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Function of SOD1, SOD2, and PI3K/AKT signaling pathways in the protection of propofol on spinal cord ischemic reperfusion injury in a rabbit model

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ABSTRACT

Aims: To verify that co-application of propofol preconditioning and postconditioning protects spinal cord from ischemia/reperfusion injury by enhancing the different subtypes of SOD activity, which is related to PI3K/AKT signal pathway.

Materials and methods: 60 rabbits were randomly equally assigned to 3 groups: Group S, sham-operation group; Group I/R., ischemia/reperfusion group; Group P, ischemia/reperfusion group with propofol treatment. Four rabbits per group were randomly executed at the time-points: days 1, 2, 3, 5, and 7 post-surgery. Spinal cord tissues at L3 to L4 levels were harvested. The bioactivities of SOD_1 and SOD_2, and the mRNA expression levels of SOD_1, SOD_2, PI3K, and AKT were detected.

Key findings: On day 1, the bioactivity of SOD_1 increased significantly in Group I/R or Group P compared with Group S ($P < 0.05$). On day 2, compared with Group S, the bioactivity of SOD1 increased significantly in Group P ($P < 0.05$). On days 3, 5, and 7, the bioactivity of SOD_1 decreased significantly respectively in Group I/R compared with Group S ($P < 0.05$). On all timepoints, the bioactivity of SOD_2 decreased significantly in Group I/R compared with Group S ($P < 0.05$). There was a positive correlation between the SOD_1 activity and the respective mRNA expression of SOD_1, PI3K, and AKT.

Significance: Co-application of propofol preconditioning and postconditioning resulted in potent protective effects against spinal cord ischemia/reperfusion injury, which was associated with the increased expression of SOD_1 in spinal cord tissues by activating PI3K/AKT signal pathway.

1. Introduction

Spinal cord injury remains a devastating complication of thoracic and thoraco-abdominal aortic interventions at present, with a reported incidence ranging from 2.7% to 28% [1–2]. To prevent and understand this complication, various methods, such as administering pharmacologics to protect the spinal cord, have been suggested. Among all pharmacologics, propofol is well-studied [3–4]. The co-application of propofol pre- and post-ischemic conditioning was seen to possibly increase the superoxide dismutase (SOD) activity of the spinal cord during the spinal cord ischemia–reperfusion injury (SCIRI) in the rabbit model in our previous study [5]. However, the underlying mechanisms remain unclear.

Superoxide dismutase (SOD) is an active oxygen free radical scavenger that can specifically catalyze active oxygen free radicals in the body, cause oxidation disproportionation reaction, and consequently transform these radicals into non-toxic water or oxides. SOD can be divided into three subtypes on the basis of the distribution sites and different structure forms in mammals [6]: (1) Cu/zinc–SOD located in the cytoplasm or mitochondrial clearance, namely, SOD1; (2) Mn–SOD located in the mitochondria inside, namely, SOD2; and (3) Cu/Zn–SOD (Ec–SOD) located outside the cell, namely, SOD3. At present, SOD has become a hot research topic. A large number of studies have shown that the occurrence and development of numerous diseases are closely related to the activity of SOD. Sasaki and Yan BC et al. found that SOD1 and SOD2 presented different degrees of expression and change in the experiment of cerebral ischemia–reperfusion injury [6–7].

Considering that the central nervous system (CNS) is composed of the spinal cord and the brain together, we speculate that SOD1 and SOD2 in the spinal cord tissues may present corresponding expression levels in the SCIRI process. In addition, we speculate that the co-application of propofol pre- and post-ischemic conditioning can impact the activity of SOD1 or SOD2 of spinal cord tissues during SCIRI and produce a protective effect. Whether SOD1 or SOD2 is more directly related to the protection has yet to be clarified and needs further research.

Wand HY discovered that the intraperitoneal injection of large doses of propofol provided brain protection through activating the phosphatidylinositol 3-kinase (PI3-K)/serine/threonine protein kinase (AKT) signal transduction pathway [8]. Previous studies have also

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demonstrated that propofol can fight against heart ischemia–reperfusion injury through activating the PI3K/AKT signal pathway and thus decreasing the myocardial apoptosis [9–10].

Based on the above research, we hypothesize in our experiment that the co-application of propofol pre- and post-ischemic conditioning protects the spinal cord from ischemia–reperfusion injury by enhancing different subtypes of SOD activity, which is related to the PI3-K/AKT signal pathway. We will examine the expression of spinal cord SOD1 and SOD2 proteins in a rabbit model and evaluate the mRNA expression of SOD1, SOD2, PI3-K, and AKT to determine the relationship between the expression of SOD1/SOD2 and the PI3K/AKT signal pathway.

2. Materials and methods

2.1. Design

A randomized controlled animal experiment.

2.2. Time and setting

This experiment was carried out in the Anesthesiology Laboratory of Renmin Hospital of Wuhan University, China, from June 2012 to September 2012.

2.3. Materials

Sixty healthy male adult Japanese white rabbits aged 3 months and weighing 2.0 kg to 2.5 kg that were supplied by the Experimental Animal Institute of Wuhan University (certification No. SCXK2008-003) were selected and randomly divided into 3 groups ($n = 20$), namely, sham-operated group (Group S), ischemia–reperfusion group (Group I/R), and ischemia-reperfusion group with propofol treatment (Group P). All animals were fed in standard cages, and all experimental treatments of animals were in strict accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, as formulated by the Ministry of Science and Technology of the People's Republic of China [11].

2.4. Methods

2.4.1. Surgical procedure

Rabbits were intravenously infused with 3% sodium pentobarbital (15 mg/kg in 5 ml of 0.9% saline), endotracheally intubated, and connected to a small-animal respirator machine (Model DH140B; Zhejiang Medical Instrument Factory, Zhejiang, China). The respiration of rabbits was controlled at 30 breaths/min, with a 1.5:1 ratio of inspiration to expiration. The level of $PaCO_2$ was maintained at 4.67 kPa to 6.00 kPa. Ringer's solution was infused at 10 ml/kg per hour through an ear vein during the procedure, and additional doses of 3% sodium pentobarbital (0.5 ml/kg) and rocuronium (0.5 mg/kg) were administered at regular intervals throughout the experiment. After intravenous heparin (3 mg/kg) administration, the right femoral artery was exposed, and a catheter with an arterial line connected to a pressure/heart transducer (LIFESCOPEE9; Japan Optical Co., Ltd., JO BLDG, Japan) was inserted for continuous monitoring of the arterial pressure. Arterial pressures, both distal and proximal to the cross clamp, were measured, and the mean arterial pressure (MAP) was calculated. Rectal body temperature was maintained at close to 38 °C with the aid of a heating pad.

2.4.2. Aortic occlusion

To initiate ischemia/reperfusion (I/R) injury by aortic occlusion [12], we incised the rabbit's skin along the lateral vertical side of the erector spinal muscle below the left costal verge. The abdominal aorta was exposed outside the peritoneum, and a silicone plastic tube with a small diameter was placed around the abdominal aorta at the distal side at 1 cm below the left artery. Aortic occlusion was induced by pulling and clamping the surrounding plastic tubing until the distal MAP reached 0 mm Hg. (The proximal MAP was increased by approximately 10% to 15% after aortic occlusion in both Groups I/R and P, but no significant difference was observed between the two groups.)

2.4.3. I/R injury and propofol treatment

Spinal cord ischemia was induced by an infra-renal aortic cross-clamp for 40 min in Groups I/R and P. Immediately after occlusion, distal blood pressure decreased, and the pulse disappeared. Reperfusion was initiated by removing occlusion and was continued for 7 days.

Propofol (Disoprofol, Ltd., Shanghai, China; Lot. No. JC843) was intravenously infused twice (30 mg/kg; equivalent to clinical dosage of 2 mg/kg to 3 mg/kg) into rabbits in Group P. Propofol in 30 ml of 0.9% sodium chloride at a rate of 3 ml/min was administered twice: once at 10 min prior to aortic clamping and once at the onset of reperfusion. The animals in Group I/R underwent standard aortic occlusion and intravenous injection of the same volume of 0.9% sodium chloride without propofol and under conditions identical to propofol injection. Sham-operated rabbits were subjected to surgical dissection without aortic occlusion and underwent intravenous injection of the same volume of 0.9% sodium chloride without propofol under identical conditions to that of propofol injection. An antibiotic (400,000 U penicillin) was administered intramuscularly immediately after operating procedures. The wounds were then sutured, and the rabbits were returned to their home cages for observation.

2.4.4. Biopsy samples of spinal cord tissues

Four rabbits from each group were terminated via artery exsanguination at 1, 2, 3, 5, and 7 days after surgery. Spinal cord tissues from levels L3 to L4 were rapidly harvested. Spinal cord tissues were washed twice using cold serum physiological solutions and placed in labeled glass bottles. The bottles were stored in a deep freezer (−80 °C) until processing.

2.4.5. Determination of the SOD1 and SOD2 activities

Spinal cord tissues of about 1 cm long from around the injured region at the third lumbar vertebra (L3) to the fourth lumbar vertebra (L4) were sampled, washed twice with cold phosphate buffer saline, and then stored at −80 °C until analysis to determine endogenous enzymatic activities. SOD1 activity was measured according to the procedure provided by the manufacturer by using sandwich ELISA kits for SOD1 (WuHan HuaMei, CSB-EL022397RB China). SOD2 activity was measured according to the procedure provided by the manufacturer by using sandwich ELISA kits for SOD2 (WuHan HuaMei, CSB-EL022398RB China). All samples were assayed in duplicate.

2.4.6. Determination of mRNA expression for SOD1, SOD2, PI3K, and AKT

To demonstrate the change of SOD1 mRNA, SOD2 mRNA, PI3K mRNA, and AKT mRNA, we harvested and homogenized fresh spinal tissues from each group. After the supernatant was harvested, the concentrations of RNA samples were measured with a Nanodrop spectrophotometer (ND-1000) (4 μg per experiment). Real-time polymerase chain reaction (RT-PCR) was used to amplify products, and β-actin was used as an internal control. For RNA amplification, first-strand cDNA was synthesized from 4 μg of total RNA and using Revert AidTM First Strand cDNA Synthesis Kit (Fermentas, USA). PCR was then carried out using the PCR MasterMix Kit (Fermentas, USA) for 30 cycles, which consisted of denaturation at 94 °C for 1 min, annealing for 1 min, and extension at 72 °C for 1 min. Gene primers were synthesized by Invitrogen Biotechnology Co., LTD (China). The primer sequences are shown in Table 1. RT-PCR products were electrophoresed in 1% agarose gel, stained with ethidium bromide, and visualized by using an ultraviolet gel imager (Bio-Rad, USA). The optical density (OD) of each product band, including the objective gene and β-actin, was obtained, and the