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Effect of propofol and ketamine anesthesia on cognitive function and immune function in young rats

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ABSTRACT

Objective: To investigate the effects of propofol and ketamine on the cognitive function and immune function in young rats. **Method:** A total of 80 young rats were randomly divided into four groups: Control group, ketamine group (experimental group A), propofol group (experimental group B), ketamine and propofol group (experimental group C). All rats had continuous injection for three times, serum IL-2, IL-4 and IL-10 and whole brain IL-1β level, hippocampal neuronal apoptosis level were measured. The cognitive ability in rats was tested by water maze. **Results:** Water maze test showed on the 1st d, the maze test latency of the control group, the experimental group B and the experimental group C water were decreased gradually; Compared with the control group after 3 days, the latency of the experimental group A, experimental group B and experimental group C were all decreased, the crossing circle times were also reduced. Hippocampal neuron apoptosis were (2.3±1.7)%, (14.7±6.9)%, (4.2±3.3)%, (10.2±4.8)% in control group, experimental group A, experimental group B and experimental group C, respectively. The neurons apoptosis of experimental group A was significantly increased. The serum IL-4 and IL-10 of the experimental group A, experimental group B and experimental group C after anesthesia were significantly higher than the control group. The whole brain IL-1β of the experimental group A, experimental group B and experimental group C were significantly lower than the control group. **Conclusions:** Propofol can reduce anesthesia effect of ketamine on the cognitive function and immune function in the young rats.

1. Introduction

Intravenous general anesthetics not only has good analgesic effect, but also has no significant side effects on inhibiting respiration, which is widely used in clinical surgical treatment[1]. The clinical application showed that the use of general anesthesia can cause recent cognitive impairment and mental ill effect and other adverse reactions. Neonatal and infant's central nervous system and immune system are still in the developmental stage and particularly sensitive to the external environment. The mechanisms of anesthesia effects on the central nervous system and immune system are still not very clear[2]. Therefore, this experiment aims to provide the basis for clinical by the application of ketamine and the composite application of propofol and ketamine on the cognitive function and immune function in young rats.

2. Materials and methods

2.1. Animals

A total of 80 healthy 7-day-old SD rats, male or female, weighing 12-18 g were selected. All animals were provided by XX University Experimental Animal Center, and were kept in a constant temperature 25 ℃, constant humidity 40% -50% environment, and had freely drank autoclaved water.

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2.2. Main reagents and instruments

Optical microscope was purchased from Japanese Nikon company,German Leica Microtome was purchased from Dalian Dajian Medical Devices Co., Ltd., Micro pipette and homogenizer were purchased from the German Eppendorf Company, −80 ℃ refrigerator were purchased from China Haier Company. TUNEL assay kit was purchased from Roche Company, IL-4, IL-1β and IgE radioimmunoassay kit were purchased from Wuhan Boster Biological Engineering Co., Ltd., Ketamine (100 mg/10 mL) were purchased from Jiangsu Hengrui Limited Company, propofol injection (200 mg/20 mL) were purchased from Sichuan Shule Pharmaceutical Corporation. Experimental animal cages, precision electronic balance, 0.9% saline solution, hematoxylin, eosin staining solution were provided by the laboratory.

2.3. Experimental methods

2.3.1. Experimental animal model and grouping methods

A total of 80 young rats were randomly divided into four groups (the control group, experimental group A, experimental group B, experimental group C) (n=20). All young rats received the adaptive breeding for 1 week in animal room. The animals in the control group received 0.9% saline l mL by intraperitoneal injection every 2 h, continuous for 3 times. The animals in experiment group A received 80 mg/kg ketamine l mL by intraperitoneal injection every 2 h, continuous for 3 times. The animals in experiment group B received 80 mg/kg propofol 1 mL by intraperitoneal injection every 2 h, continuous for 3 times. The animals in experiment group C received 80 mg/kg ketamine and propofol 1 mL by intraperitoneal injection every 2 h, continuous for 3 times. The injection volume was 1 mL, and if it was less than l mL it was supplemented by saline. Half of rats in each group were randomly sacrificed after 15 min of anesthesia, the other half underwent Morris water maze test 3 weeks later. All died or abandoned animals in midway were supplemented by modeling again.

2.3.2. Immune parameters detection

Using heparinization disposable 5 mL sterile syringe, 2 mL blood was obtained by percutaneous puncture at the point of maximal impulse and then it was injected into sterile EP tube. After 30 min at 4℃, it was centrifuged at 3 000 r/min at low temperature for 10 min. Serum was separated and stored at −80℃ for the test. Serum IL-2, IL-4 and IL-10 levels were detected by ELISA.

2.3.3. Brain tissue specimen collection, preparation and indicators test

After blood collection, half of the young rats were randomly selected. The heart was exposed by thoracotomy, the perfusion needle was inserted to the ascending aorta from the left ventricle, and fixed. The right auricle was cut. It was washed at 4 ℃ saline by perfusion needle until the effluent of the right atrium was clear. Then it was fixed by 4% paraformaldehyde phosphate buffer. Hippocampal was isolated from the brain tissue when the body tissues and organs were hard, they were paraffin-embedded and cut. Neuronal apoptosis detection was performed by terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) method. TUNEL-positive cells showed brown particles in the nucleus. Six horizons were randomly selected and average optical density was measured. Positive intensity and the apoptotic index were calculated. The formula was as follow: apoptotic index (AI) = MOD × Area% × 100, MOD represents the average gray level; area% represents the percentage of the total positive nucleus area in the total nucleus area. The other half young rats cerebral was obtained quickly by sterile opening cranium, and brain tissue was mixed with ice normal saline by homogenizer. 10% brain homogenate was prepared at 4 ℃, and centrifuged at 3 000 r/min for 15 min. The supernatant was stored at −80 ℃ for test. Whole brain IL-1β levels were detected by ELISA.

2.3.4. Morris water maze test

Behavior of rats was observed by Morris water maze[3]. Round tank has four quadrants. A black platform was fixed at the fourth quadrant, located 1 cm underwater. The rats were put into the water of a randomly select quadrant, swim tracks of the rats were recorded with a camera. How long rats find the platform is the latency. After this test, the platform was removed and the rats were put into water from the same water-entering point, the times of crossing the former platform were measured.

2.4. Statistical analysis

Data were expressed as mean±SD values and analyzed with SPSS 13.0 software. After the variance test, the difference between two groups was compared with single factor analysis of variance. P<0.05 was considered as statistical significant difference.

3. Results

3.1. Serum IL-2, IL-4 and IL-10 and whole brain IL-1β level

The serum IL-2 in each groups showed no significant

difference (P>0.05). The serum IL-4 and IL-10 of the experimental group A, experimental group B and experimental group C were significantly higher than the normal control group (P<0.05). There was significant difference in the serum IL-4 and IL-10 levels between experimental group C and experimental group A (P<0.05). The whole brain IL-1β level of the experimental group A, experimental group B and experimental group C were significantly lower than the normal control group (P<0.05). There was significant difference in whole brain IL-1β level between experimental group C and experimental group A (P <0.05) (Table 1).

3.2. Hippocampal neurons apoptosis

Hippocampal neurons apoptosis of the experimental group A was (14.7±6.9)%, which was significantly increased than that of the control group [(2.3±1.7)%] (P<0.05); The apoptosis rate of the experimental group B was (4.2±3.3)%, which had no significant difference from NS group (P>0.05), but significantly lower than that of experimental group A (P<0.05); The apoptosis rate of the experimental group C was (10.2±4.8)%, which was significantly increased compared with the control group (P<0.05), but significantly decreased compared with the experimental group A (P<0.05).

3.3. Rat behavior observation

With the changes of time, the latency of control group, experimental group B and experimental group C were decreased gradually, there was significant difference between that on 1st d and 3rd d (P<0.05). The latency of experimental group A was not changed significantly (P>0.05); The latency after 3 days gradually decreased in the experimental group A, experimental group B and experimental group C, which was statistically significant different from that of the control group (P<0.05). Compared with the experimental group A, the latency on the 3rd day was significant different from that of the experimental group B and experimental group C (P<0.05). Compared with the control group, there was significant difference in the crossing circle times from experimental group A, experimental group B and experimental group C (P<0.05). Compared with the experimental group A, the crossing circle times of the there was significant difference in the crossing circle times from experimental group B and experimental group C (P<0.05) (Table 3).

4. Discussion

Postoperative cognitive dysfunction attracts increasing attention. Numerous studies have shown the use of narcotic drugs is closely related the understanding dysfunction. Hippocampal neuron is considered to be major neurons which involved in long-term memory. Damage in the neurons synapse structure can significantly affect the ability of learning and memory in rats. Postsynaptic membrane receptor also involved in cognitive function of rats as an important information carrier, and that the pathogenesis of Alzheimer's patients is related with the decreased expression of receptor [4-11]. The immune changes under anesthetized stress of rats also attract researchers' attention. Anesthetic ketamine and propofol were involved in the regulation of the central nervous system by inhibiting postsynaptic membrane receptors, but the changes of the cognitive function and immune function and the mechanism is still not clear. Thus we anesthetized the young rats in this

Table 1

Serum IL-2, IL-4 and IL-10 and whole brain IL-1β level of rats.

Indexes	Control group	Experimental group A	eExperimental group B	Experimental group C
IL-2 (ng/mL)	0.5±0.1	0.4±0.1	0.4±0.1	0.4±0.1
IL-4 (ng/mL)	0.4±0.1	1.5±0.5*	1.3±0.5*	1.0±0.3*△
IL-10 (ng/mL)	0.7±0.2	3.1±0.8*	2.6±0.6*	1.8±0.5*△
IL-1β (ngL)	115.4±15.3	93.1±10.2*	97.1±18.6*	112.8±18.4*△

Note: * Compared with the control group, P <0.05, △Compared with experimental group A, P <0.05.

Table 2

Water maze test results.

Indexes	Latency(s)			Crossing circle times
	1 d	2 d	3 d	
Control group	112.7±24.4	89.5±15.4	53.2±12.1*	6.2±2.3
Experimental group A	115.0±24.5	105.9±20.6	104.3±17.3△	1.2±0.6△
Experimental group B	114.2±26.2	92.6±16.8	65.0±13.1*△	3.5±2.1△
Experimental group C	109.9±25.3	90.3±20.4	72.4±11.6*△	3.2±2.2△

Note: * Compared with the control group, P <0.05, △Compared with experimental group A, P <0.05.

study and then explore the effect of ketamine and propofol on the cerebral development and the immune system.

As a classical neurological behavior methods, morris water maze has become the standard mode to study the memory mechanism[12]. Therefore, this experiment also chose this classic mode to explore the effect of anesthetics on the cognitive function in rats. This study showed that the latency at three days is not significantly shorter after the use of anesthetics ketamine, and the times of cross the flat is far less than the normal control group, which suggested the ketamine anesthesia can reduce the spatial learning and memory ability in young rats .The latency at three days is significantly shorter than 1 day of young rats after the use of anesthetics propofol, but the exploration time was significantly longer than the normal control group. The times of cross the flat is significantly increased than the normal control group, which suggested the ketamine anesthesia can reduce the spatial learning and memory ability in young rats, but the degree is significantly less than the ketamine group.

Studies have found that ketamine abusers have a long damaged memory, and its mechanism is related to damaged hippocampal neurons, which prompt some neuronal apoptosis of rats. Once propofol does not have an impact on cognitive function in rats , but the use of many drugs can cause propofol nerve degeneration and affect the brain development[13-18]. The spatial learning and memory ability in young rats of ketamine & propofol group is similar to the propofol group, the latency is significantly shorter than the ketamine group, and the times of cross the flat is also significantly increased, which suggested that the ketamine & propofol anesthesia can reduce the effect ketamine on cognitive function. The apoptosis rate of ketamine & propofol group is significantly lower than the ketamine group by the study of hippocampal neuronal apoptosis, which is accordance with the change of spatial learning and memory ability in young rats. That showed propofol can reduce ketamine-induced apoptosis in hippocampal neurons. These results indicate that the mechanism that propofol can reduce the effect of ketamine anesthesia on rats' cognitive lies in hippocampal neuronal apoptosis. With the increase in hippocampal neuronal apoptosis, the long-term learning and memory dysfunction are more obvious[19-23]. Numerous studies also believes that the use of propofol in humans and animals have the cerebral protective effect. Its protective mechanism may be related with reducing cerebral metabolic rate of oxygen and intracranial pressure and reducing excitatory amino acid glutamate neurotransmitter release, blocking glutamate pathways, reducing neurotoxicity induced pathological damage, the lipid peroxidation effect, preventing protein denaturation and release of inflammatory mediators and preventing secondary damage neuronal cells; reducing the formation of free radicals[24-26].

Immunity is one of the main effects of the stress response. Immune function in young rats still in the developmental stage, which is sensitive the stimulation of external factors. Generally it is believed that the impact of stress on the immune system is mainly suppression and regulation. T lymphocytes are the most important component which constitute the immune system. Good immune function of the body needs to maintain a moderate level of response and homeostasis. Th1 cells and Th2 cells is critical in maintaining the balance of immune function. Once there is Th cell subsets imbalance, that is Thl/Th2 ratio and functional imbalance, will lead to immune dysfunction[27,28], which usually expressed as the abnormal secretion of the activation factor of various inflammatory cell. This study suggests that the anesthetic of each group has little effect on IL-2 secretion, but the serum IL-4 and IL-10 were significantly increased, while the whole brain IL-1β were significantly decreased. Studies suggest that stress response of the adult rats is different from that in the young rats. Adult rats have a strong adaptability,while the young rats have long-lasting effects to the stress response and have a continuing influence. IL-1β is a cytokines produced by a variety of cells in a infection and inflammation state, which have a wide range of physiological effects. It often called the central stress-mediated factors.When there is systemic stress responses, central IL-1β may showed high expression[29,30].

In summary, ketamine can inhibit the cognitive function in young rats and has toxic effects on hippocampal neurons. In combination with propofol, it can reduce neurotoxicity and protect the brain tissue. Ketamine can inhibit the immune function in young rats, while propofol can reduce the ketamine's inhibition to the immune function .However, there are still some gaps between the animal studies and human clinical trials. If it is consistent with human trials still need further research.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Chen S. Clinical comparative study of propofol and isoflurane on elderly patientscognitive function after surgery. *Strait Pharmaceutical J* 2012; **24**(6): 127-128.

[2] Gan RH, Xu LL, Xie AQ, Tian S, Yang ZY. Influence of isoflurane

and propofol on cognitive of aged patients. *Western Med* 2011; **23**(6): 1104-1106.

[3] Sun HJ, Liang SJ, Zhang LW. Effects of propofol and isoflurane anesthesia on elderly patients cognitive function after surgery. *J Knotty* 2010; **9**(10): 773-774.

[4] Marsden KC, Shemesh A, Bayer KU, Carroll RC. Selective translocation of Ca^{2+}/calmodulin protein kinase IIalpha (CaMK Ⅱ alpha) to inhibitory synapses. *Proc Natl Acad Sci USA* 2010; **107**(47): 20559-20564.

[5] Gustin RM, Shonesy BC, Robinson SL, Rentz TJ, Baucum AJ, Jalan-Sakrikar N, et al. Loss of Thr286 phosphorylation disrupts synaptic CaMK Ⅱ α targeting, NMDAR activity and behavior in pre-adolescent mice. *Mol Cell Neurosci* 2011; **47**(4): 286-292.

[6] Chen NM, Ding L. Propofol to the elderly colon cancer S100 β expression and cognitive function after radical surgery. *Chin Rural Med* 2012; **19**(8): 13-14.

[7] Xu XD, Zhang LC. Experimental research of propofol on learning and memory function. *Chin Pract Med* 2013; **8**(12): 7-9.

[8] Wu D. The influence of Sevoflurane and propofol general anesthesia on elderly patients after anesthesia emergence time and cognitive function. *Chin Pharmaceutical* 2013; **22**(5): 95-96.

[9] Meyer P, Langlois C, Soëte S, Leydet J, Echenne B, Rivier F, et al. Unexpected neurological sequelae following propofol anesthesia in infants: Three case reports. *Brain Dev* 2010; **32**(10): 872-878.

[10]Xiao J,Zheng LM, Wang ML, et al. Effects of disoprofol and isoflurane on postoperative cognitive dysfunction in the elderly. *Chin Modern Doctor* 2010; **48**(11): 80-81.

[11]Sharma S, Rakoczy S, Brown-Borg H. Assessment of spatial memory in mice. *Life Sci* 2010; **23**(17-18): 521-536.

[12]Jiang Y, Zhou ZJ, Chen P, Huang F, Xian YS, Wang YL, et al.Effects of multiple doses of propofol on cognitive function in neonatal rats. *Chin J Anesthesiol* 2008; **28**(11): 1007-1009.

[13]Jiang Y, Gao J, Chen P, Li QY. Effects of propofol on pyramidal neuron apoptosis in hippocampus of neonatal rats. *J Chongqing Med Univ* 2009; **34**(3): 304-307.

[14]Xiao B, Han F, Shi YX. Expression changes of CaMKII α and pCaMKII in the PTSD rat amygdala. *Progr Anatomical Sci* 2009; **15**(4): 396-399.

[15]Chen YF. The effect of propofol on cell apoptosis after freezing injury rats. *Xianning Coll J* 2007; **21**(6): 474-475.

[16]Kuai JK, Han LC, Chai W. The long-term changes and mechanism of cognitive function after ketamine anesthesia in neonatal rats. *Chin Med Sci* 2007; **16**(9): 789-791.

[17]Song SL, Wang JM. Experimental research about the effect of monosodium gutamate on the ability of learning and memory and the behaviour in open field in rats. *Guangzhou Med* 2008; **39**(1): 1-3.

[18]Li YC, Li Y, Wang YS, Li JQ, Li ZX, Li EY, et al. Protective effects of preconditioning with different doses of propofol given once or repeatedly on the brain against global cerebral ischemia-reperfusion injury in rats. *Chin J Anesthesiol* 2006; **26**(8): 720-723.

[19]Zhao DM, Li QC, Diao HL, Liu HF, Wang LM, Huang F. Effects of propofol pretreatment on learning and memory ability and hippocampus injury of Alzheimer′s disease-like model rats. *J Binzhou Med School* 2007; **30**(6): 407-411.

[20]Erasso DM, Camporesi EM, Mangar D, Saporta S. Effects of isoflurane or propofol on postnatal hippocampal eurogenesis in young and aged rats. *Brain Res* 2013; **1530**: 1-12.

[21]Mirski MA, Lewin JJ, Ledroux S, Thompson C, Murakami P, Zink EK, et al. Cognitive improvement during continuous sedation in critically ill, awake and responsive patients: the Acute Neurological ICU Sedation Trial (ANIST). *Intensive Care Med* 2010; **36**(9): 1505-1513.

[22]Erasso DM, Chaparro RE, Quiroga Del Rio CE, Karlnoski R, Camporesi EM, Saporta S. Quantitative assessment of new cell proliferation in the dentate gyrus and learning after isoflurane orpropofol anesthesia in young and aged rats. *Brain Res* 2012; **2**; 1441: 38-46.

[23]Schoen J, Husemann L, Tiemeyer C, Lueloh A, Sedemund-Adib B, Berger KU, et al. Cognitive function after sevoflurane-*vs*. propofol-based anaesthesia for on-pump cardiac surgery: a randomized controlled trial. *Br J Anaesth* 2011; **106**(6): 840-850.

[24]Soukup J, Selle A, Wienke A, Steighardt J, Wagner NM, Kellner P. Efficiency and safety of inhalative sedation with sevoflurane in comparison to an intravenous sedation concept with propofol in intensive care patients: study protocol for a randomized controlled trial. *Trials* 2012; **10**(13): 135.

[25]Lewis LD, Weiner VS, Mukamel EA, Donoghue JA, Eskandar EN, Madsen JR, et al. Rapid fragmentation of neuronal networks at the onset of propofol-induced unconsciousness. *Proc Natl Acad Sci USA* 2012; **109**(49): E3377-E3386.

[26]Ye X, Lian Q, Eckenhoff MF, Eckenhoff RG, Pan JZ. Differential general anesthetic effects on microglial cytokine expression. *PLoS One* 2013; **8**(1): e52887.

[27]Erdogan MA, Demirbilek S, Erdil F, Aydogan MS, Ozturk E, Togal T, et al. The effects of cognitive impairment on anaesthetic requirement in the elderly. *Eur J Anaesthesiol* 2012; **29**(7): 326-331.

[28]Luo J, Min S, Wei K, Li P, Dong J, Liu YF. Propofol protects against impairment of learning-memory and imbalance of hippocampal Glu/GABA induced by electroconvulsive shock in depressed rats. *J Anesth* 2011; **25**(5): 657-665.

[29]Rezaei F, Nasseri K, Esfandiari GR, Sadeghi SM, Fathie M, Gharibi F. Remifentanil added to propofol for induction of anesthesia can reduce reorientation time after electroconvulsive therapy in patients with severe mania. *J ECT* 2012; **28**(2): 124-127.