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Original Article

Study on the protective effect of dexmedetomidine preconditioning on brain in rats with experimental subarachnoid hemorrhage and its mechanism of action

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Abstract: Objective: To study the protective effect of dexmedetomidine on early cerebral injury in rats with subarachnoid hemorrhage. Methods: A total of 60 SD rats were divided into three groups: sham operation group (Sham group, n=8), subarachnoid hemorrhage group (SAH group, n=16) and the group pretreated with dexmedetomidine + subarachnoid hemorrhage (Dex+SAH group, n=36). Dex+SAH group was divided into three subgroups according to different concentrations of dexmedetomidine, namely low dosage group (Dex-L+SAH group, 10 μg), medium dosage group (Dex-M+SAH group, 50 μg) and high dosage group (Dex-H+SAH group, 100 μg), with 12 rats for each group. Mortality of all the SD rats in each group was recorded within 72 h after the operation; levels of IL-1β, IL-6 and TNF-α were detected and their neurological function scores, subarachnoid hemorrhage scores and severity of brain tissue edema were recorded within 24 h after the operation (there were 8, 10, 10, 10, 12 rats in Sham, SAH, Dex-L+SAH, Dex-M+SAH and Dex-H+SAH group received examination 24 h after the surgery, respectively); Results: The mortality of the rates in SAH group was higher than that of Sham group and Dex-H+SAH group (P=0.022, P=0.039). The neurological function scores of subarachnoid hemorrhage of the rats in SAH group were lower than those of other fours groups. The neurological function scores of the rates in Dex-H+SAH group were higher than those of Dex-L+SAH group and Dex-M+SAH group. The subarachnoid hemorrhage scores of the rats in SAH group were higher than those of other fours groups. The subarachnoid hemorrhage scores of the rates in the Dex-H+SAH group were lower than those of Dex-M+SAH group and Dex-L+SAH group. As for severity of brain tissue edema, the water content of brain tissue in the rats of SAH group was higher than that of other fours groups; the water content of brain tissue of the rats in Dex-H+SAH group was lower than that of Dex-M+SAH group and Dex-L+SAH group. The levels of IL-1β, IL-6 and TNF-α in the rats of SAH group increased significantly compared with Sham group; levels of IL-1β, IL-6 and TNF-α in the rats of Dex-H+SAH group was lower than those of Dex-M+SAH group and Dex-L+SAH group with statistically significant differences. Conclusions: Dexmedetomidine preconditioning generates certain protective effect on brain tissue of rats with subarachnoid hemorrhage possibly through reducing brain tissue edema, subarachnoid hemorrhage and anti-inflammatory effect; the effect of high-dose dexmedetomidine is better than that of medium dose and low dose.

Keywords: Subarachnoid hemorrhage, dexmedetomidine, brain protection

Introduction

Subarachnoid hemorrhage is commonly seen in the Department of Neurosurgery and is often acute, which can cause serious cerebral injury to patients once the onset, so it results in high lethality rate and disability rate [1, 2]. Progress of medical technology has made such mortality substantially reduced, but the brain damage caused therefrom makes the later living quality of the patient greatly decreased [3]. The study results indicated that the main causes of early brain injury resulting from subarachnoid hemorrhage include increased intracranial pressure, decreased cerebral blood flow, decreased cerebral perfusion pressure, total cerebral ischemia, inhibition of aerobic respiration, destruction of blood-brain

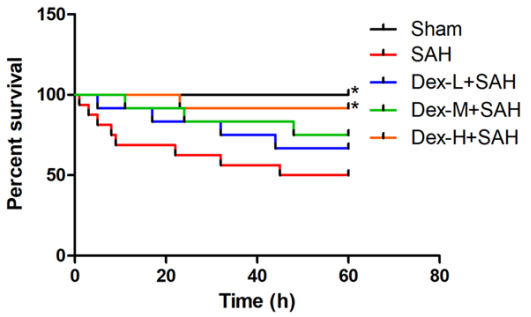

Figure 1. Mortality of rats in each group. Compared with SAH group, *P<0.05.

barrier, encephaledema and neuronal death. Relevant data indicated that about half of patients couldn't take care of themselves after therapy [4, 5]. Therefore, it is of great significance to reduce the patient's early cerebral injury caused by subarachnoid hemorrhage. Atorvastatin and valproic acid are commonly used for cerebral protection clinically, of which valproic acid reduces cerebral injury mainly through reducing brain tissue edema and destruction of blood-brain barrier, while atorvastatin protects the brain from damage mainly through inhibiting the apoptosis of neurons. The drugs with different mechanisms of action generate different protective effects on different injuries.

Dexmedetomidine is a commonly used sedative drug clinically, which is also a potent and highly selective α2 adrenergic agonists [6]. It has a strong effect on the inhibition of the release of noradrenaline in the neuroeffector junction as well as anti-hyperalgesia induced by neuropathic injury [7]. Previously study has proved that dexmedetomidine has positive effects on the brain damages and subarachnoid hemorrhage [8, 9]; therefore, in this study, dexmedetomidine was used to pretreat the rats and its effect of cerebral protection for the experimental rats with subarachnoid hemorrhage was studied.

Materials and methods

Experimental animals

A total of 60 healthy SD male rats were selected as the experimental animals. All the rats were provided by the Animal Experiment Center of our hospital, and they were approved by the Ethics Committee for Laboratory Animals. The age of rats was within 7-10 weeks, and their weight was within 180-250 g.

The 60 SD rats were divided into three groups: sham operation group (Sham group, n=8), subarachnoid hemorrhage group (SAH group, n=16) and the group pretreated with dexmedetomidine+subarachnoid hemorrhage (Dex+SAH group, n=36). Dex+SAH group was divided into three subgroups according to different concentrations of dexmedetomidine, namely low dosage group (Dex-L+SAH group, 10 μg), medium dosage group (Dex-M+SAH group, 50 μg) and high dosage group (Dex-H+SAH group, 100 μg), with 12 rats for each group. Scores of the nervous function, levels of subarachnoid hemorrhage, scores of brain tissue edema and levels of serum inflammatory factors of rats in each group were evaluated within 24 h after the models were established.

Experimental methods

Establishment of subarachnoid hemorrhage model: Firstly, the rats were anaesthetized with 1% pentobarbital sodium (50 mg/kg), and the right common carotid artery, external carotid artery and internal carotid artery were separated and exposed for ligation, so that the anastomosis between the external carotid artery and the internal carotid artery was cut off. Secondly, the external carotid artery was ligated and cut off to form a straight line with the internal carotid artery. Thirdly, a nylon wire with a length of 50 mm was extended into the external carotid artery, then directly into the intracranial segment through the internal carotid artery, with a depth of 18.5 mm; at this moment, a certain resistance could be felt when inserting, continue extending it into 3 mm to pierce the blood vessel, and then withdrew it quickly after 15 s. Silk thread was used to ligate the stump of external carotid artery, recover the blood flow and suture the neck incision before the model of subarachnoid hemorrhage was established. For the Sham group, extension was stopped after extended into 18.5 mm, so that it did not pierce the blood vessel. An electric heater was given for keeping warm before the rats awoke. The rats are fed in separate cages and kept warm.

Table 1. Comparison of water content in brain tissue of rats in each group

Group	Water content (%)	P (vs Sham group)	P (vs SAH group)	P (vs Dex-L+SAH group)	P (vs Dex-M+SAH group)
Sham group (n=8)	49.07±4.29				
SAH group (n=10)	103.29±5.17	<0.001			
Dex-L+SAH group (n=10)	82.93±4.93	<0.001	<0.001		
Dex-M+SAH group (n=10)	62.38±5.92	<0.001	<0.001	<0.001	
Dex-H+SAH group (n=12)	51.04±5.22	0.388	<0.001	<0.001	0.001

Pretreated with dexmedetomidine: The three groups to be pretreated with dexmedetomidine were pretreated with dexmedetomidine immediately through intravenous injection before the models were established. A total of 10 μg was injected into the low dosage group within 30 min before ligation, 50 μg for the medium dosage group within 30 min before ligation and 100 μg for the high dosage group within 30 min before ligation.

Outcome measures

Mortality of the rats in each group within 72 h after the establishment of models was record.

Water content of brain tissue: The brain tissues were removed immediately after the living rats' heads were cut after 72 h; the wet weight of brain tissues was measured directly and then the dry weight was measured after dried out, thus calculating the water content of the rats' brain tissue. The percentage calculation was ((wet weight-dry weight)/wet weight) * 100%.

Neurological deficit score: Neurological deficit of the rats was scored within 24 h after the operation by using Garcia scoring criteria, including the ability of autonomic activities, symmetry of limbs motion, stretching and climbing of front feet, reaction to body touching and tentacle reaction, scoring with 0-3 for each item from severity to normality to calculate the total scores [10, 11].

The scores on subarachnoid hemorrhage of the living rats within 24 h after the operation were recorded. The brain was divided into six parts, and the amount of bleeding of each part was measured respectively; summed them up to get the total scores of hemorrhage. If there is no hemorrhage, marked 0 point; if the amount of bleeding was extremely less, marked 1 point; if the amount of bleeding was medium with blood clots, marked 2 points; if the blood clots covered the cerebral tonic vessels of this part, marked 3 points [12].

Levels of IL-1β, IL-6 and TNF-α tested within 24 h after the operation were detected. The venous blood of rats was taken for test with ELISA kit (purchased from Beyotime Biotechnology Co., Ltd.).

Statistical criteria

SPSS21.0 was used to conduct statistics on the study results; the enumeration data were expressed in n (%); Chi-square test and Fisher's exact test were carried out for comparison among groups; the enumeration data were expressed in mean and standard deviation; t test was carried out for the comparison of two independent samples. A P<0.05 meant statistically significant differences.

Results

Comparison of mortality of rats in each group

The mortality of Sham group was 0/8; the mortality of SAH group was 50% (8/16), and 6 rats died within 24 h after the operation; the mortality of Dex-L+SAH group was 33% (4/12), and 2 rats died within 24 h after the operation; the mortality of Dex-M+SAH group was 25% (3/12), and 2 died within 24 h after the operation; the mortality of Dex-H+SAH group was 8.3% (1/12), and no rat died within 24 h after the operation.

The multiple comparison results showed that the mortality of rats in SAH group was higher than that of the Sham group (P=0.022), which presented statistically significant differences. The mortality of Dex-H+SAH group was obviously reduced than that of SAH group, which presented statistically significant differences (P=0.039). See **Figure 1**.

Table 2. Comparison of neurological function scores of rats in each group

Group	Neurological function score (point)	P (vs Sham group)	P (vs SAH group)	P (vs Dex-L+SAH group)	P (vs Dex-M+SAH group)
Sham group (n=8)	22.58±2.39				
SAH group (n=10)	5.49±1.28	<0.001			
Dex-L+SAH group (n=10)	12.93±2.21	<0.001	<0.001		
Dex-M+SAH group (n=10)	17.32±3.11	0.001	<0.001	0.002	
Dex-H+SAH group (n=12)	19.43±2.19	0.007	<0.001	<0.001	0.077

Table 3. Subarachnoid hemorrhage scores of rats in each group

Group	Subarachnoid hemorrhage score (point)	P (vs Sham group)	P (vs SAH group)	P (vs Dex-L+SAH group)	P (vs Dex-M+SAH group)
Sham group (n=8)	0				
SAH group (n=10)	17.48±2.11	<0.001			
Dex-L+SAH grpup (n=10)	14.58±1.92	<0.001	0.005		
Dex-M+SAH group (n=10)	12.94±1.39	<0.001	<0.001	0.042	
Dex-H+SAH group (n=12)	11.33±2.03	<0.001	<0.001	0.001	0.046

Comparison of water content of brain tissue in 30 rats

The water content of brain tissue in Sham group presented statistically significant differences with other groups (all P<0.001), but there was no statistically significant difference between Dex-H+SAH group and Sham group (P=0.388). The water content of brain tissue of the rats with SAH was most, which presented statistically significant difference with that of the groups pretreated with different dosage of dexmedetomidine (all P<0.001). See **Table 1**.

Comparison of neurological function scores of rats in each group

The neurological function score of Sham group presented statistically significant differences with that of other groups (all P<0.01); the neurological function score of the rats in SAH group was lowest, which presented statistically significant difference with that of the groups pretreated with different dosage of dexmedetomidine (P<0.001). See **Table 2**.

Subarachnoid hemorrhage scores of rats in each group

The subarachnoid hemorrhage score of Sham group presented statistically significant differences with that of other groups (all P<0.001); the rats in SAH group had the highest subarachnoid hemorrhage score (all ranged 16.5-18 scores), which presented statistically significant differences with that of the groups pretreated with different dosage of dexmedetomidine (all P<0.005). See **Table 3**.

Comparison of levels of inflammatory factors of rats in each group

Levels of three inflammatory factors TNF-α, IL-1β and IL-6 of the rats in SAH group obviously increased than those of Sham group (all P<0.01). The average TNF-α, IL-1β and IL-6 of the rats in each group pretreated with dexmedetomidine were lower than those of SAH group (all P<0.01). TNF-α, IL-1β and IL-6 of Dex-H+SAH group were lower than those of Dex-M+SAH group and Dex-L+SAH group (all P<0.01), which presented statistically significant differences. See **Figure 2** and **Table 4**.

Discussion

In the current research, the best method which can simulate the human subarachnoid hemorrhage is the intravascular acupuncture in rats [13]. Therefore, this method was used in this study to research the effect of protection on early cerebral injury of subarachnoid hemorrhage after pretreated by dexmedetomidine. As can be seen from the study results, mortality of the rats increased after subarachnoid hemorrhage; obvious encephadelema and neurological deficits occurred in the rats; levels of severe inflammatory factors increased. These indicated that subarachnoid hemorrhage brought

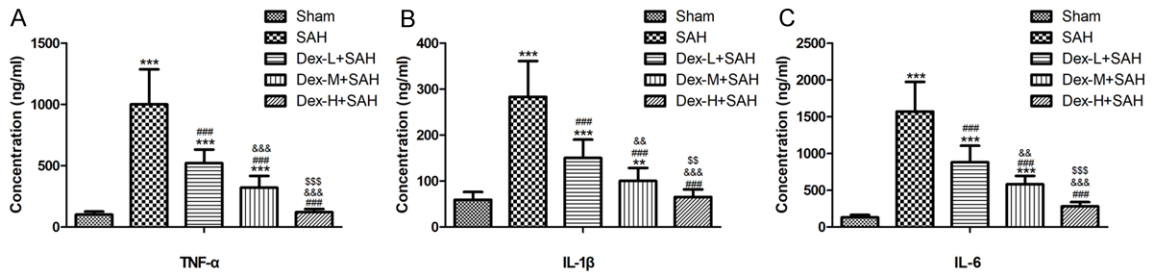

Figure 2. Comparison of levels of serum inflammatory factors TNF-α, IL-1β and IL-6 of rats in each group. Compared with Sham group, $^{**}P<0.01$, $^{***}P<0.001$; compared with SAH group, $^{\#\#\#}P<0.001$; compared with Dex-L+SAH group, $^{\&\&}P<0.01$, $^{\&\&\&}P<0.001$; compared with Dex-M+SAH group, $^{\$\$}P<0.01$, $^{\$\$\$}P<0.001$.

Table 4. Comparison of levels of inflammatory factors of rats in each group

Group	Sham group	SAH group	Dex-L+SAH group	Dex-M+SAH group	Dex-H+SAH group
TNF-α	100.2±25.7	1,000.4±286.52	520.5±111.8	320.5±96.0	120.5±26.0
IL-1β	59.2±16.9	283.1±78.0	150.2±39.9	100.2±28.3	65.2±16.6
IL-6	129.3±34.7	1,569.4±404.0	879.2±225.2	579.2±115.0	279.2±60.3

severe damage to the body such as severe inflammatory reaction and encephaledema, resulting in neurological deficits, which had a certain disability and may lead to death in severe cases. The mortality of all the rats pretreated with dexmedetomidine decreased [14-17]; as can be seen from the study results, high dosage of dexmedetomidine of 100 μg achieved the best effect. However, it presented no statistical significance for the severity of subarachnoid hemorrhage of the rats pretreated with different dosages of dexmedetomdine. It can be inferred that dexmedetomidine did not protect the brain through reducing severity of subarachnoid hemorrhage, but relevant similar research and evidence were not found and it was opposite of our hypothesis, which might be caused by small sample size in this study; therefore, further studies need to be carried out to figure out the exact mechanisms [18, 19].

Dexmedetomidine is commonly used as a sedative drug clinically, and it is of high selection used as a kind of adrenergic agonist [20]. The analysis results showed that the effect of Dexmedetomidine on early cerebral injury may be related to the following aspects: (1) early cerebral injury of the patients with subarachnoid hemorrhage has a certain correlation with sympathetic nervous excitement and dysfunction of vascular endothelial cells, while Dexmedetomidine can reduce the excitability of sympathetic nerves and adjust the stability of vascular endothelial cells, thereby generating the effect of protection on cerebral vessels and brain [21]; (2) dexmedetomidine generates an effect on stability of hemodynamics, so that the stability of hemodynamics and cerebral circulation can be guaranteed when subarachnoid hemorrhage occurs in a rat [22, 23]; (3) dexmedetomidine also has the effect of anti-anxiety and sedation in addition to its effect on stability of vessels; such kind of comprehensive effect can keep body stable for all kinds of indicators under the influence of disease, so the influence of subarachnoid hemorrhage on body will decrease. The dosage of 100 μg achieved the best effect, which indicated that the higher the dosage of dexmedetomidine is, the better effect achieved will be. However, the highest dosage of this study was 100 μg, which was unable to determine the effect of higher dosage of the drug. This study did not further study the mechanism of action through the whole study. In combination of the expression of inflammatory factors, it could also be inferred that dexmedetomidine may play a protective role by reducing the release of inflammatory factors. The mechanism of action of dexmedetomidine on early cerebral protection can be further studied in the future from the prospective of gene expression.

In summary, dexmedetomidine preconditioning to the rats with subarachnoid hemorrhage can play a certain role in protecting brain of

the rats; the expressions of inflammatory factors decreased, and the effect of 100 μg was better than that of 10 μg and 50 μg.

Disclosure of conflict of interest

None.

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