DEXMEDETOMIDINE ATTENUATES SPINAL CORD ISCHEMIA-REPERFUSION IN RATS BY INHIBITING PROINFLAMMATORY FACTORS AND ATTENUATING LIPID PEROXIDATION IN SPINAL CORD TISSUE

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ABSTRACT

Objective: *To study the mechanism through which dexmedetomidine alleviates spinal cord ischemia-reperfusion in rats.*

Methods: *A total of 72 clean, healthy, male Sprague-Dawley (SD) rats were randomly selected and divided into three groups containing 24 rats each: sham operation group, model group, and dexmedetomidine group. The spinal cord ischemia-reperfusion rat model was established. Rats in the sham operation group only underwent laparotomy and threading. Rats in the dexmedetomidine group were given a tail-vein injection of 4 μg/kg dexmedetomidine. The Tarlov score was used to evaluate the neurobehavioral functions of rats at each time point. Hematoxylin and eosin (HE) staining was used to determine the pathological changes in the spinal cords of rats. Enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of tumor necrosis factor-α (TNF-α) and interleukin-1α (IL-1α) in the spinal cord tissue of rats from each group. The xanthine oxidation method was used to determine changes in superoxide dismutase (SOD) activity, and the thiobarbital method was used to determine the levels of malondialdehyde (MDA) in rats from each group. The number of normal neurons in the anterior horn of the spinal cord was observed by microscope.*

Results: *Compared with the sham operation group, the Tarlov score, SOD levels, and the number of normal neurons in the anterior spinal horn of rats in the model group were significantly reduced, and the levels of TNF-α, IL-1α, and MDA were significantly increased (P<0.05). Compared with the model group, the Tarlov score, SOD levels, and the number of normal neurons in the anterior horn of the spinal cord in the dexmedetomidine group were significantly increased, and the levels of TNF-α, IL-1α, and MDA were significantly reduced (P<0.05). The nerve cells in the sham operation group presented clear outlines, the cytoplasm contained the same distribution of Nissl bodies, the nuclear morphological structure was typical, and the nucleoli were large and obvious. In contrast, the neuronal contours of the rats in the model group disappeared or were unclear; the cytoplasm of the rats was indifferent, the nuclear morphology was abnormal, and the nucleoli were reduced or disappeared. The contours of the nerve cells in the dexmedetomidine group disappeared, with slight edema, and some of the cytoplasmic and nucleoli morphology were blurred.*

Conclusion: *Dexmedetomidine can significantly reduce spinal cord ischemia-reperfusion injury and improve neurological function in rats, which may be associated with the inhibition of pro-inflammatory factors and the reduction of lipid peroxidation in spinal cord tissue.*

Keywords: *Dexmedetomidine, pro-inflammatory factors, lipid peroxidation, spinal cord ischemia-reperfusion.*

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Introduction

Spinal cord ischemia-reperfusion injury represents a critical obstacle to recovery following spinal cord injury, which often results in serious injury to nerve cells. Spinal cord ischemia-reperfusion injury is a primary cause of severe disability and death in patients after spinal surgery and aneurysm surgery. The incidence of spinal cord ischemia-reperfusion injury following spinal injuries has been reported to be approximately 3%-18%[(1)].

Currently, spinal cord ischemia-reperfusion injury prognosis is poor, and paralysis and death are common outcomes, which have large impacts on patients' physical and mental health and can affect the patient's family and the social economy.

With the rapid development of new surgical techniques, the number of patients who undergo upward thoracoabdominal aortic aneurysm surgery has increased. However, due to the difficulty of performing such surgical techniques, the thoracic and abdominal aorta must be temporarily blocked for a long time during the surgery, which increases the incidence of spinal cord ischemia-reperfusion injury[2]. Therefore, the development of methods and measures that can effectively prevent and treat spinal cord ischemia-reperfusion injury is critical. Dexmedetomidine is a highly selective α_2 adrenergic receptor agonist that has been commonly used in clinics in recent years.

Dexmedetomidine has powerful sedative and hypnotic effects, a short half-life and action time, with minimal inhibition of respiration. In addition, dexmedetomidine has stable hemodynamic properties and significantly reduces the incidence of postoperative restlessness, nausea, and vomiting[3]. With the increasingly widespread use of dexmedetomidine in clinical practice, studies have found that dexmedetomidine can significantly reduce the myocardial infarction area after ischemia-reperfusion injury.

In addition, the occurrence of pulmonary edema and pleural exudation in rats with acute lung injury can be alleviated, the inflammatory response of the lungs can be inhibited, and the lungs of rats can be protected[4-5]. The protective mechanism may occur through the inhibition of the inflammatory response and oxidative stress, which results in the inhibition of neuronal apoptosis and the release of excitatory amino acids[6].

In addition, some reports have suggested that dexmedetomidine may play a role in the protection against spinal cord ischemia-reperfusion injury. The purpose of this study was to investigate the effects of dexmedetomidine on spinal cord ischemia-reperfusion injury in rats and to explore the underlying mechanism.

Materials and methods

Experimental animals

A total of 72 clean, healthy, male Sprague-Dawley (SD) rats [SCXK (Shanghai) 2016-0001], weighing 213±12g, were randomly selected (provided by Shanghai Ruitimos Biotechnology Co., LTD., production license). Rats were maintained under conditions of 24±3°C, at 55±15% humidity, with a 12-h/12-h light/dark cycle, for 1 week.

Main instruments and reagents

Biosafety cabinet (Beijing Donglian Har Instrument Manufacturing Co., LTD., model: class II B2); low-temperature high-speed centrifuge (Shanghai Luxiangyi Centrifuge Instrument Co., LTD., model no.: TGL-16.5M); biological microscope (Suzhou Jingtong Instrument Co., LTD., model: XBL-10A); electronic balance (Shanghai Shunyu Hengping Scientific Instrument Co., LTD., model: AE323); –80°C ultra-low-temperature refrigerator (Jinan Zhuolong Biotechnology Co., LTD., model: BDF-86H50); incubator (Beijing Fuyilian Medical Equipment Co., LTD., model: FYL-YS-66L); small animal laboratory (Beijing Heli Science and Technology Development Co., LTD., model: JPT-S); hematoxylin and eosin (HE) staining kit (Shanghai Weiao Biotechnology Co., LTD.); superoxide dismutase (SOD) detection kit (Beijing Baiolaibo Technology Co., LTD.); malondialdehyde (MDA) detection kit (Hefei Lisle Biotechnology Co., LTD.); tumor necrosis factor-α (TNF-α) test kit (Shanghai Jiwen Industry Co., LTD.); interleukin-1α (IL-1α) test kit (Shanghai Xinyu Biotechnology Co., LTD.); and dexmedetomidine (Sichuan Guorui Pharmaceutical Co., LTD, Production batch number: 20170097, specification: 2 ml: 0.2 mg).

Experimental grouping and methods

To establish a rat model of spinal cord ischemia-reperfusion, the rats were anesthetized, and a scalp needle was used to puncture the tail vein. The rat was placed in the supine position, the abdominal skin was prepared, and the abdominal cavity was opened along the abdominal line.

The intestines were removed to the left, and the abdominal aorta was isolated and exposed. The renal artery was identified and threaded between the bilateral renal arteries. The abdominal aorta segment between the renal arteries was blocked by a non-invasive artery clamp, and blood perfusion was restored after ischemia for 45 min. The abdominal cavity was closed layer by layer, and 5 U penicillin were injected intraperitoneally. Particular attention was paid to maintaining the body temperature of the rat during the operation.

Rats were randomly divided into a sham operation group, a model group, and a dexmedetomidine group, with 24 rats in each group. The rats in the sham operation group underwent only laparotomy and threading without the abdominal aorta being clipped. Rats in the sham operation group and model group were both injected with 4

ml/kg normal saline by tail vein, and the abdominal aorta was clipped after 5 min in the model group. The rats in the dexmedetomidine group were given a tail-vein injection of 4 g/kg dexmedetomidine, and the abdominal aorta was clipped after 5 minutes.

Observation indicators

Measurement of neurobehavioral score

The Tarlov score was used to evaluate the neurobehavioral function of rats in each group following surgery, 12, 24, and 48 h after reperfusion. The Tarlov score ranges from 0-4 points.

A score of 0 indicates that hind limb movement is not detected; a score of 1 indicates that weak hind limb movement is detected, but not against gravity; a score of 2 indicates that the hind limb is able to resist gravity, but the rat cannot stand; a score of 3 indicates that the rat is able to stand and walk but cannot jump normally; and a score of 4 indicates that hind limb function is fully restored and the animal can jump normally. 0-1 of them were assessed as amputation.

At 48 h after ischemia-reperfusion, HE staining was used to measure the pathological changes in the spinal cords of each group. At 48 h after spinal cord ischemia-reperfusion, 0.2 g spinal cord tissue was extracted from the rats in each group. The levels of TNF-α and IL-1β in the spinal cord were determined by enzyme-linked immunosorbent assay (ELISA). SOD activity was determined using the xanthine oxidation method, and MDA levels were determined using the thiobarbital method.

A spinal cord anterior horn of rats was randomly selected, and a straight line was made from the fixed point of the anterior horn to the central point of the central tube. Along this line, images of 4 fields were successively collected, moving from the outside to the inside. The numbers of neurons in the spinal cord anterior horns of rats in each group were observed by microscope.

Normal neurons feature a nucleus that round and centered, with clear contours and obvious nucleoli, with nisite in the cytoplasm, and a cell body diameter of approximately 30-60 meters.

Statistical methods

SPSS, version 21.0, software package was used to perform statistical analysis in this study. Measurement data are described as the mean ± standard deviation ($\bar{x}\pm s$) and were compared among many groups with single factor multivariate mean. An independent-samples t-test was used between the two groups. The Tarlov score was used to evaluate the neurobehavioral functions of rats at each time point. HE staining was used to determine the pathological changes in the spinal cords of rats.

ELISA was used to determine the levels of TNF-α and IL-1α. Xanthine oxidation was used to determine changes in SOD activity, and thiobarbital was used to determine changes in MDA levels in rats. The number of normal neurons in the anterior horn of the spinal cord was observed by microscope. The results were considered significant if $P<0.05$.

Results

Comparison of neurobehavioral scores among rats in each group

Compared with the sham operation group, the mean Tarlov score of rats in the model group was significantly reduced ($P<0.05$). Compared with the model group, the mean Tarlov score of the dexmedetomidine group was significantly increased ($P<0.05$, Table 1).

Group	n	Awake	12 hours after reperfusion	24 hours after reperfusion	48 hours after reperfusion
Sham operation group	24	3.88±0.36	3.88±0.36	3.88±0.36	3.88±0.36
Model group	24	0.38±0.75[a]	0.35±0.73[a]	0.24±0.71[a]	0.23±0.69[a]
Dexmedetomidine group	24	1.38±0.92[b]	1.52±0.91[b]	1.51±0.93[b]	1.63±1.07[b]
F		152.10	156.17	163.99	139.46
P		< 0.001	< 0.001	< 0.001	< 0.001

Table 1: Comparison of the neurobehavioral scores of the rats in each group ($\bar{x}\pm s$).

Note: compared with the control group, [a]P<0.05; compared with the model group, [b]P<0.05.

Pathological changes in the spinal cords of rats in each group

The rat nerve cells in the sham operation group were observed to have clear contours, the cytoplasm contained uniformly distributed nishenite, and the nuclei were large and obvious, with typical morphologies and structures.

In the model group, the nuclear contours disappeared or were not clear, the cytoplasmic nissite was indifferent, the nucleus morphology was abnormal, and the nucleoli reduced or disappeared. The contours of the nerve cells in the dexmedetomidine group disappeared, and the cells displayed slight edema.

The morphologies and structures of some cytoplasmic components and the nucleoli were fuzzy, as shown in Figure 1.

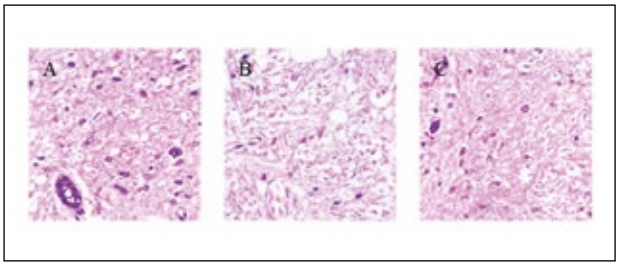

Figure 1: Pathological changes observed in the spinal cord of rats in each group.
A: sham operation group; B: model group; C: dexmedetomidine group.

Comparison of rat elements in each group

Compared with the sham operation group, the number of normal neurons in the anterior horn of the spinal cord was significantly reduced in the model group (P<0.05).

Compared with the model group, the number of normal neurons in the anterior horn of the spinal cord was significantly increased in the dexmedetomidine group (P<0.05, Table 2).

Group	n	Number of normal neurons in the spinal cord anterior horn
Sham operation group	24	17.96±1.85
Model group	24	4.32±1.99[a]
Dexmedetomidine group	24	10.34±3.12[b]
F		196.54
P		< 0.001

Table 2: Comparison of the number of normal neurons in the anterior horn of the spinal cord for each group ($\bar{x}\pm s$).
Note: compared with the control group, [a]P<0.05; compared with the model group, [b]P<0.05.

Changes in TNF-α, IL-1α, SOD, and MDA levels in the rats in each group

Compared with those in the sham operation group, the levels of TNF-α, IL-1α, and MDA in the spinal cord tissue of rats in the model group significantly increased, whereas the level of SOD was significantly reduced (P<0.05).

Compared with those in the model group, levels of TNF-α, IL-1α, and MDA were significantly reduced in the rats in the dexmedetomidine group, whereas SOD levels were significantly increased (P<0.05, Table 3).

Group	n	TNF-α (pg/mg)	IL-1α (pg/mg)	SOD (U/mg)	MDA (nmol/mg)
Sham operation group	24	147.92±12.19	42.66±1.19	276.30±14.48	2.47±0.48
Model group	24	533.75±17.88[a]	142.22±6.75[a]	163.19±16.30[a]	9.13±0.49[a]
Dexmedetomidine group	24	229.33±15.75[b]	85.35±5.73[b]	212.40±15.74[b]	5.73±0.64[b]
F		4157.12	2250.62	320.26	907.30
P		< 0.001	< 0.001	< 0.001	< 0.001

Table 3: Changes in the TNF-α, IL-1α, SOD, and MDA levels in the rats of each group ($\bar{x}\pm s$).
Note: compared with the control group, [a]P<0.05; compared with the model group, [b]P<0.05.

Discussion

Spinal cord ischemia-reperfusion injury is a severe complication that can occur after spinal cord injury or following thoracic or abdominal aortic aneurysm resection, which may lead to neurological dysfunction, such as paraplegia and lower extremity palsy, and can have serious impacts on patients' rehabilitation and quality of life. Studies suggest that the mechanism of spinal cord injury can be divided into two types: primary injury and secondary injury[7]. The primary injury process is usually transient, unpredictable, and unavoidable. Secondary injury typically occurs after the primary spinal cord injury, and the process is longer than that for primary injury. According to relevant reports, the mechanisms of secondary spinal cord injury include inflammation, calcium ion overload, ischemia-reperfusion, and other factors[8]. To date, no effective treatment for primary spinal cord injury exists.

In recent years, clinicians have used various methods, such as deep hypothermia, to inhibit circulation and cerebrospinal fluid drainage to protect the spinal cord during surgery; however, the occurrence of postoperative paraplegia appears to be inevitable[9]. Therefore, the identification of drugs that may provide protective effects against ischemia-reperfusion injury remains necessary. Dexmedetomidine is a highly selective lumedine-2 receptor agonist, which can produce significant sedation, analgesia, and anti-anxiety effects without respiratory depression. In addition, dexmedetomidine can also reduce blood pressure and inhibit sympathetic excitation. Recently, studies have found that dexmedetomidine not only has anesthetic effects but also has significant multi-organ protective effects[10]. In the present study,

a spinal cord ischemia-reperfusion injury model was established in rats to investigate the role and mechanism of action for dexmedetomidine in spinal cord ischemia-reperfusion injury.

The spinal cord consists of gray matter and white matter. Gray matter contains various nerve cells and can be divided into anterior, posterior, and lateral segments based on location within the spinal cord. The anterior horn contains a large number of motor neurons, which are responsible for the motor function of limbs. When spinal cord ischemia-reperfusion injury occurs, the gray matter in the anterior horn is injured first, followed by the posterior horn and the lateral horn[11]. Therefore, the number of normal neurons in the anterior horn of the spinal cord is significant for the evaluation of spinal cord injury severity. The Tarlov score is a standard method used to evaluate the nerve functions of rat hind limbs, which can indirectly reflect the degree of spinal cord injury in rats[12]. The results of this study showed that after spinal cord ischemia-reperfusion injury, the nerve function and the number of normal neurons in the spinal cord anterior horn were significantly reduced and that dexmedetomidine demonstrated a protective effect against spinal cord ischemia-reperfusion injury.

Studies have shown that spinal cord neurons are rich in lipids and unsaturated fatty acids, making them more vulnerable to attack by oxygen free radicals. When spinal cord ischemia occurs, spinal cord tissue produces large quantities of oxygen free radicals and peroxide products[13]. MDA is the most important peroxide product and exerts toxic effects against the cell membrane, resulting in the loss of the physiological function of the membrane. MDA can reflect the degree of lipid peroxidation in the body, which can indirectly reflect the oxygen free radical content. SOD is the primary free radical scavenger in the body, which can prevent cells from being damaged by free radicals. Therefore, SOD activity can reflect the ability of the body to scavenge oxygen free radicals. According to relevant reports, increased SOD activity and reduced MDA contents are associated with reduced oxidative stress in vivo[14]. The results of the present study suggest that dexmedetomidine can increase SOD activity, reduce MDA levels, and inhibit the oxidative stress response of the spinal cord.

The inflammatory response is a primary mechanism associated with spinal cord ischemia-reperfusion injury. After spinal cord ischemia-reperfusion injury occurs, neutrophils enter the spinal cord and bind to selectin ligands on the surfaces of spinal vascular endothelial cells, resulting in the adherence and aggregation of endothelial cells, which can result in the mechanical obstruction of spinal microcirculation, reducing blood supply to the spinal cord and further aggravating spinal cord injury[15]. TNF-α and IL-1α are all pro-inflammatory factors. The results of this study indicated that dexmedetomidine could significantly inhibit the expression of pro-inflammatory factors. This effect may be due to the inhibition of mononuclear macrophage by dexmedetomidine, which reduces the infiltration and aggregation of neutrophils and inhibits the expression of TNF-α and IL-1α[16].

In summary, dexmedetomidine can significantly reduce spinal cord ischemia-reperfusion injury in rats and improve neurological function, which may be associated with the inhibition of pro-inflammatory factors and the reduction of lipid peroxidation in spinal cord tissue.

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