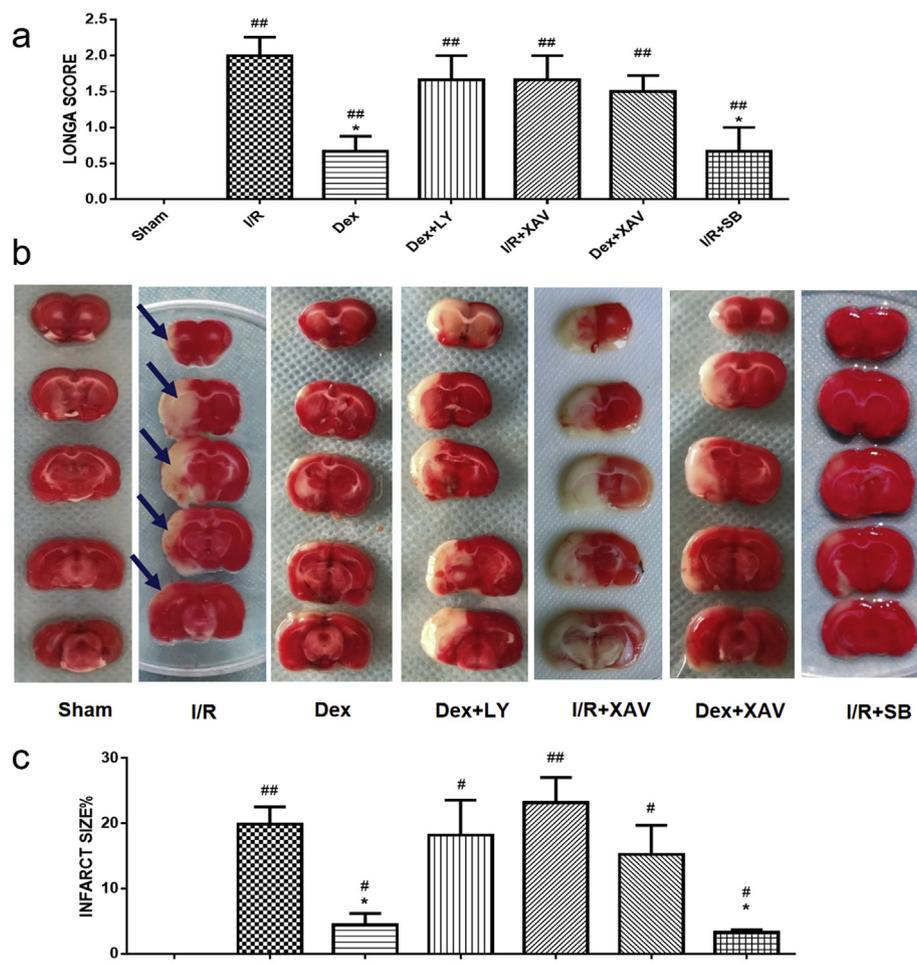


Fig. 2. Effect of dexmedetomidine on neurological deficit score (Longa score) (a) and cerebral infarct size (b); upper panel: TTC staining of representative sections (arrow pointing to representative infarcted); lower panel: quantification of infarct size (c) at 24 h reperfusion in rats exposed to MCAO-induced ischemia/reperfusion (I/R). Data are expressed as MEAN ± SD, n = 6 (Compared with I/R group, *p < 0.05; Compared with the sham group, #p < 0.05, ##p < 0.01).

within the penumbra cortex. Compared with the sham group, the number of neurons in other groups were decreased to different extents. Compared with the sham group, the number of survival neurons in the I/R group was significantly decreased (I/R: 9.17 ± 6.65 vs. Sham: 42.50 ± 6.29 , one-way ANOVA, $p < 0.001$, Fig. 3a and b) and showed cell shrinkage, as well as increased cell gap and glial cell proliferation. The number of survival neurons in the Dex group and I/R + SB group was significantly higher than in the I/R group (Dex: 27.83 ± 7.68 vs. I/R: 9.17 ± 6.65 , one-way ANOVA, $p < 0.05$; I/R + SB: 25.17 ± 6.11 vs. I/R: 9.17 ± 6.65 , one-way ANOVA, $p < 0.05$, Fig. 3a and b), and the margins of nerve cells were clear. In the Dex + LY, I/R + XAV and Dex + XAV groups, cell shrinkage was obvious, with increased cell gaps and glial cell proliferation, and the number of survival neurons was not significantly different compared with the I/R group.

3.3. Effect of Dex on the expression of pAKT, pGSK3β, β-catenin in cytoplasm and β-catenin in nuclei in the ischemic penumbra of rats subjected to focal cerebral I/R

As shown in Fig. 4, compared with the I/R group, expressions of pAKT, pGSK3β, β-catenin in cytoplasm and β-catenin in nuclei of Dex group significantly increased (pAKT, Dex: 0.61 ± 0.12 vs. I/R: 0.18 ± 0.04 , one-way ANOVA, $p < 0.05$, Fig. 4a; pGSK3β, Dex: 0.80 ± 0.26 vs. I/R: 0.17 ± 0.07 , one-way ANOVA, $p < 0.01$, Fig. 4b; β-catenin in cytoplasm, Dex: 0.66 ± 0.12 vs. I/R: 0.30 ± 0.14 , one-way ANOVA, $p < 0.01$, Fig. 4c; β-catenin in nuclei, Dex: 1.94 ± 0.35

vs. I/R: 0.91 ± 0.41 , one-way ANOVA, $p < 0.01$, Fig. 4d). Compared with the I/R group, expressions of pAKT, pGSK3β and β-catenin in cytoplasm and β-catenin in nuclei of Dex + LY group showed no significant difference. Compared with the I/R group, protein expression of pAKT, pGSK3β and β-catenin in cytoplasm of Dex + XAV group significantly increased (pAKT, Dex + XAV: 0.56 ± 0.14 vs. I/R: 0.18 ± 0.04 , one-way ANOVA, $p < 0.05$, Fig. 4a; pGSK3β, Dex + XAV: 0.63 ± 0.26 vs. I/R: 0.17 ± 0.07 , one-way ANOVA, $p < 0.05$, Fig. 4b; β-catenin in cytoplasm, Dex + XAV: 0.57 ± 0.10 vs. I/R: 0.30 ± 0.14 , one-way ANOVA, $p < 0.05$, Fig. 4c), but the expression of β-catenin in nuclei showed no significant difference. Compared with the I/R group, the expression of pAKT in I/R + SB group showed no significant difference, but expressions of pGSK3β, β-catenin in cytoplasm and β-catenin in nuclei were significantly higher than in the I/R group (pGSK3β, I/R + SB: 0.63 ± 0.27 vs. I/R: 0.17 ± 0.07 , one-way ANOVA, $p < 0.05$, Fig. 4b; β-catenin in cytoplasm, I/R + SB: 0.67 ± 0.16 vs. I/R: 0.30 ± 0.14 , one-way ANOVA, $p < 0.01$, Fig. 4c; β-catenin in nuclei, I/R + SB: 2.01 ± 0.48 vs. I/R: 0.91 ± 0.41 , one-way ANOVA, $p < 0.01$, Fig. 4d).

4. Discussion

In this study, treatment of rats exposed to I/R with Dex not only reduced the neurologic deficit score and cerebral infarct size, but also improved survival of neuron in the ischemic penumbra ($P < 0.01$). This protection by Dex against cerebral I/R injury was accompanied by the up-regulation of pAKT, pGSK3β, β-catenin in cytoplasm and β-

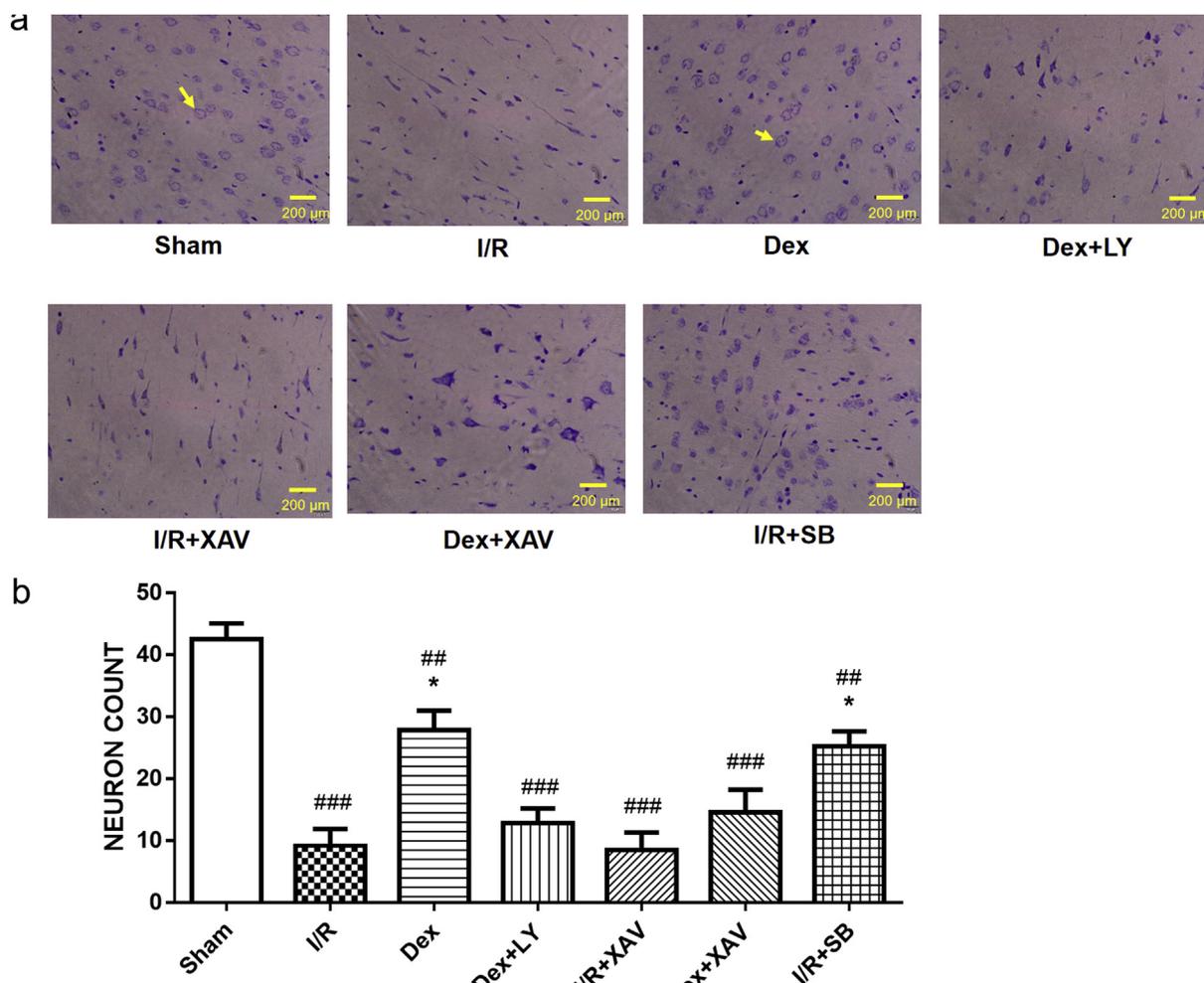


Fig. 3. Effect of dexmedetomidine (Dex) on neuron survival in cortex (ischemic penumbra) from rats exposed to 2 h MCAO-induced ischemia and 24 h reperfusion (I/R). upper panel: Representative Nissl staining in cortex; normal neurons showed large cell bodies and clear Nissl bodies (arrows pointing), and dead or dying neurons were shrunken with condensed nuclei and sparse Nissl bodies. lower panel: Quantification of surviving neurons; surviving neurons per high power field were calculated. Photomicrographs are $\times 400$. Data are expressed as MEAN \pm SD, n = 6 (Compared with I/R group, *p < 0.05; Compared with sham group, ##p < 0.01, ###p < 0.001).

catenin in nuclei. All the results indicate that PI3K/AKT/GSK3 β and Wnt/ β -catenin pathways are activated in this process. However, this protection by Dex against cerebral I/R injury was inhibited by PI3K inhibitor LY294002 and β -catenin inhibitor XAV939 respectively, indicating that the PI3K/AKT/GSK3 β and Wnt/ β -catenin pathways were all involved in the brain protection of Dex (Fig. 5).

The inhibitory effect of XAV939 on β -catenin was shown in the nucleus. XAV939 can stabilize axin to promote β -catenin degradation by blocking tankyrase1/2 and inhibit the formation of transcriptional complexes, thereby inhibiting the transcription regulation of β -catenin. In this study, we observed that the application of XAV939 did not significantly affect the expression of pAKT and pGSK caused by Dex. In other words, XAV939 only inhibited the transcription regulation of Wnt/ β -catenin pathway and did not affect the activation of PI3K/AKT/GSK3 β pathway caused by Dex. However, the up-regulation of pAKT, pGSK, β -catenin in cytoplasm and β -catenin in nuclei caused by Dex was significantly inhibited by LY294002, indicating that the activation of PI3K/AKT/GSK3 β and Wnt/ β -catenin pathways were blocked by LY294002. This suggested that the PI3K/AKT/GSK3 β pathway should be located upstream of the Wnt/ β -catenin pathway and regulate the Wnt/ β -catenin pathway in the protection by Dex against cerebral I/R injury.

In I/R + SB group, GSK3 β inhibitor SB216763 was administered to inhibit the activity of GSK3 β and promote the accumulation of β -

catenin in cytoplasm and nuclei, activating the Wnt/ β -catenin pathway. According to the result, the neurologic deficit score and cerebral infarct size were reduced and the survival of neuron in the ischemic penumbra was improved significantly compared with I/R group, proved that the activation of Wnt/ β -catenin signaling pathway in cerebral ischemia/reperfusion process may have a protective effect, consistent with previous studies [19]. But it must be noted, however, that GSK3 β is a multifunctional enzyme that is not only associated with the Wnt/ β -catenin pathway but also related to other processes, such as mitochondrial apoptotic signaling [23,24]. Therefore, the protective effect of GSK3 β inhibitor SB216763 against cerebral I/R injury may not only be related to the activation of Wnt/ β -catenin pathway, but also related to other mechanisms, which need to be further studied.

In addition, according to the body surface area normalization method which was recommended by the Food and Drug Administration [25], the dose of Dex used in our experiment was equivalent to an approximate dose of 0.6 μ g/kg/h in humans, which is considered the usual dose of clinical practice. Therefore, to patients with high risk of ischemic stroke, Dex might be an optimal option for perioperative medication.

Our experiment has some deficiencies. First, in this study, we observed the activation of these two pathways in the ischemic penumbra only at 2 h of reperfusion and the brain injury was assessed only at 24 h of reperfusion. The long-term effects of Dex on cerebral I/R injury and

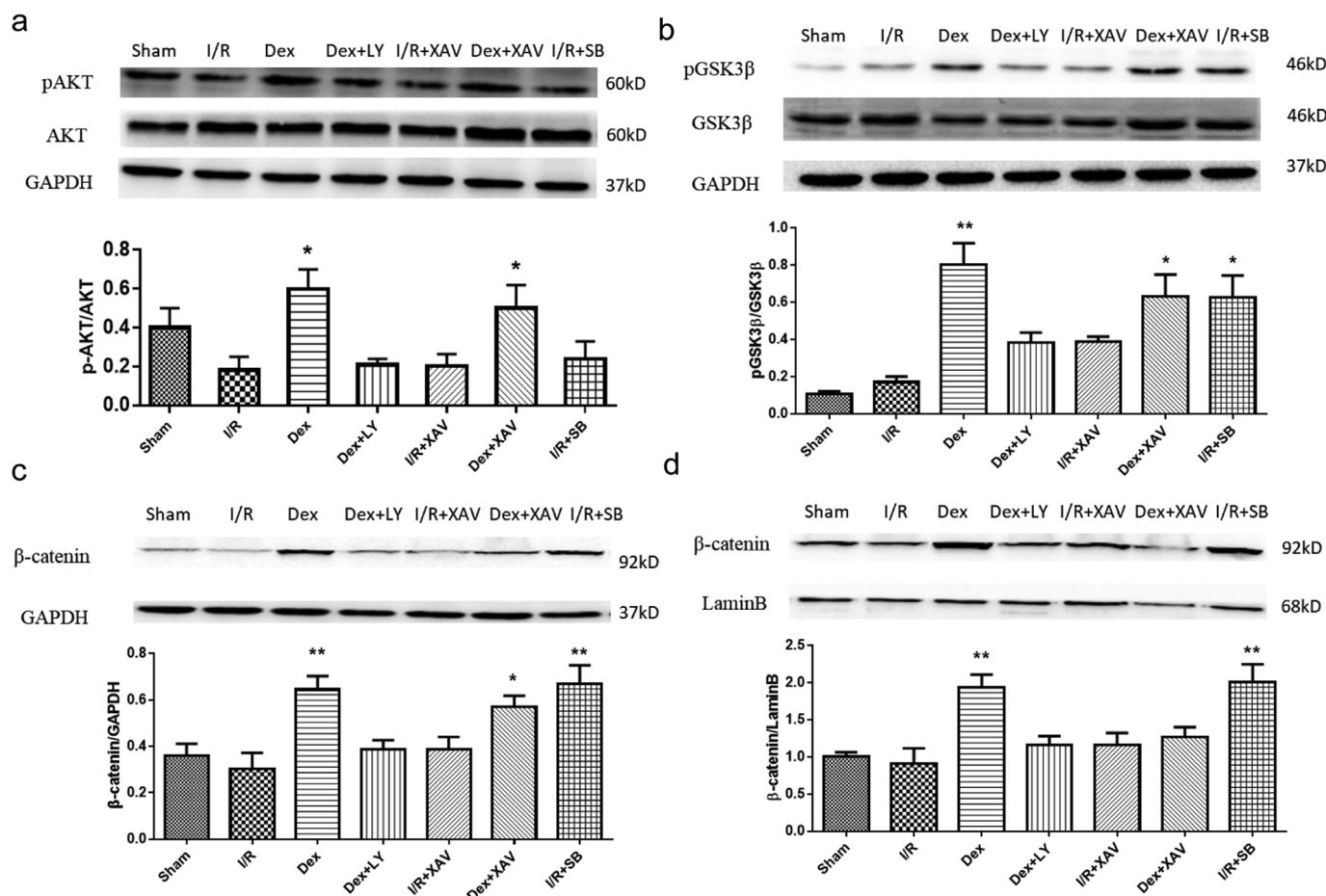


Fig. 4. Effect of Dex on expression of pAKT (a), pGSK3β (b), β-catenin in cytoplasm (c) and β-catenin in nuclei (d) in the ischemic penumbra of rats exposed to 2 h MCAO-induced ischemia and 2 h reperfusion (I/R). Relative optical density (O.D.) of pAKT, pGSK3β, β-catenin was calculated. Data are expressed as MEAN ± SD, n = 6 (Compared with I/R group, *p < 0.05, **p < 0.01).

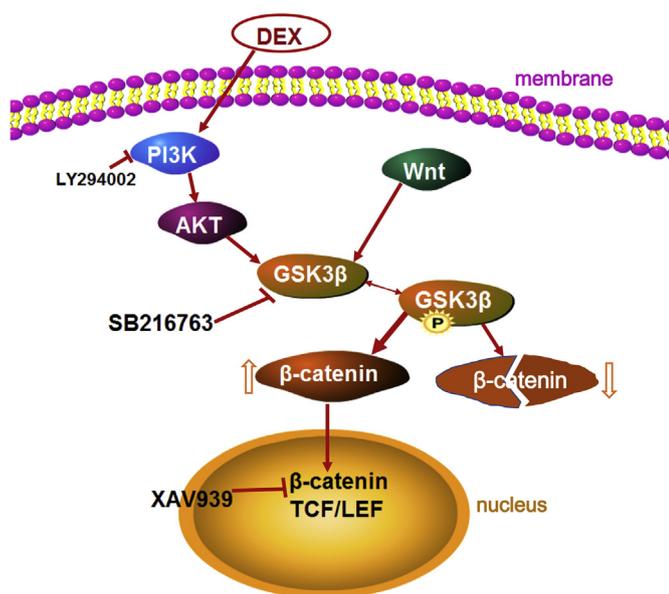


Fig. 5. The roles of PI3K/AKT/GSK3β and Wnt/β-catenin pathway in the protection process by dexmedetomidine against cerebral ischemia/reperfusion injury.

the duration of activation of these two pathways remain to be further studied, since previous studies have shown that the duration of

activation of Wnt/β-catenin pathway may have different effects on ischemia/reperfusion organs [16,26]. Second, our experiment suggested that Wnt/β-catenin pathway played an important role in the protection by Dex against cerebral I/R injury, however the downstream mechanism of β-catenin requires further study.

In conclusion, our data demonstrated that treatment with Dex reduced cerebral injury in rats exposed to transient focal ischemia/reperfusion, and this was mediated by the activation of the PI3K/AKT/GSK3β pathway as well as the activation of downstream Wnt/β-catenin pathway. And the Wnt/β-catenin pathway may play an important part in the protection against transient focal cerebral ischemia/reperfusion injury in rats.

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Author contributions

Ping Li and Hongtao Liu designed the experiments. Ping Li performed the experiments and acquired the data. Ping Li and Yongfang Zhang analyzed the data and wrote the manuscript under the guidance of Hongtao Liu. All authors approved the manuscript.

Declaration of competing interest

We declare that we have no conflicts of interest to this work.

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