Influence of Dexmedetomidine on Cognitive Function and Inflammatory Factors in Rats and Analysis of Its Molecular Mechanism after Cardiac Surgery under Cardiopulmonary Bypass

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ABSTRACT

The study aimed to explore the influence of Dexmedetomidine (Dex) on cognitive function and inflammatory factors in rats after cardiac surgery under cardiopulmonary bypass (CPB). For this purpose, 30 healthy male SD rats were reared in a quiet and clean environment with alternating light for 12 hours. They were rolled randomly into 3 groups, each with 10 rats, namely the control (Ctrl) group, the experimental group, and the Dex group. The rats in the Ctrl were not treated, and the rats in the experimental group were intraperitoneally injected with 50μg/kg saline. After that, cardiac surgery was performed under CPB. Rats in the Dex group were injected with 50 μg/kg Dex intraperitoneally and underwent cardiac surgery under CPB. The Morris water maze (MWM) experiment was performed to test the learning and memory abilities and spatial positioning abilities of SD rats. Enzyme-linked immunosorbent assay (ELISA method) was adopted to detect the contents of TNF-α, IL-6, and IL-1β. Fluorescence quantitative PCR was applied to determine the mRNA expression levels of TNF-α, IL-6, and IL-1β in the hippocampus. Results showed that in the MWM experiment, in contrast with the Ctrl, the escape latency of the experimental group and the Dex group after surgery were prolonged (*P*<0.05), and the times they crossed platforms reduced (*P*<0.05). In contrast with the experimental group, the escape latency of the Dex group shortened, and the times they crossed platforms increased. ELISA suggested that in contrast with the experimental group, the concentrations of TNF-α, IL-6, and IL-1β in the Ctrl decreased (*P*<0.05), and those in the Dex group decreased slightly. In the fluorescence quantitative PCR experiment, in contrast with the experimental group, the mRNA expression levels of TNF-α, IL-6, and IL-1β in the Ctrl increased, and those in the Dex group decreased slightly. Then Dex can improve the cognitive dysfunction of rats undergoing cardiac surgery under CPB, and its molecular mechanism may be to reduce the inflammation around the heart and hippocampus.

Introduction

According to the latest statistics, the elderly population in China reached 160 million in 2019, accounting for 22.97% of the world's elderly population. The aging population in China is becoming increasingly severe (1), and the increase in the proportion of elderly retired employees drives the increase in medical expenses (2). The number of elderly patients undergoing surgery is on the rise. Postoperative cognitive dysfunction (POCD) is a common central nervous system complication after surgical anesthesia (3). POCD not only affects the patient's mental state and quality of life, but also prolongs the patient's hospital stay, increases the patient's medical expenses, and brings an economic burden to the family. Therefore, with the advent of an aging society, our country must study the molecular mechanism of POCD (4). Cognitive function refers to the ability of the human brain to process, store, and extract information, including language information, intellectual skills, and cognitive strategies. Cognitive dysfunction includes language, perception, memory, calculation, comprehension, and judgment obstacles (5). POCD refers to patients who have no mental illness before surgery, and their cognitive function deteriorates slightly after surgical anesthesia, which is mainly manifested as learning difficulties, memory decline, and mental difficulties (6). At present, the molecular mechanism of postoperative cognitive impairment is still unclear, and many scholars believe

that it is likely to be closely related to the contents of inflammatory factors in the nervous system (7).

During surgical treatment of neurodegenerative diseases, anesthesia and surgical trauma will increase the expression of pro-inflammatory genes. Besides, the contents of inflammatory factors will increase, which will lead to inflammation and reduce the learning and memory ability of animals. Therefore, the contents of inflammatory factors in the nervous system may be the most likely factor leading to postoperative cognitive impairment (8). Inflammatory factors hinder the function of mitochondria by triggering oxidative stress and activating glial cells, which leads to neuroinflammation. As a result, it in turn affects the normal metabolism of brain cells, induces neuropathological mechanisms, and changes the transmission of neurotransmitters (9). Studies have shown that even after non-brain surgery, the mRNA expression of TNF-α, IL-6, and IL-1β is still obviously increased. It indicates that pro-inflammatory factors can enter the brain tissue through the blood-brain barrier, thereby affecting the brain and nervous system. Terrando once found through research that TNF-α destroys the blood-brain barrier and drives macrophages into the hippocampus (10). The hippocampus tissue is an important part of the limbic system of the brain. It plays a role in learning, memory, and spatial positioning. It is affected by inflammatory factors, which damage the cognitive function of animals. Dex Hydrochloride (HCL) is an α2-adrenergic receptor agonist (11). Clinical experience in the United States has shown that Dex HCL can produce a stable and long-lasting sedative effect. Preoperative injection of Dex HCL can reduce the amount of opioid or non-opioid analgesics before and after surgery (12).

In summary, a CPB system was established in the study. Then, cardiac surgery was performed on SD rats to simulate the postoperative cognitive impairment model, which was caused by clinical myocardial ischemia-reperfusion surgery. Through experimental comparison, whether Dex can improve the postoperative cognitive impairment was analyzed, so as to further study the molecular mechanism of Dex on the body.

Materials and Methods
Experimental animals

30 healthy male Sprague Dawley (SD) rats were selected. They were 18 months old, weighing 450-550g. They were reared in the breeding room, and the environment was quiet and clean, well ventilated, with 12h alternating lighting. The breeding temperature was (20-28°C), relative humidity was about 50%, and adequate feed and drinking water was ensured during the experiment.

Experiment grouping

The adaptation training was carried out in the feeding room for 5 days before the experiment started. Through the random number table method, all rats were equally rolled into 3 groups, each with 10 rats, namely the Ctrl, the experimental group, and the Dex group. Ctrl: no injection of any drugs without CPB cardiac surgery. Experimental group: intraperitoneal injection of 50μg/kg normal saline 1 hour before surgery, followed by anesthesia and cardiac surgery under CPB. Dex group: intraperitoneal injection of 50μg/kg Dex HCL 1 hour before surgery, followed by CPB cardiac surgery after anesthesia.

MWM experiment

MWM is an experimental method designed by British psychologist Morris to study the learning and memory of the brain (Figure 1). In the experiment, the water aversion of rats was used to stimulate SD rats to swim in the water, so as to find platforms hidden in the water. Then, the learning and memory ability of the rats was tested in spatial positioning (13). The experiment included a cylindrical pool with a diameter of 1.5m and a height of 0.5m and a rat platform with a diameter of 12cm and a height of 30cm. Preparation of swimming pool. The cylindrical pool was divided into four quadrants with the center of the circle as the origin. According to the counterclockwise direction, it was divided into four quadrants of I, II, III, and IV from the upper right. Then, the platform was placed vertically at the I quadrant (35, 35) of the pool. The pool was filled with tap water, and the water temperature should be controlled at about 25°C. It should be noted that the temperature difference did not exceed one degree. When the platform was flooded, the highest point of the platform was 1cm from the level of the pool.

Then, milk powder was added to make the water turbid and make sure that the position of the platform can't be seen clearly.

After that, adaptive exercises started. A quadrant was selected at random as the starting position. The rat was held with the hand so that its tail touched the water surface first. The SD rat's head was toward the pool wall. It was gently put into the water without the head being pressed. Then, the trajectory of the swimming, the time spent in each quadrant, the speed of movement, and the time required to find the platform (escape latency) was recorded with the smart video analyzer. During each training process, if the rat did not find the platform within the 60s, it was guided to find the platform by hand and made to stay on the platform for 10s.

Subsequently, the rat was removed and wiped. Then, they were put in a warm cage. Each rat had 4 practices a day, and the rat started from a different quadrant in each practice with an interval of 20 minutes each time for 5 consecutive days. Positioning navigation test before surgery. One day before the cardiac surgery, the rat was put in the pool and the data were recorded. If the rat did not find the platform within the 60s, it needed to be guided to find the platform, and stay on the platform for 10s. Then, they were taken back into the cage. The training result was recorded in the 60s. Postoperative MWM experiment. 1 day, 5 days, and 10 days after CPB cardiac surgery, the rats were subjected to an MWM experiment. Each rat practiced 4 times each time. In each test, the rats started from a different quadrant with an interval of 20 minutes. External conditions such as platform location remain unchanged. Various data were recorded with an intelligent video analyzer. A space exploration experiment was performed after the operation. After the positioning and navigation test of each rat was over, the platform in the pool was taken away. On one day, 5 days, and 10 days after the operation, a space exploration experiment was performed and various data were recorded.

Cardiac surgery under CPB

A rat CPB model was established in the study. Then, after the cardiac surgery on rats, the CPB was terminated, and the needle tube was pulled out in turn. When the heartbeat of the rat was restored, the infection was simulated after clinical human heart surgery. The experiment was guaranteed to be in a sterile environment, and the surgical incision was sutured after the experiment was over. After successful anesthesia, the rat was in the supine position, and an oxygen mask was installed. Then, the left inguinal skin of the rat was cut, the heart was separated, and the left femoral artery was ligated (Figure 2). Subsequently, a 24G intravenous indwelling needle was injected into the proximal end. After the ligation was fixed, heparin solution was injected. Then, the monitor was connected through a transducer, to detect the level of arterial blood pressure in rats. The abdomen of the proximal end of the tail was incised from the middle, a 20G intravenous indwelling needle was injected, and the arterial perfusion was performed. The front end of the 16G intravenous indwelling needle was modified to have multiple side holes. The right neck of the rat was incised, and the venous jugular vein was separated. Then, the distal end was ligated, and the indwelling needle was injected into the proximal end. The needle was taken as a venous drainage tube and placed at the venous orifice of the right atrium. After rats were subjected to myocardial ischemia-reperfusion surgery (14), the surgical incision was sutured. After the rats recovered, they were returned to the cage for normal feeding.

Execution of rats and material extraction

On the 10th day after the operation, all rats were sacrificed after the MWM experiment was finished. Then, the brain tissue was separated (Figure 3) and placed on ice. After the intact hippocampus tissue was obtained, the hippocampus tissue was marked in liquid nitrogen and put in a -80°C refrigerator, to prepare materials for subsequent fluorescence quantitative experiments.

ELISA method to determine the concentration of TNF-α

The slats needed were taken out from the sealed bag in the refrigerator at 4°C, and the remaining slats and desiccant were put back into the aluminum foil bag. Then, the sealed bag was put back in the refrigerator. After standards and specimen diluents were added to the blank wells of the slat, specimens and standards of different concentrations (100μL/well) were added to the remaining corresponding wells.

Then, the reaction wells were sealed with sealing tape and placed in a constant temperature incubator at 36°C for 90 minutes. The biotinylated antibody working solution was prepared 20 minutes in advance. Then, the plate was washed 5 times. The biotinylated antibody diluent was added to the blank wells, and a biotinylated working solution (100μL/well) was added to the remaining wells. After that, the reaction wells were sealed with new sealing tape and placed in a 36°C constant temperature incubator for 60 minutes. The enzyme conjugate working solution was prepared 20 minutes in advance and stored at room temperature 22-25°C away from light. The plate was washed 5 times. After enzyme conjugate diluent was added to the blank wells, enzyme conjugate working solution (100uL/well) was added to the remaining wells. The reaction wells were sealed with new sealing tape and placed in a constant temperature incubator at 36°C for 30 minutes in the dark. Then, the micro-plate reader was turned on to warm up the instrument. At the same time, the detection program was set. The plate was washed 5 times. After 100uL/well of tetramethylbenzidine (TMB) was added, the plates were put in a 36°C constant temperature incubator. The incubator was placed in the dark for 15 minutes. After 100uL/well of stop solution was added and mixed well, OD_{450} value was measured immediately (completed within 3 minutes). The result was kept in the instrument and printed out for later use. After the experiment was over, the instrument and unused reagents were returned. Finally, the concentration of TNF-α was calculated by drawing a standard curve (15).

Determination of the concentration of IL-6 and IL-1β

The concentrations of IL-6 and IL-1β were determined in the same way as above. The operations were performed strictly according to the kit operating instructions.

Trizol method to extract RNA from hippocampus

The hippocampus tissue was taken out of the -80°C low-temperature refrigerator, placed in a grinder for grinding, and the ground tissue was transferred into a sterile 1.5mL EP tube. Then, 1 mL Trizol was added to the EP tube. According to the Trizol instructions (16), it was placed at room temperature for 5 minutes, and on ice for 10 minutes (not invert and shake). Subsequently, 200L chloroform was added and shaken vigorously for 15 seconds. After the sample was put at room temperature for 5 minutes, it was centrifuged at 12000×g at 4°C, for 10 minutes. Then, the upper layer of water was absorbed and transferred to another EP tube. It should be noted not to absorb the middle and lower layers. After 0.5 mL of isopropanol was added to the upper water EP tube and mixed well, the sample was placed at room temperature for 15 m and centrifuged at 12000×g for 10 minutes at 4°C. Then, the supernatant was aspirated and discarded. After 1 mL of 75%, absolute ethanol was added and mixed by vertexing, the sample was centrifuged at 500×g for 5 minutes at 4°C. Then, 1mL of 75% absolute ethanol was added to wash the precipitate again. After the supernatant was aspirated and discarded, the sample was dried at room temperature for 5 minutes. Then, 40μL of DEPC was used to completely dissolve the RNA. At the same time, a spectrophotometer was adopted to detect the RNA concentration.

Fluorescence quantitative PCR

When primers were designed, the cDNA of TNF-α, IL-6, and IL-1β was found in the gene bank. Then, they were designed through DNAMAN. The upstream primer of TNF-α had 20 bases and the downstream primer had 21 bases. The upstream primer of IL-6 had 23 bases, and the downstream primer had 21 bases. The upstream primer of IL-1β had 19 bases, and the downstream primer had 22 bases. After the primers were designed, BLAST detection was performed on them. If it was not complementary to other genes, the experiment can be started. Samples were added according to the instruction method. With reference to the operating steps of the fluorescence quantitative PCR instrument, the sample cDNA was synthesized, and stored in the refrigerator at -80°C for later use. The sample cDNA was put in a quantitative PCR reaction system. Then, amplification was performed in a fluorescent quantitative PCR machine. The reaction program was set as 120s pre-denaturation at 93°C, followed by 60s denaturation at 93°C, 60s at 60°C, and 60s at 71°C, with 40 cycles in total. Finally, there were 7 minutes of extension at 71°C.

Statistical methods

The calculation data in this experiment were expressed by the mean ± variance ($\bar{x}\pm s$), and data were processed by SPSS (22.0), and *P*<0.05) was considered statistically significant.

Results and discussion
Results of MWM

Before surgery, rats were trained for five consecutive days. With the increase in the number of practices, the rats' learning and memory abilities continued to strengthen. It indicated that the difference in escape latency between the three groups was obvious (*P*<0.05) (Table 1, Figure 1). After cardiac surgery, in contrast with the Ctrl, the escape latency of the experimental group was increased (*P*<0.05), and the times the rats crossed the platform decreased (Table 2, Table 3, Figure 2, Figure 3). In contrast with the Ctrl, the escape latency of the Dex group increased and the times the rats crossed the platform decreased. However, in contrast with the experimental group, there was an obvious improvement (Table 2, Table 3, Figure 2, and Figure 3).

Table 1. Escape latency comparison before surgery (s, $\bar{x}\pm s$)

Group	n	5 days before surgery	4 days before surgery	3 days before surgery	2 days before surgery	1 day before surgery
Ctrl	10	32.7±12.1	20.8±6.8	19.4±7.1	12.1±2.4	12.3±1.8
Experimental group	10	33.7±11.5	21.3±7.1	20.1±6.9	13.5±2.1	13.4±1.7
Dex group	10	31.9±10.9	20.7±7.5	20.5±6.5	13.2±1.8	12.5±1.9

Note: the incubation period was gradually shortened, and the difference within the group was obvious (*P*<0.05)
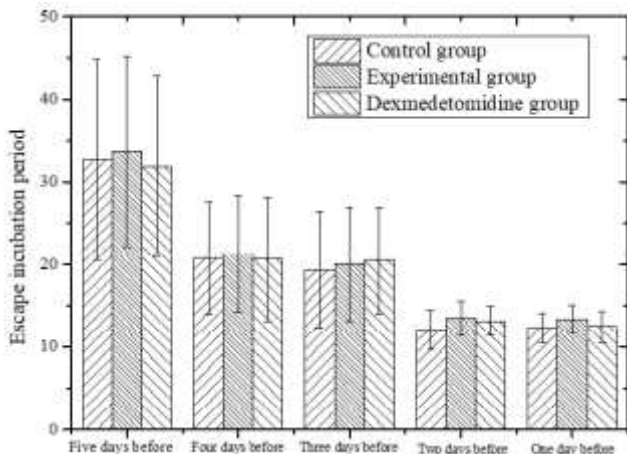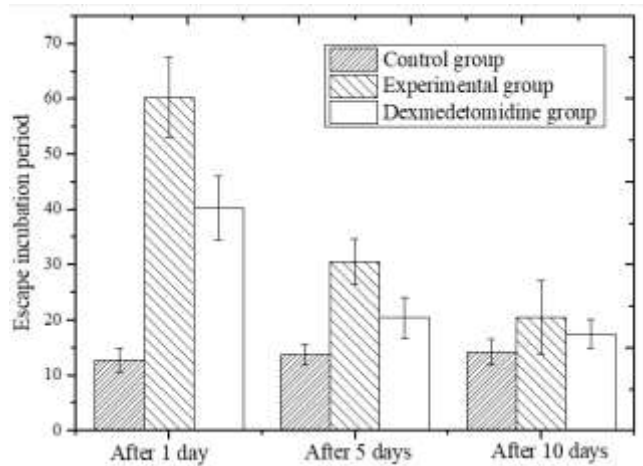

Figure 1. Comparison of preoperative escape latency in rats

Table 2. Escape latency comparison after surgery (s, $\bar{x}\pm s$)

Group	n	1 day after surgery	5 days after surgery	10 days after surgery
Ctrl	10	12.7±2.12	13.7±1.93	14.1±2.32
Experimental group	10	60.23±7.25ab	30.47±4.15ab	20.43±6.71
Dex group	10	40.21±5.72a	20.35±3.75a	17.45±2.56

Note: in contrast with the Ctrl, a: *P*<0.05; in contrast with the Dex group, b: *P*<0.05.


Figure 2. Comparison of escape latency after surgery

Table 3. Comparison of the number of times rats crossed the platform after surgery (times, $\bar{x}\pm s$)

Group	n	1 day after surgery	5 days after surgery	10 days after surgery
Ctrl	10	3.2±0.65	3.4±1.21	3.8±1.05
Experimental group	10	1.2±0.31c	1.4±0.52c	1.7±0.43c
Dex group	10	2.1±0.52cd	2.3±0.82cd	2.4±0.75cd

Note: in contrast with the Ctrl, c: *P*<0.05, in contrast with the experimental group, d: *P*<0.05, the difference was obvious.
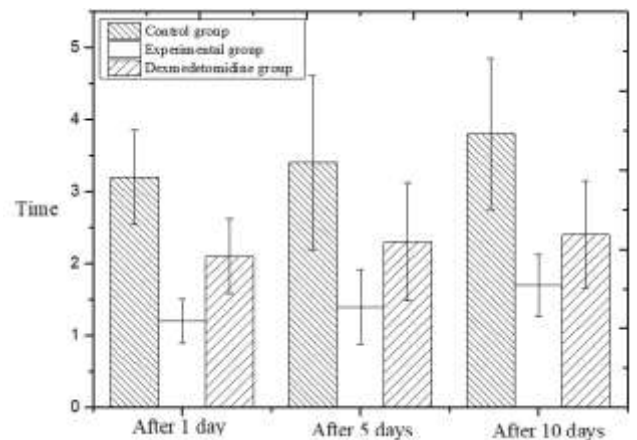
**Figure 3.** Comparison of the number of times the rats crossed the platform after surgery

Results of fluorescence quantitative PCR

In contrast with the Ctrl, the mRNA expression levels of TNF-α, IL-6, and IL-1β in the experimental group and the Dex group were increased ($P<0.05$). In contrast with the Dex group, the mRNA expression levels of the three factors in the Ctrl decreased, and those in the experimental group increased (Table 4, Figure 4).

Table 4. Comparison of mRNA expression levels of three inflammatory factors in hippocampus (pg/mL, $\bar{x}\pm s$)

Group	n	TNF-α	IL-6	IL-1β
Ctrl	10	15.15±3.12	78.97±12.75	54.01±21.32
Experimental group	10	24.21±5.28[e]	110.27±3.45[e]	65.45±24.85[e]
Dex group	10	18.27±4.51[ef]	94.95±11.65[ef]	61.54±21.78[ef]

Note: in contrast with the Ctrl, e: $P<0.05$, in contrast with the experimental group, f: $P<0.05$, the difference was obvious.
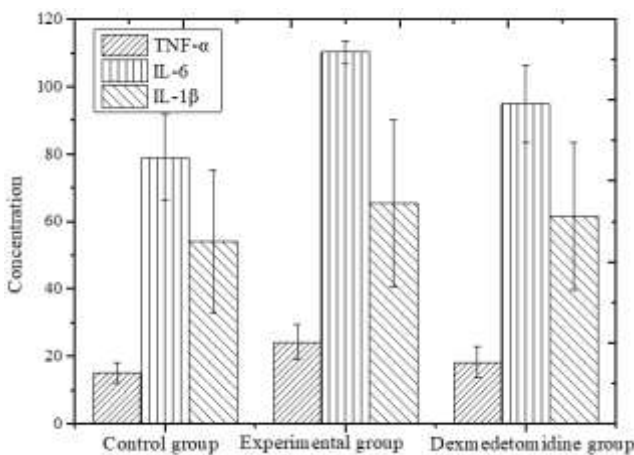
Figure 4. Comparison of mRNA expression levels of three inflammatory genes in the hippocampus

In this study, rats were injected with Dex before surgery. Then, the cognitive dysfunction and inflammatory factor concentrations were measured after CPB cardiac surgery, so as to verify the molecular mechanism of Dex on the inflammatory response.

Fong et al. (2006) pointed out that POCD was the result of patient's factors, drugs, and various reasons during and after surgery (17). From the data of MWM, it was evident that the escape latency of rats in the experimental group and the Dex group was longer at 1, 5, and 10 days after cardiac surgery than that before the operation ($P<0.05$). In contrast with the Ctrl, the intra-group difference between the experimental group and the Dex group after the operation was obvious ($P<0.05$), and the times rats

crossed platforms were also obviously reduced. Therefore, cardiac surgery under CPB in this experiment would affect the cognitive function of rats. In the MWM, in contrast with the experimental group, the Dex group had shorter escape latency at 1, 5, and 10 days after surgery ($P<0.05$), and the times rats crossed platforms increased ($P<0.05$). It indicated that Dex can reduce the incidence of cognitive dysfunction under CPB cardiac surgery. Dex can improve immunity and reduce inflammation (18-20). After cardiac surgery under CPB, the complement system was activated, which stimulated the release of inflammatory factors, and caused inflammation. ELISA method suggested that in contrast with the Ctrl, the concentrations of TNF-α, IL-6, and IL-1β in the experimental group and Dex group were increased ($P<0.05$), and the concentration in the Dex group was lower than that in the experimental group ($P<0.05$). It suggested that injection of Dex before surgery can improve the inflammatory response caused by surgery in rats. Fluorescence quantitative PCR indicated that in contrast with the Ctrl, the mRNA expression levels of TNF-α, IL-6, and IL-1β in the hippocampus of the experimental group and the Dex group were increased ($P<0.05$). The mRNA expression of the three inflammatory genes IN in the Dex group was lower than that of the experimental group ($P<0.05$). It proved that Dex can not only act on the inflammatory response around cardiac surgery but also improve the inflammation in the nervous system.

Conclusions

In this study, the MWM experiment was used to compare the escape latency of rats. In contrast with the experimental group, the escape latency of the Ctrl shortened, and the escape latency of the Dex group decreased. It was evident that Dex can improve the POCD of rats. As for the determination of concentrations of inflammatory factors and mRNA expression level, in contrast with the experimental group, the inflammatory factor concentration and mRNA expression level in Ctrl and the Dex group decreased. It was evident that Dex can inhibit the release of inflammatory factors, decrease the mRNA expression levels, and reduce inflammation. In summary, Dex can reduce inflammation and improve postoperative cognitive impairment.

Acknowledgments

Conflict interest

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