Influence of Sevoflurane Posttreatment on TLR4 and TRAF6 Expression in Brain Tissues of Rats with Cerebral Ischemia Reperfusion

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***Abstract*—Objective: To explore the influence of sevoflurane posttreatment on TLR4 and TRAF6 expression in brain tissues of rats with cerebral ischemia reperfusion. Method: The period from January 2015 to March 2018 was chosen as the research period. Besides, healthy male rats were selected in the research period as the sham operation group, ischemia reperfusion group, 1.0MAC sevoflurane posttreatment group and 1.5MAC sevoflurane posttreatment group. After treatment of rat models respectively, neurologic impairment scoring was implemented, and the influence of sevoflurane was judged. Results: Neurologic impairment score of ischemia reperfusion group was maximum (P<0.05). TLR4 and TRAF6 expression in brain tissues of rats in sham operation group was minimum, and TLR4 and TRAF6 expression in brain tissues of rats in ischemia reperfusion group was maximum (P<0.05). Conclusion: Sevoflurane posttreatment can improve rats' neurological function and reduce TLR4 and TRAF6 expression in brain tissues of rats, so sevoflurane has application value.**

***Index Terms*—sevoflurane; brain tissue; TLR4; TRAF6; cerebral ischemia reperfusion**

Ischemia reperfusion means blood perfusion returns to normal again after brain tissue ischemia happens. Toll-like receptor 4 (TLR4) and tumor necrosis factor receptor-associated factor 6 (TRAF6) are reaction markers of brain tissue health or inflammatory degree. This study mainly aims at the influence of sevoflurane application on cerebral ischemia reperfusion. The rats were chosen as the samples for detailed analysis to summarize relevant experience for clinical reference. Now, the research details are sorted out and reported as follows.

I. Data and Method

A. General data

The period from January 2015 to March 2018 was chosen as the research period. Besides, healthy male rats were selected in the research period as the sham operation group (all rats in this group were male, with the weight of 230-280g and average weight of (254.3±0.23)g), ischemia reperfusion group (all rats in this group were male, with the weight of 230-275g and average weight of (254.7±0.17)g), 1.0MAC sevoflurane posttreatment group and 1.5MAC sevoflurane posttreatment group (all rats in the two groups were male, with the weight of 230-270g and average weight of (254.5±0.34)g). Each group had 20 rats, and basic conditions of rats were close (P>0.05), so inter-group statistical contrast could be implemented.

B. Method

The reperfusion model of focal cerebral ischemia was established according to Zea Longa suture method. Firstly, the rats were weighed, and supinated on the operating table. Intraperitoneal injection of 3.5mg/kg chloral hydrate (concentration 6%) was conducted for the rats, and anesthesia and fixation were done well for the rats. Orotracheal intubation and connection with ALC-V9 animal ventilator were conducted to control rats' normal breathing[1]. The operative incision was implemented in the middle of neck. Meanwhile, rats' left common carotid artery, furcation, internal carotid and external carotid artery were separated. At the same time, rats' external carotid artery was ligatured, and the far end of internal common carotid and left carotid artery were occluded. The suture line was inserted from the external carotid artery and through internal carotid, and the suture line went deep upward to make it pass through the starting point of rats' cerebral common artery. The suture line ended when resistance encountered and the anterior artery of brain opened. Then, the skin was sutured. After ischemia for 2h, the suture line was pulled out for cerebral ischemia reperfusion[2].

Ischemia-reperfusion modeling operation of sham operation group was same with the above steps, but suture line insertion operation was not implemented for the rats. After cerebral ischemia for 2h in the ischemia reperfusion group, cerebral perfusion was conducted for the rats. For 1.0MAC sevoflurane posttreatment group and 1.5MAC sevoflurane posttreatment group, after ischemia for 2h, the volatilization pot of sevoflurane was well connected between the respirator and oxygen hose. Besides, the breathing pot was adjusted to make the inhalation concentration keep at 1.0MAC (concentration 2.4%) or 1.5MAC (concentration 3.6%). After inhalation for 15min, sevoflurane supply stopped.

C. Observational indexes

After treatment of rat models respectively, neurologic impairment scoring was implemented, and the inter-group contrast was conducted to judge the influence of sevoflurane. Zea Longa scoring standard was used for neurologic impairment scoring. 0 score means there is no obvious neurologic impairment symptom, and 5 scores means death. 4 scores means the rats cannot walk by themselves, and have consciousness loss phenomenon. 3 scores means contralateral dumping phenomenon happens to the rats during walking. 2 scores means the rats can walk, but they have contralateral rotation phenomenon. 1 score means the rats cannot completely stretch contralateral forepaw. If neurologic impairment score is 5 or 0, this means modeling fails, and modeling is required again for the standby rats. Meanwhile, neurologic impairment scoring is required again, too[4-6].

In addition, immunohistochemical method was applied to test TLR4 and TRAF6 in brain tissues of rats in each group, and records were made for each group. The mean values were recorded and compared[7].

D. Statistical analysis

For the research data, measurement data statistics was conducted with ($\bar{X} \pm S$), and tested with t test. $P<0.05$ means the difference between the two groups is significant, and there is statistical significance. Data processing software was SPSS25.0.

II. RESULTS

A. Neurologic impairment scoring for each group

Neurologic impairment score of rats in 1.5MAC sevoflurane posttreatment group was relatively low, followed by 1.0MAC sevoflurane posttreatment group. The score of the two groups was lower than that of ischemia reperfusion group. The data comparison of each group is shown in Tab.1.

TAB.1 NEUROLOGIC IMPAIRMENT SCORE OF EACH GROUP ($\bar{X} \pm S$)

Group	6h	12h	24h	48h
Sham operation group	0	0	0	0
Ischemia reperfusion group	2.24±0.29	2.33±0.34	2.51±0.43	2.34±0.41
1.0MAC sevoflurane posttreatment group	1.85±0.32	1.96±0.36	2.21±0.41	2.05±0.37
1.5MAC sevoflurane posttreatment group	1.44±0.26	1.54±0.32	1.71±0.33	1.53±0.32
F	96.32	84.16	82.37	16.34
P	<0.05	<0.05	<0.05	<0.05

B. TLR4 and TRAF6 expression in brain tissues of rats

TLR4 and TRAF6 expression in brain tissues of rats in the sham operation group was minimum, followed by 1.5MAC sevoflurane posttreatment group and 1.0MAC sevoflurane posttreatment group. TLR4 and TRAF6 expression in brain tissues of rats in the ischemia reperfusion group was maximum. This indicates that sevoflurane influences TLR4 and TRAF6 expression. The data of each group are shown in Tab.2 and Tab.3.

TAB.2 TLR4 AND TRAF6 EXPRESSION IN BRAIN TISSUES OF RATS ($\bar{X} \pm S$)

Group	TLR4 6h	12h	24h	48h
Sham operation group	2.67±0.36	3.12±0.45	3.17±0.55	2.87±0.42
Ischemia reperfusion group	16.47±1.63	21.94±2.27	26.35±2.62	22.14±2.31
1.0MAC sevoflurane posttreatment group	12.63±1.39	14.21±1.59	18.67±1.96	14.58±1.52
1.5MAC sevoflurane posttreatment group	7.62±1.17	9.62±1.63	12.26±1.84	9.36±1.28
F	136.92	96.37	158.37	162.58
P	<0.05	<0.05	<0.05	<0.05

TAB.3 TRAF6 EXPRESSION IN BRAIN TISSUES OF RATS ($\bar{X} \pm S$)

Group	TRAF6 6h	12h	24h	48h
Sham operation group	4.86±0.69	5.08±0.81	5.76±0.73	4.93±0.75
Ischemia reperfusion group	26.65±2.94	38.24±3.64	43.68±4.54	35.36±3.59
1.0MAC sevoflurane posttreatment group	19.35±2.09	27.54±2.36	35.64±3.49	26.78±2.68
1.5MAC sevoflurane posttreatment group	15.38±1.67	18.37±1.86	26.38±2.58	19.31±2.09
F	115.28	160.39	18.67	19.67
P	<0.05	<0.05	<0.05	<0.05

III. DISCUSSION

Cerebrovascular disease has high morbidity clinically, and will lead to cerebral ischemia and brain tissue injury to different degrees, thus endangering patients' health and even life safety. Thus, cerebrovascular disease has high disability rate and fatality rate[8].

At present, there are few clinical researches on application of relevant medicines in ischemia reperfusion. In this study, sevoflurane was applied for detailed analysis. The results showed that, neurological function of rats in 1.5MAC sevoflurane posttreatment group and 1.0MAC sevoflurane posttreatment group were relatively good, and TLR4 and TRAF6 expression in brain tissues was

relatively low. The inter-group difference was significant ($P<0.05$). Sevoflurane is a common anesthesia medicine. As a kind of volatile liquid, the medicine is well and widely applied in general anesthesia, induction and maintenance. Its action time for patients is short, and its induction is fast. Besides, it inhibits respiratory tract little, and can maintain haemodynamics stability of the receptor. Meanwhile, sevoflurane can activate extracellular signal adjustment. Through effective adjustment, human kinase transduction pathway can be intervened to protect the myocardial function[9]. In the process of ischemia reperfusion, the brain tissues will be affected to different degrees, and injury and even apoptosis will appear. Thus, body stress response is caused, and inflammatory reaction is aggravated. The application of sevoflurane in ischemia reperfusion model can inhibit autophagosome clearance and damage in the period of reperfusion, and protect human cardiac muscle cells, thus reducing cell death after ischemia[10]. TLR4 and TRAF6 expression is a symbolic substance of inflammatory reaction level. In this study, TLR4 and TRAF6 expression of rats in sevoflurane groups is relatively good, so sevoflurane has certain application value. But, its detailed mechanism needs further researches and discussions.

In conclusion, sevoflurane posttreatment can improve rats' neurological function and reduce TLR4 and TRAF6 expression. So, its clinical influence is positive and it deserves promotion.

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