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Original Article

Protective effect and mechanism of hydrochloride dexmedetomidine on the severe craniocerebral trauma in rats

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Abstract: To investigate the cerebral protection effect of dexmedetomidine (DEX) in severe craniocerebral injury rat model. A total of 120 male SD rats were equally randomly divided into DEX group, sham group, and control. Rat acute severe craniocerebral injury model was established using hydraulic blow method. The rats received sedative treatment at 2 h after injury. Rat behavior changes before and after modeling were observed. Serum S-100β, neuron specific enolase (NSE), and myelin basic protein (MBP) levels on pre-operation (T1), day 1 post operation (POD1, T2), day 3 post operation (POD3, T3), day 5 post operation (T4), and day 7 post operation (T5) were detected by ELISA. Rat hippocampus was extracted for pathological section on T2, T3, T4, and T5. Neuronal cell death was observed upon Nissl's staining. NF-κB and Caspase-3 expression in hippocampus were tested by immunohistochemistry. Serum S-100β, NSE, and MBP levels significantly decreased on T2, T3, T4, and T5 in DEX group compared with control group ($P < 0.05$). Neuronal cell death, NF-κB, and Caspase-3 expression markedly reduced on T2, T3 (except NF-κB), T4, and T5 in DEX group compared with control group ($P < 0.05$). DEX can protect brain upon improving cerebral oxygen metabolism and alleviating cerebral ischemia hypoxia in severe craniocerebral injury.

Keywords: Severe craniocerebral injury, dexmedetomidine, cerebral metabolism, cerebral protection

Introduction

Following the development of economy and traffic transportation industry, the incidence of craniocerebral trauma shows rising trend year by year. As one of the more common car accident injuries in clinic, craniocerebral trauma and its complications has a serious influence on the central nervous system [1]. According to the Glasgow scoring system, patients with craniocerebral trauma can be grouped into mild (13-15 points), medium (9-12 points), heavy (6-8 points), and severe (3-5 points). Among them, patients in heavy and severe types have the most severe injury and complex treatment because of the interaction of injury factors, treatment methods, and individual differences, leading to the total fatality rate remains at 30%-50% [2, 3]. Heavy and severe craniocerebral injury may cause cerebral edema, nonphysical brain blood oxygenation and perfusion, local cerebral ischemia, cerebral embolism, and inflammatory cascade reaction, which can induce central nervous system damage [4]. Thus, application of sedative with central nervous system protection can alleviate cerebral function damage after severe craniocerebral injury, thus has a practical value and clinical significance.

Dexmedetomidine (DEX) is a kind of α_2 adrenergic agonists that commonly used as auxiliary medication is clinical anesthesia. It has the effect of sedation, analgesia, and anti-anxiety, and is usually used for sedation during tracheal intubation and mechanical ventilation in general anesthesia [5, 6]. It was found that DEX could be used in surgical operation of acute craniocerebral injury as it can inhibit the effect of perioperative inflammatory reaction and cerebral protection [7, 8]. Up to now, however, there is still lack of report about DEX adoption in the seda-

tive treatment after severe craniocerebral injury. This study intends to explore the cerebral protection effect of DEX in severe craniocerebral injury through the establishment of rat severe craniocerebral injury model.

Materials and methods

Experimental animals

Male SD rats at 6-week old and weighted 250~300 g were purchased from laboratory animal center of Wuxi Medical College.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of PLA 101 Hospital.

Main reagents and instruments

Serum S-100β, neuron-specific enolase (NSE), and myelin basic protein (MBP) ELISA kits were got from R&D. Bio-Rad 680 microplate reader was bought from Bio-Rad. Claudin 5 antibody was got from Abcam. CD31 antibody was purchased from Invitrogen (USA). Fluorescence microscope was from Olympus.

Rat acute severe craniocerebral injury model preparation

The rats in DEX group and control were anesthetized by 10% chloral hydrate (3.0 ml/kg) intraperitoneal injection. The head was fixed on stereotactic frame and disinfected. A straight incision was made on the top to expose parietal bone. A bone window with diameter at 4 mm was opened on 3 mm after bregma and 2 mm on the right side of sagittal suture. The endocranium was kept integrity. The skull connecting pipe was adhered to bone window and sterile saline solution was injected through the lumen. After consciousness recovery, the hydraulic blow apparatus was connected to the connecting pipe to perform hydraulic blow with the striking force at 3.2~3.5 atm. The rat in sham group received same procedure without Control operation with before, but no hydraulic blow. The rats were kept in single cage after spontaneous breathing recovery.

Experimental animals grouping and treatment

A total of 120 male SD rats were equally randomly divided into DEX group, sham group, and control. Serum S-100β, neuron-specific enolase (NSE), and myelin basic protein (MBP) levels on pre-operation (T1), POD1 (T2), POD3 (T3), POD5 (T4), and POD7 (T5) were detected by ELISA. Rat hippocampus was extracted for pathological section on T2, T3, T4, and T5. Neuronal cell death was observed upon Nissl's staining. The rats in DEX group received 30 ug/kg DEX intraperitoneal injection at 12 h after modeling, while the rats in control received equal amount of normal saline.

Blood sample collection

A total of 2 ml peripheral venous blood was extracted in EDTA anticoagulated tube and centrifuged to extract supernatant. The serum was stored at -80°C.

Serum S-100β, NSE, and MBP detection

Serum S-100β, NSE, and MBP levels were detected according to the ELISA manual.

Hippocampus pathological examination

The rats were killed on POD1 and POD7 after hydraulic injury. The brain tissue was fixed in 4% paraformaldehyde and then incubated in 30% sucrose solution. After embedding, the tissue was coronary serial sectioned at 10 μm thickness from 0.7~2.3 mm after bregma for immunofluorescent staining. Classical immunofluorescent staining was applied for CD31 and Claudin 5 staining. After washed by PBS for three times, the slice was blocked by normal rabbit or mouse serum for 30 min. Next, the slice was incubated in rabbit anti rat Claudin 5 antibody (1:500) and mouse anti rat CD31 antibody (1:500) at 4°C overnight. After washed by PBS for three times, the slice was further incubated in Alexa Fluor 488-conjugated goat anti rabbit antibody (1:1000) and Mexa Fluor 568-conjugated goat anti mouse antibody (1:1000) at 20-37°C for 1 h. At last, the slice was stained by DAPI avoid of light for 5 min. After sealed by anti-fluorescence quenching agent, the slice was observed under the fluorescence microscope. The data was analyzed by the Image J software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All data analysis was performed on IBM SPSS 19.0 software. Measurement data was depicted as mean ± standard deviation and compared by t test. Enumeration data was com-

Table 1. Serum S-100β, NSE, and MBP levels comparison (x±s, μg/L)

Group	n	Index	T1	T2	T3	T4	T5
Control	40	S-100β	0.072±0.002	0.245±0.055*,#	0.334±0.002*,#	0.567±0.050*,#	0.735±0.015*,#
		MBP	7.34±0.02	12.45±2.15*,#	21.45±1.15*,#	23.56±0.15*,#	36.45±3.78*,#
		NSE	8.35±0.45	21.46±2.55*,#	30.84±5.25*,#	34.56±5.74*,#	36.45±3.78*,#
Sham	40	S-100β	0.073±0.001	0.066±0.001	0.056±0.154	0.056±0.001	0.045±0.005
		MBP	7.35±0.05	8.31±0.03	10.15±0.05	11.56±1.05	19.55±3.56
		NSE	8.23±0.55	11.45±2.55	16.56±3.46	17.45±5.67	19.55±3.56
DEX	40	S-100β	0.071±0.001	0.197±0.056*,#,&	0.234±0.002*,#,&	0.324±0.001*,#,&	0.335±0.004*,#,&
		MBP	7.35±0.05	10.15±0.15*,#,&	17.35±1.05*,#,&	18.45±0.07*,#,&	21.45±0.15*,#,&
		NSE	8.33±0.46	16.34±2.45*,#,&	20.84±1.13*,#,&		26.45±3.45*,#,&

*P < 0.05, compared with T1; #P < 0.05, compared with sham group; &P < 0.05, compared with control group. T1: pre-operation; T2: 1 day post the operation; T3: 3 day post the operation; T4: 5 day post the operation; T5: 7 day post the operation.

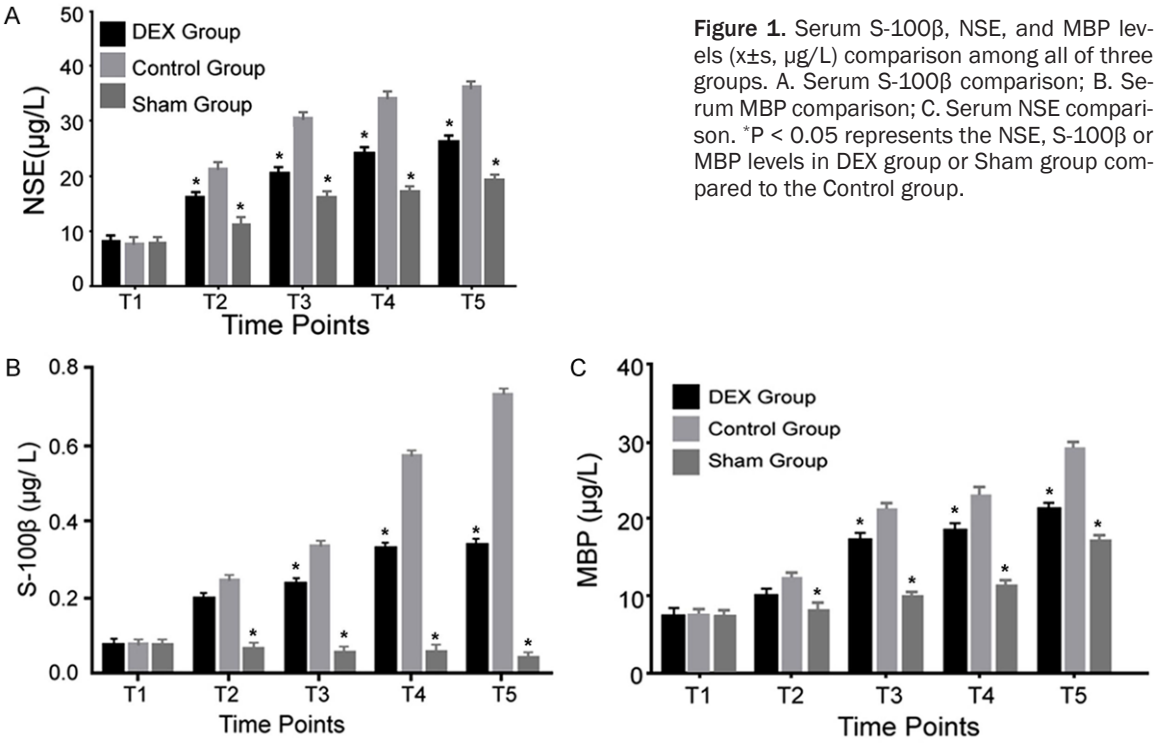

Figure 1. Serum S-100β, NSE, and MBP levels (x±s, μg/L) comparison among all of three groups. A. Serum S-100β comparison; B. Serum MBP comparison; C. Serum NSE comparison. *P < 0.05 represents the NSE, S-100β or MBP levels in DEX group or Sham group compared to the Control group.

pared by chi-square test. Correlation analysis was adopted upon Spearman rank analysis. Multivariate analysis was applied using Logistic regression analysis. P < 0.05 was presented as statistical significance.

Results

Serum S-100β, NSE, and MBP levels comparison

Serum S-100β, NSE, and MBP levels in T1 showed no statistical significance among three groups (P > 0.05). Serum S-100β, NSE, and MBP levels in T2, T3, T4, and T5 were obviously higher than T1 in DEX group and control (P < 0.05). Serum S-100β, NSE, and MBP levels significantly increased on T2, T3, T4, and T5 in DEX group and control compared with sham group (P < 0.05). Their levels in DEX group were obviously lower than the control (P < 0.05) (**Table 1**; **Figure 1**).

Hippocampal neuron cell death comparison

Cellular morphology in hippocampus was normal in sham group. The cells in control group arranged disorder, appeared edema and number reduce on T2, and showed Nissl body disin-

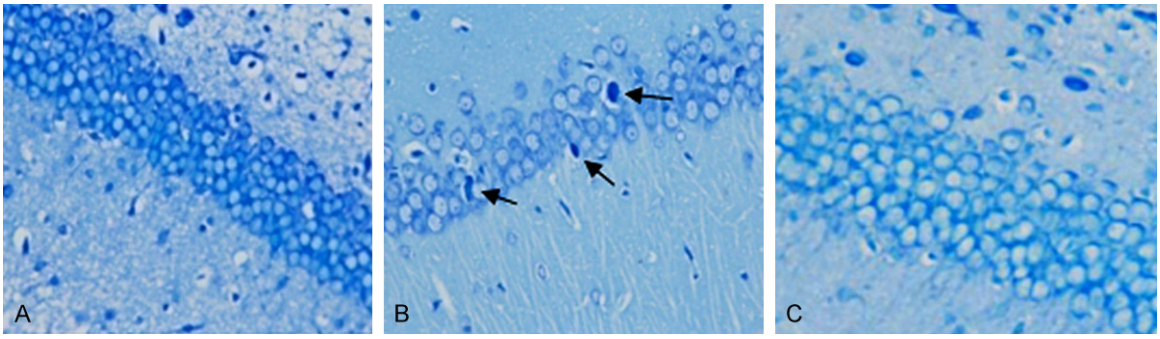

Figure 2. Hippocampal neuron cell death comparison in all groups. A. Sham group; B. Control group; C. DEX group. The arrows represent the Nissl body in the tissues.

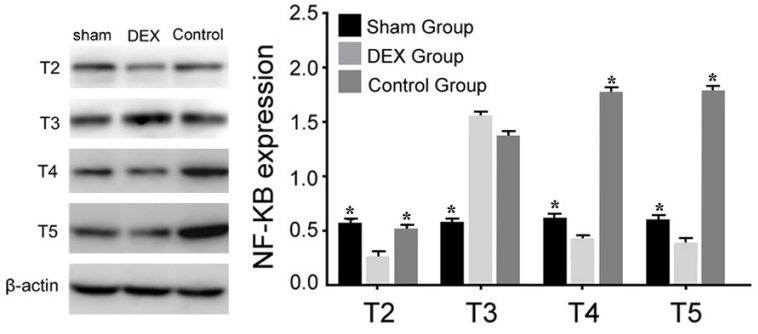

Figure 3. Hippocampal neuron cells NF-κB expression comparison in all groups. *P < 0.05 represents the NF-κB levels in Control group or Sham group compared to the DEX group.

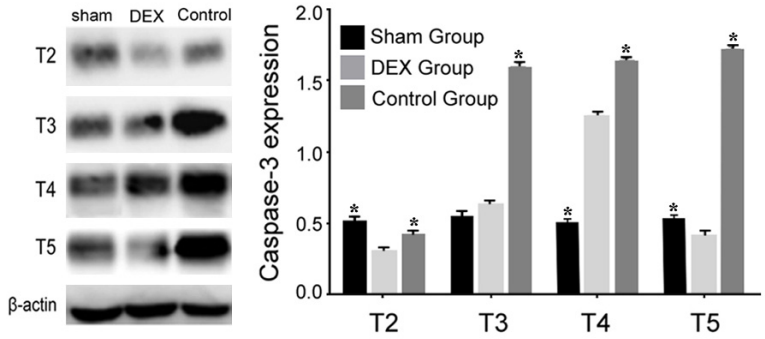

Figure 4. Hippocampal neuron cells Caspase-3 expression comparison in all groups. *P < 0.05 represents the caspase-3 levels in control group or sham group compared to the DEX group.

tegration and lost, together with karyopyknosis, karyorrhexis, and karyolysis on T5. Cell damage in DEX group were obviously slighter than that in control at the same time point (**Figure 2**).

NF-κB expression in hippocampal neuron cells

Western blot revealed that NF-κB showed no statistical difference in sham group among different time points (P > 0.05). NF-κB expression in T2 was lower in DEX compared with sham group (P < 0.01). It gradually elevated and reached top in T3, which was obviously higher than that in sham group (P < 0.01). It then reduced, which was lower than that of sham group in T4 and T5 (P < 0.01). NF-κB level in T2 was lower in DEX group compared with sham and control (P < 0.05). It showed no statistical difference in T3 compared with control while higher than that in sham group. NF-κB kept in relative low level in T4 and T5 at DEX group, which was significantly lower than that in sham and control (P < 0.01) (**Figure 3**).

Caspase-3 expression in hippocampal neuron cells

Western blot revealed that Caspase-3 showed no statistical difference in sham group among different time points (P > 0.05). Caspase-3 expression in T2 was lower in DEX compared with sham group (P < 0.01). It gradually elevated and reached top in T4, which was obviously higher than that in sham group (P < 0.01). It then reduced, which was lower than that of sham group in T5 (P < 0.01). Caspase-3 level in T2 was lower in DEX group compared with sham and control (P < 0.05). Caspase-3 kept

in relative low level in T5 at DEX group, which was significantly lower than that in sham and control ($P < 0.01$) (**Figure 4**).

Discussion

Heavy and severe craniocerebral injury induced nonphysical brain blood oxygenation and perfusion, local cerebral ischemia, cerebral embolism, and inflammatory cascade reaction can trigger central nervous system damage [9-11]. Searching for correct cerebral protective measures can alleviate cerebral function damage after severe craniocerebral injury.

Ehsan Z showed that 1 μg/kg DEX infusion before vein anesthesia induction and 0.4 μg/kg DEX injection till finish operation can reduce the perioperative inflammatory response in patients with acute craniocerebral injury [12]. Therefore, this study selected DEX for investigation. S-100β, MBP, and NSE proteins are mainly located in glial cells of the central nervous system. They can be detected in blood during glial cell damage and blood brain barrier dysfunction [13, 14]. Their protein concentrations in the blood reflect the particular sensitive index of brain damage. This study applied the classical ELISA method to detect serum S-100β, MBP, and NSE protein concentrations to ensure the examination accuracy. Our results demonstrated that serum S-100β, MBP, and NSE levels decreased in DEX group, suggesting that DEX produced cerebral protection after severe craniocerebral injury.

It was revealed that cell apoptosis is the major type of delayed neuron death caused by cerebral hypoxia ischemia, involving the mechanisms of (1) energy metabolism disorder; (2) intracellular calcium overload; (3) lipid peroxidation; (4) the toxic effects of excitatory amino acids; (5) free radical damage; (6) inflammatory response [15-18]. It was found that excitatory amino acids play a critical role in the pathogenesis of cell death induced by neuron toxic effect [19]. Glu is the major part of excitatory amino acids that acts the excitatory neurotransmitter effect to maintain normal brain function. However, cerebral ischemia hypoxia induces a large number of substances, such as reactive oxygen species, leading to GLT concentration at 10 times in synaptic cleft and extracellular glutamate. Excessive glutamate releases to be a potential neurotoxin. Once the glutamate content in synaptic cleft exceeds the GLT adjusting range and breaks the physiological balance between excitatory and inhibitory amino acids, it may over stimulate ionic glutamate receptor on cell membrane, such as N-methyl-D-aspartic acid (NMDA) receptor. It further leads to a series of diseases, including early Na^+, Cl^-, H_2O internal flow induced cell edema and Ca^{2+} overload, and late apoptosis induced secondary nerve injury, leading to "exitotoxicity" [20].

In this study, Western blot revealed that NF-κB expression was lower in DEX compared with sham group. It gradually elevated and reached top in T3 and then reduced, which was lower than that of sham group and Control group. NF-κB level in T2 was lower in DEX group compared with sham and control. NF-κB kept in relative low level in T4 and T5 at DEX group, which was significantly lower than that in sham and control. It suggested that DEX may enhance excitatory amino acids Glu transport, alleviate nerve excitability toxicity effect, improve brain hippocampus tissue pathology, and reduce neuron apoptosis through down-regulating NF-κB expression, thus to play an important role in neuroprotection.

Caspase-3 widely expressed in the body to regulate cell apoptosis and proliferation. Page VJ discovered that Caspase-3 activation upregulated GLT-1 expression in Astrocytes and enhanced the ability to scavenge extracellular Glu [21]. Piva S observed that sustained activated Caspase-3 transfected to Astrocytes increased GLT-1 expression with time and dose dependence [22]. On the other side, suppression of Caspase-3 and NF-κB reduced GLT-1 level in Astrocytes [23]. Western blot demonstrated that Caspase-3 showed no statistical difference in sham group among different time points ($P > 0.05$). Caspase-3 expression in T2 was lower in DEX compared with sham group ($P < 0.01$). It gradually elevated and reached top in T4, which was obviously higher than that in sham group ($P < 0.01$). It then reduced, which was lower than that of sham group in T5 ($P < 0.01$). Caspase-3 level in T2 was lower in DEX group compared with sham and control ($P < 0.05$). Caspase-3 kept in relative low level in T5 at DEX group, which was significantly lower than that in sham and control ($P < 0.01$). It suggested that DEX can alleviate nerve

excitability toxicity effect, improve brain hippocampus tissue pathology, and reduce neuron apoptosis via downregulating Caspase-3 expression.

Conclusion

DEX can protect brain upon improving cerebral oxygen metabolism and alleviating cerebral ischemia hypoxia in severe craniocerebral injury through downregulating NF-κB and Caspase-3 levels.

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Disclosure of conflict of interest

None.

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