DEXMEDETOMIDINE ALLEVIATES HIPPOCAMPAL NEUROINFLAMMATION AND COGNITIVE IMPAIRMENT INDUCED BY ANESTHESIA THROUGH SIRT1

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ABSTRACT

Objective: *To investigate the effect and mechanism of dexmedetomidine (DEX) on hippocampal neuroinflammation and cognitive impairment induced by anesthesia in aged rats.*

Methods: *Sixty 18-month-old SD rats were randomly divided into a control group, a model group and a DEX group. Splenectomies were performed in the model group and the DEX group, while no treatment was given in the control group. Dexamethasone was injected into the tail vein of the dexamethasone group before the operation, and a Y maze test was performed at 9 AM on the first, third and seventh days after operation. The pH value, PaO_2, $PaCO_2$ and other blood gas analysis indexes of mice in each group under anesthesia were detected, and the serum IL-1 was detected by enzyme-linked immunosorbent assay β,TNF- α Level. A western blot was used to detect SIRT1 and NF in the hippocampus- ϰ B protein expression.*

Results: *There was no significant difference in pH, PaO_2 and $PaCO_2$ among the three groups (P>0.05). There was no significant difference in shuttle times among the three groups on the first, third, or seventh day after the operation (P>0.05). On the first and third days after the operation, the alternation score rates of the model group and the DEX group were significantly lower than that of the control group, and the alternation score rate of the DEX group was significantly higher than that of the model group (P<0.05). The levels of IL-1 in the model group and the DEX group were significantly increased on the first and third days after the operation β. The level of IL-1 in the DEX group was significantly higher than that in the control group β. The level was significantly lower than that of the model group (P<0.05). On the first and third days after the operation, TNF - α in the model group and the DEX group decreased- α. The level of TNF in the DEX group was significantly higher than that in the control group- α. The level was significantly lower than that of the model group (P<0.05). The SIRT1 protein in the model group and the DEX group was significantly lower than that in the control group- ϰ. The expression of the SIRT1 protein in the DEX group was significantly higher than that in model group- ϰ. Protein B was significantly lower than that in model group (P<0.05).*

Conclusion: *DEX can inhibit the inflammatory reaction and improve the postoperative cognitive function of aged rats after anesthesia, which may be related to the regulation of SIRT1 pathway related proteins by DEX.*

Keywords: *Dexmedetomidine, SIRT1, anesthesia, aged rats, hippocampal nerve, inflammation, cognitive function.*

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Introduction

Postoperative cognitive dysfunction (POCD) is one common complication of the central nervous system after surgery. It often manifests as changes in personality, memory, attention, mental activity, language comprehension and social activities after surgery, which is closely related to the inflammatory reaction induced by surgery and anesthesia(1). POCD is common in elderly patients, which is related to central nervous system degeneration(2). With the recent prolongation of the life span and decrease in the birth rate, the problem of social aging has become increasingly significant. In recent years, the increasing probability of surgical operations and anesthesia has raised the incidence of POCD higher and higher, which seriously affects patients' quality of life after an operation(3). In recent years, studies into

Parkinson's disease, Alzheimer's disease and others have found that reducing the central inflammatory response can improve patients' cognitive function[4].

Dexmedetomidine (DEX) is a common drug in clinic α2 adrenoceptor agonist, with sedative, analgesic and other effects[5]. Recent studies have shown that DEX has a certain anti-inflammatory effect. Xie Fang et al[6]. have shown that DEX can inhibit the inflammatory reaction in septic mice. In this study, sixty 18-month-old SD rats were selected as observation objects to analyze the effect and mechanism of DEX on hippocampal neuroinflammatory response and cognitive impairment induced by anesthesia in aged rats.

Methods

General information

Experimental animals

Sixty 18-month-old SD rats with an average body weight of 550±50 g were provided by the experimental animal center of Sun Yat-sen University (license number: SCXK(Yue)2009-0011). The rats were fed in the animal room at 23-24°C and a 12-h light cycle. The rats were fed and allowed to drink freely by special personnel. The experimental operations on the animals were carried out in accordance with the relevant standards of the regulations for the administration of experimental animals.

Experimental drug

DEX, provided by Jiangsu Hengrui Pharmaceutical Co., Ltd.

Methods

Establishment of experimental model and medication

All mice were randomly divided into a control group, a model group and a DEX group. Splenectomies were performed in the model group and the DEX group. First, anesthetized mice were intraperitoneally injected with 2% pentobarbital sodium. The incision was routinely prepared and disinfected. A transverse incision of about 2 cm was made along the skin 1 cm below the left costal margin. The subcutaneous tissue was separated layer by layer to enter the abdominal cavity. The spleen related blood vessels were ligated and severed. The spleen was removed. After bleeding stopped, the abdomen was closed and disinfected again. The whole operation was sterile. The control group was not treated. The rats in the DEX group were punctured in the tail vein and pumped with 10 μg/kg DEX in 10 minutes, and with 10 μg/kg/h continuously; rats in the model group were injected with normal saline.

Observation indexes

Y-maze test

A Y-maze test was performed at 9 AM on the first, third and seventh days after the operation. The whole maze was made of PVC board and divided into three arms, marked A, B and C, respectively. The angle between the three arms was 120° and the background of the inner wall of each arm was different. The three arms of the Y maze were randomly set as the starting arm, the new arm, and the other arm. Before the test, the new arm was blocked by a partition, and the rats were put in from the starting arm and allowed to move freely within the starting arm and the other arm.

Then, to start the test, the new arm was opened. The rats were placed in the starting arm and allowed to move freely throughout the three arms. The shuttle path was recorded and videoed. The movement path of the rats during the test was recorded, and the shuttle times and alternate score rate were calculated. Supposing that the new arm is A, the starting arm is B, and the other arm is C, during the test, the motor path of the rats was BACABCBAB. During the experiment, a total of 9 arms were passed, and the shuttle time was 9. In this case, the patterns were CBA, ABC, CAB, BAC; that is, the alternate score was 4, and the alternate score rate = (alternate score/(total number of arms - 2)) × 100%.

Blood gas analysis

Each group was selected for blood gas analysis, the abdomens of the rats were cut under anesthesia, the heart was exposed, the left ventricle was punctured with an arterial blood gas needle, and an i- START blood gas analyzer was used for blood gas analysis.

Detection of inflammatory indexes

The blood was decapitated and put into a micro centrifuge tube, which was left at room temperature for 30 min, then centrifuged at 1500 r / min for 5 min, the supernatant was taken, and the specimens were stored in a refrigerator at -80°C. The serum levels of IL-1β, and TNF-α were detected by ELISA.

Western blot

The hippocampal tissue was taken out and put into a glass homogenizer. The tissue was cut into pieces; RIPA and PMSF were added according to the ratio of 1ml RIPA+ 10 μL PMSF / 100ml tissue and homogenized for 30 min. The supernatant was obtained by centrifugation at 4°C for 5 min. The BCA method was used to determine the protein content, SDS-PAGE electrophoresis, membrane transfer, blocking, incubating the first antibody, incubating the second antibody, and ECL developer analysis, and the genesnap imaging analysis system was used to analyze the integral absorbance value of the target protein band.

Statistical methods

The data collected in this study were analyzed using the SPSS20.0 software package. All the measurement data in accordance with a normal distribution were compared by ($\bar{x}\pm s$). The comparison between multiple groups was carried out using one-way ANOVA, and the pairwise comparison was done using a SNK-q test.

The counting data were expressed as a percentage, and the comparison between groups was performed using a χ^2 test. Statistical results considered P<0.05 as statistically significant.

Results

Comparison of blood gas analysis indexes of mice in each group

As seen in Table 1, there was no significant difference in pH, PaO_2 and $PaCO_2$ among the three groups (P>0.05).

Group	pH value	PaO_2 (mmHg)	$PaCO_2$ (mmHg)
Control group	7.43±0.02	106.52±2.85	42.56±1.65
Model group	7.39±0.08	105.39±3.46	43.58±3.25
DEX group	7.41±0.06	104.69±3.15	44.25±2.64
F	2.31	1.700	2.150
P	0.108	0.191	0.126

Table 1: Comparison of blood gas analysis indexes of mice in each group ($\bar{x}\pm s$).

Comparison of shuttle times of mice in each group

As seen in Table 2, there was no significant difference in shuttle times among the three groups on the first, third and seventh days after the operation (P>0.05).

Group	Shuttle times of rats		
	1d after operation	3d after operation	7d after operation
Control group	13.25±2.16	12.85±1.96	13.64±2.51
Model group	14.25±2.61	12.97±2.15	13.46±1.85
Dex group	13.89±2.41	13.26±1.87	12.94±1.25
F	0.890	0.220	0.660
P	0.416	0800	0.519

Table 2: Comparison of shuttle times of mice in each group ($\bar{x}\pm s$).

Comparison of alternate score rate of mice in each group

On the first and third days after operation, the alternation score rate of the model group and the DEX group was significantly lower than that of the control group, and the alternation score rate of the DEX group was significantly higher than that of the model group (P<0.05). See Table 3.

Group	Alternate score rate (%)		
	1d after operation	3d after operation	7d after operation
Control group	82.61±5.64	82.64±10.25	83.64±9.61
Model group	38.95±6.34[a]	62.58±4.68[a]	82.65±10.25
Dex group	56.39±10.25[ab]	75.62±5.38[ab]	83.64±7.54
F	243.972	39.881	0.080
P	<0.001	<0.001	0.925

Table 3: Comparison of alternate score rate of mice in each group ($\bar{x}\pm s$).

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

IL-1β Level of mice in each group comparison

The levels of IL-1β in the model group and the DEX group were significantly higher than that in the control group. The level of IL-1β was significantly lower than in the model group, and the difference was statistically significant (P<0.05). See Table 4.

Group	IL-1β (pg/ml)		
	1d after operation	3d after operation	7d after operation
Control group	89.65±10.52	89.62±6.52	85.62±6.34
Model group	286.35±25.64[a]	263.95±20.14[a]	86.64±5.25
Dex group	241.36±10.56[ab]	136.95±15.26[ab]	85.21±3.26
F	724.550	716.011	0.420
P	<0.001	<0.001	0.662

Table 4: IL-1β of mice in each group horizontal comparison ($\bar{x}\pm s$).

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

TNF-α Level of mice in each group comparison

On the first and third days after the operation, the level of TNF- α in the DEX group was significantly higher than that in control group. The level of TNF- α was significantly lower than in the model group, and the difference was statistically significant (P<0.05). See Table 5.

Group	TNF-α (pg/ml)		
	1d after operation	3d after operation	7d after operation
Control group	42.95±3.16	40.61±3.58	35.64±3.85
Model group	132.79±5.28[a]	100.25±6.39[a]	36.84±2.15
Dex group	75.98±6.52[ab]	75.64±8.52[ab]	35.97±3.16
F	1541.48	426.94	0.780
P	<0.001	<0.001	0.461

Table 5: TNF of mice in each group- α Horizontal comparison ($\bar{x}\pm s$).

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

Comparison of SIRT1 pathway related proteins in mice in each group

The SIRT1 protein in the model group and the DEX group was significantly lower than that in the control group. The expression of the NF-ϰB protein was significantly higher than that in the control group. The SIRT1 protein in the DEX group was significantly higher than in the model group. The expression of NF-ϰB protein was significantly lower than that in the model group. The difference was statistically significant (P<0.05). See Figure 1.

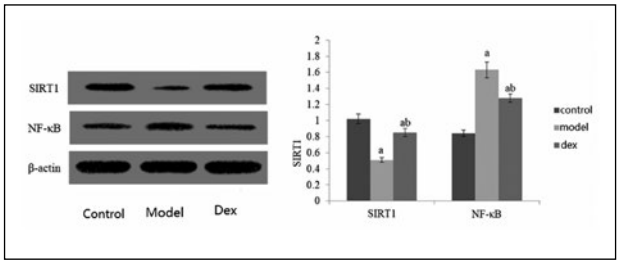

Figure 1: Comparison of SIRT1 pathway related proteins in mice of each group.

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

Discussion

POCD is a common postoperative complication in elderly patients. According to previous statistics, the probability of POCD in non-cardiac surgery patients over 60 years old is as high as 26% one week after surgery, and the probability of POCD in the three months after surgery is about 10%, which not only affects the quality of life of elderly patients, but also results in a heavy economic burden for families and society[7]. At present, the cause of POCD is not completely clear, but most studies indicate that POCD is related to the release of inflammatory factors[8]. POCD is often characterized by spatial learning and memory impairment. DEX is a common α2 receptor agonist at present, and research by Cheng Shao et al[9]. shows that DEX has the functions of maintaining hemodynamic stability, acting as an anti-inflammatory, and inhibiting blood glucose rise in patients with neurosurgery surgery, and it can reduce inflammatory response and stress response and promote the recovery of patients after neurosurgery. Recent studies suggest that the anti-inflammatory effect of DEX may be related to the central parasympathetic effect and activation of the cholinergic anti-inflammatory pathway, but its related mechanism is not fully understood[10]. In this experiment, the alternation score rates of the model group and the DEX group were significantly lower than that of the control group, and the alternation score rate of the DEX group was significantly higher than that of the model group on the first and third day after the operation. It is suggested that DEX can significantly improve the cognitive function of POCD rats, which is similar to the findings of Dong Lijuan et al[11].

An inflammatory reaction is one of the common causes of nerve injury, and it is also considered to be a promoter of POCD[12]. Inflammatory factor IL-1β, TNF-α can promote the release of toxic substances, change the morphology of neurons, and eventually lead to neuronal dysfunction[13]. Compared to those in other areas in the brain, neurons in the hippocampus are more vulnerable to damage, and because the hippocampus is closely related to learning and memory, damage to the hippocampus will negatively impact the learning and memory abilities of mice[14]. In this experiment, IL-1β and TNF- α in the model group and the DEX group were significantly higher than in the control group, and the levels of IL-1β and TNF- α in the DEX group were significantly lower than those in the model group on the first and third days after the operation. These results suggest that DEX can significantly reduce the inflammatory response of POCD rats and improve the cognitive function of mice.

NF-ϰ B is a nuclear transcription factor that widely exists in the nuclei of many kinds of cells, and it is a common regulatory pathway of

proinflammatory transcription factor expression[15]. Mu Xingguo et al[16]. showed that NF- ϰ B also participates in the inflammatory reaction of the nervous system, and it affects the cognitive function of patients by aggravating the neuron damage of an ischemic stroke. Chen Ling et al[17]. showed that the SIRT1/NF-ϰ B signaling pathway is the key pathway through which resveratrol can improve cognitive dysfunction in neonatal rats with hypoxic-ischemic brain damage. SIRT1 is a nicotinamide adenine dinucleotide dependent protein deacetylase, which is often expressed in hippocampal neurons. It plays a role in the occurrence and development of neurodegenerative diseases such as neuronal regeneration, learning and memory, and AD. Hu Yanhui et al[18]. showed that the SIRT1 signaling pathway is involved in the process of SGB reducing POCD in aged rats. Studies by Hu et al[19]. indicate that the SIRT1/NLRP3 pathway may be a key mechanism of quercetin neuroprotection in diabetic encephalopathy. In this experiment, the SIRT1 protein in the model group and the DEX group was significantly lower than in the control group, and NF-ϰ B was significantly higher than in the control group. The expression of the SIRT1 protein in the DEX group was significantly higher than that in the model group, and the NF-ϰ B protein was significantly lower than in the model group. It is suggested that DEX can improve the cognitive function of POCD rats, which may be related to DEX regulating SIRT1 pathway related proteins and inhibiting an inflammatory response.

In conclusion, DEX can inhibit the inflammatory response and improve the postoperative cognitive function of aged rats after anesthesia, and its mechanism may be related to the regulation of SIRT1 pathway related proteins by DEX.

References

1) Ma J, Williams J, Eastwood D, Lin S, Qian X, et al. High-dose Propofol Anesthesia Reduces the Occurrence of Postoperative Cognitive Dysfunction via Maintaining Cytoskeleton. Neuroscience 2019; 421: 136-143.
2) Daiello LA, Racine AM, Yun Gou R, Marcantonio ER, Xie Z, et al. Postoperative Delirium and Postoperative Cognitive Dysfunction: Overlap and Divergence. Anesthesiology 2019; 131(3): 477-491.
3) Lu Y, Xu X, Dong R, Sun L, Chen L, et al. MicroRNA-181b-5p attenuates early postoperative cognitive dysfunction by suppressing hippocampal neuroinflammation in mice. Cytokine 2019; 120: 41-53.
4) Lv M, Zeng H, He Y, Zhang J, Tan G. Dexmedetomidine promotes liver regeneration in mice after 70% partial hepatectomy by suppressing NLRP3 inflammasome not TLR4/NFϰB. Int Immunopharmacol 2018; 54: 46-51.
5) Kang K, Gao Y, Wang SC, Liu HT, Kong WL, et al. Dexmedetomidine protects against lipopolysaccharide-induced sepsis-associated acute kidney injury via an α7 nAChR-dependent pathway. Biomed Pharmacother 2018; 106: 210-216.
6) Hong J, Chen Q, Wang Y, Lin S, Su G. Dexmedetomidine alleviates smoke-induced bronchial and alveolar epithelial cell injury. Gen Physiol Biophys 2020; 39(3): 293-300.
7) Li WX, Luo RY, Chen C, Li X, Ao JS, et al. Effects of propofol, dexmedetomidine, and midazolam on postoperative cognitive dysfunction in elderly patients: a randomized controlled preliminary trial. Chin Med J (Engl) 2019; 132(4): 437-445.
8) Mao L, Zeng Q, Su W, Song M, Li J, et al. Elevation of miR-146a Inhibits BTG2/BAX Expression to Ameliorate Postoperative Cognitive Dysfunction Following Probiotics (VSL3) Treatment. Mol Neurobiol 2021; 58(7): 3457-3470.
9) Zhou W, Zhang D, Tian S, Tan C, Ma R, Zhang J, Sun J, Zhang Z. Optimal Dose of Dexmedetomidine for Perioperative Blood Glucose Regulation in Non-Diabetic Patients Undergoing Gastrointestinal Malignant Tumor Resection: A Randomized Double-Blinded Controlled Trial. J Invest Surg. 2021 Aug;34(8):869-874.
10) Li H, Zhang X, Chen M, Chen J, Gao T, et al. Dexmedetomidine inhibits inflammation in microglia cells under stimulation of LPS and ATP by c-Fos/NLRP3/caspase-1 cascades. EXCLI J 2018; 17: 302-311.
11) Deiner S, Luo X, Lin HM, Sessler DI, Saager L, et al. Intraoperative Infusion of Dexmedetomidine for Prevention of Postoperative Delirium and Cognitive Dysfunction in Elderly Patients Undergoing Major Elective Noncardiac Surgery: A Randomized Clinical Trial. JAMA Surg 2017; 152(8): e171505.
12) Cao M, Fang J, Wang X, Wang Y, Duan K, et al. Activation of AMP-activated protein kinase (AMPK) aggravated postoperative cognitive dysfunction and pathogenesis in aged rats. Brain Res 2018; 1684:21-1629.
13) Zhao J, Zuo H, Ding K, Zhang X, Bi Z, et al. Changes in plasma IL-1β, TNF-α and IL-4 levels are involved in chemotherapy-related cognitive impairment in early-stage breast cancer patients. Am J Transl Res 2020; 12(6): 3046-3056.
14) Cohen EJ, Quarta E, Fulgenzi G, Minciacchi D. Acetylcholine, GABA and neuronal networks: a working hypothesis for compensations in the dystrophic brain. Brain Res Bull 2015; 110: 1-13.
15) Li C, Zhao B, Lin C, Gong Z, An X. TREM2 inhibits inflammatory responses in mouse microglia by suppressing the PI3K/NF-ϰB signaling. Cell Biol Int 2019; 43(4): 360-372.

16) Shyu KG, Wang BW, Chang H. Retraction: Hyperbaric oxygen activates discoidin domain receptor 2 via tumour necrosis factor-α and the p38 MAPK pathway to increase vascular smooth muscle cell migration through matrix metalloproteinase 2. Clin Sci (Lond) 2016; 130(20): 1841.
17) Tang XL, Wang X, Fang G, Zhao YL, Yan J, et al. Resveratrol ameliorates sevoflurane-induced cognitive impairment by activating the SIRT1/NF-ϰB pathway in neonatal mice. J Nutr Biochem 2021; 90: 108579.
18) Zeng Y, Shi Y, Zhan H, Liu W, Cai G, et al. Reduction of Silent Information Regulator 1 Activates Interleukin-33/ST2 Signaling and Contributes to Neuropathic Pain Induced by Spared Nerve Injury in Rats. Front Mol Neurosci 2020; 13: 17.
19) Hu T, Lu XY, Shi JJ, Liu XQ, Chen QB, et al. Quercetin protects against diabetic encephalopathy via SIRT1/NLRP3 pathway in db/db mice. J Cell Mol Med 2020; 24(6): 3449-3459.

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