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Adiponectin improves isoflurane-induced cognitive dysfunction in elderly rats via inhibiting p38-MAPK signal pathway in hippocampus

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Abstract. – OBJECTIVE: To investigate the intervention of exogenous adiponectin in the elderly rats with cognitive dysfunction induced by isoflurane through mitogen-activated protein kinase (MAPK) signaling pathway in hippocampus.

MATERIALS AND METHODS: A total of 60 healthy elder Sprague Dawley (SD) rats aged 15-20 months and weighing 400-500 g were selected. These rats were randomly divided into four groups, i.e., the control group, the anesthetic group, adiponectin intervention group, and p38-MAPK antagonist group, in which the rats in the control group were treated through inhalation of pure oxygen for 4 h at a rate of 4 L/min, while the rats in the other 3 groups were treated through inhalation of isoflurane for 4 h. During the inhalation of isoflurane, the concentration of isoflurane was 3.5% at the beginning and decreased to 2.2% at 1 h, and 1.7% between 2 h and 4 h. Then, the intraperitoneal injection of 0.5 mL normal saline was performed for the rats in the control group and the anesthetic group, while adiponectin (300 mg/kg) was injected into the rats in the adiponectin intervention group and p38-MAPK antagonist group. Simultaneously, the antagonist (20 mg/kg) diluted to 0.5 mL was given to the rats in the p38-MAPK antagonist group, once/day for 3 days. Morris water maze test was carried out respectively in the 1st, 3rd, and 7th day, and 5 rats participated in the test at each time point, during which we recorded the escape latency, as well as the length of the swimming route of rats. Reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting were employed to detect the mRNA and protein expressions of p38 in the hippocampus.

RESULTS: The escape latency and the length of the swimming route at any time point after the intervention in the anesthetic group were significantly longer than those in the control group ($p<0.05$), and they were significantly shorter in the adiponectin intervention group than those in the anesthetic group ($p<0.05$), but there were no differences between the p38-MAPK antagonist group and the anesthetic group ($p>0.05$). The mRNA and protein expressions of p38 at any time point after intervention in the anesthetic group were higher than those in the control group ($p<0.05$), and they were significantly lower in the adiponectin intervention group than those in the anesthetic group ($p<0.05$), but there were no differences between the p38-MAPK antagonist group and the anesthetic group ($p>0.05$).

CONCLUSIONS: Exogenous adiponectin can improve the cognitive dysfunction of the elderly rats after anesthesia using isoflurane, possibly by inhibiting the p38-MAPK signal pathway in hippocampus.

Key Words:
Adiponectin, Isoflurane, Cognitive dysfunction, Hippocampus, p38-MAPK signal pathway.

Introduction

The occurrence of postoperative cognitive dysfunction (POCD) in elderly patients, associated with the type and dosage of anesthetics, as well as the tolerance, can seriously affect the postoperative recovery and life quality, which has become a research priority in anesthesiology[1]. The occurrence of POCD involves various mechanisms, such as tau protein in CNS, regulation in beta-amyloid peptides, inflammatory, and stress response[2]. Adiponectin, as the endogenous cytokine with various biological activity, not only plays important roles in the regulations of glucose metabolism, lipid metabolism, insulin resistance, inflammation, and stress response[3,4], but also participates in pathophysiological process of some diseases, such as obesity, diabetes mellitus, coronary heart disease, stroke, and Alzheimer's disease[5-7]. In the current study, the intervention of adiponectin in the POCD in elderly rats anesthetized using isoflurane through mitogen-activated protein kinase (MAPK) signal pathway in the hippocampus was analyzed.

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Materials and Methods

Source of Animals

We selected a total of 60 healthy, elder, Sprague-Dawley (SD) rats ranged from 15 to 20 months in age and 400 to 500 g in weight, which were purchased from the Animal Experiment Center of Sangon Biotechnology Co., Ltd. (Shanghai, China). The experiment was carried out for 1 week to adapt to the environment. This study was approved by the Animal Ethics Committee of Guizhou Provincial People's Hospital Animal Center.

Research Methods

The rats were placed into a plexiglass box (25 cm × 45 cm × 20 cm) with an air inlet and an air outlet in both ends. The air inlet was connected to an R510-IP vaporizer of anesthetic gas (RWD Life Science Co., Ltd, Shenzhen, China) for piping the O_2 or isoflurane (Batch No: R510-25, RWD Life Science Co., Ltd, Shenzhen, China) into the box, and a 5250 monitor of anesthetic gas (Ohmeda, Madison, WI, USA) was connected to the air outlet for monitoring the concentrations of isoflurane, O_2, and CO_2. A heating blanket was placed beneath the box to maintain the temperature at (38.5±0.5)°C.

The rats were randomly divided into four groups, i.e. the control group, the anesthetic group, adiponectin intervention group, and p38-MAPK antagonist group, in which the rats in the control group were treated through inhalation of pure oxygen for 4 h at a rate of 4 L/min, and the rats in other 3 groups were treated through inhalation of isoflurane for 4 h. During the inhalation of isoflurane, the concentration of isoflurane was 3.5% at the beginning and decreased to 2.2% at 1 h, and 1.7% between 2 h and 4 h. Then, 0.5 mL of normal saline was intraperitoneally injected into the rats in the control group and the anesthetic group, while adiponectin (300 mg/kg; Beyotime Biotechnology Co., Ltd., Shanghai, China) was injected into the rats in the adiponectin intervention group and p38-MAPK antagonist group. Simultaneously, VX-702 antagonist (20 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) that was diluted to 0.5 mL was given to the rats in the p38-MAPK antagonist group, once/day for 3 days.

Observation Indexes

Morris water maze test was carried out respectively at the 1st, 3rd, and 7th day, and 5 rats participated in the test at each time point, during which we recorded the escape latency, as well as the length of swimming route of rats. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and Western blotting were employed to detect the mRNA and protein expressions of p38 in hippocampus.

Morris Water Maze Test

The test was carried out in the same period every day in a rounded pool (diameter 120 cm, height 40 cm), in which we placed a cylinder platform (diameter 10 cm, height 30 cm), and the temperature of the water was kept at (25±1)°C. In the first 4 days, 1 point was selected from 4 isometric points in four orientations, i.e., East, South, West, and North, from which the rat was placed facing the wall of the pool. The swimming videos were taken by the camera installed at 2 cm above the water and collected by the route-tracking system that was connected to the camera. Before the training, the rats were put on the platform for 20 s and then placed in the maze facing the wall of the pool. In each trial, the swimming time was limited in 90 s, and the camera stopped recording if the rat was not able to find out the platform in 90 s, and the escape latency of this rat was recorded as 90 s. Subsequently, under the guidance of the tester, the rat climbed onto the platform and took a rest for 20 s followed by the next round of training. From the 5th day, the records of rat in each point for two times were averaged as the score before the intervention.

RT-PCR

Hippocampus tissues were isolated, from which we extracted the total RNA using regular TRIzol reagent (Invitrogen, Carlsbad, CA, USA). UV spectrophotometer was applied in the detection of the concentration and purification, and the reverse-transcription kit was used for the synthesis of complementary deoxyribose nucleic acid (cDNA). The primer sequence was synthesized by Sangon Biotechnology (Shanghai) Co., Ltd. (Shanghai, China) according to the sequence in the Gene Bank: for p38-MAPK, (F) 5'-GACCCTGTCTCCGAGTCGTTC-3', (R) 5'-GATGGTTCGGTAGGTGCTGC-3'; for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), (F) 5'-CGCGAGAAGATGACCCAGAT-3', (R) 5'-GCACTGTGTTGGCGTACAGG-3'. The reaction system was set as follows: cDNA 2 μL + 3 μL upstream primer and 3 μL downstream primer + 0.5 μL Taq polymerase + 1 μL dNTPs + 3 μL $MgCl_2$ + 5 μL 10×Buffer + 2.5 μL ddH_2O_2.

The reaction conditions were set as follows: 95°C for 5 min, 95°C for 30 s, 58°C for 30 s, 72°C for 60 s, for a total of 30 cycles and ended by 72°C for 10 min. PCR product was identified through 2% agarose gel electrophoresis and imaged using the gel imaging system; then, the grayscale analysis was performed on photos taken by digital camera. The results were presented by the $2^{-\Delta\Delta Ct}$ method.

Western Blot

Radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China) was added into the homogenized tissues for the extraction of the total protein, followed by a preliminary quantitative assay using Coomassie Brilliant Blue. Before protein detection, we used the antibody of β-actin to perform the dose-normalized test for the quantity of the protein in each sample. 30 μg of total protein was taken for isolating the proteins through 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and the isolated stripes were electrically transferred to the polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), and incubated with the rabbit anti-rat p38-MAPK monoclonal antibody (1:2000; Invitrogen, Carlsbad, California, USA) overnight. Thereafter, the goat anti-rabbit polyclonal secondary antibody (1:500, Bio-Rad, Hercules, CA, USA) was added for incubation at the room temperature for 4 h. After the membrane was washed by Phosphate-Buffered Saline (PBS), the color development was carried out using enhanced chemiluminescence (ECL), and the results were preserved. The Lab Works 4.5 gel imaging software (Media Cybernetics, Rockville, MD, USA) was employed for the semi-quantitative analysis, and the results were presented as integrated optical density (IOD).

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 20.0 software (IBM Corp., Armonk, NY, USA) was applied to the statistical analysis. The measurement data were presented as mean ± standard deviation. The One-way analysis of variance (ANOVA) was performed for the intergroup comparisons, while the Least Significant Difference (LSD) *t*-test was performed for paired comparison and variance analysis of repeated measurement data for intragroup comparison. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of Escape Latency and Length of Swimming Route of Rats

The escape latency and length of swimming route at any time point after the intervention in the anesthetic group were significantly longer than those in the control group ($p<0.05$), and they were significantly shorter in the adiponectin intervention group than those in the anesthetic group ($p<0.05$), but there were no differences between the p38-MAPK antagonist group and the anesthetic group ($p>0.05$) (Table I).

Comparisons of mRNA Expression of p38 in Hippocampus

The mRNA expression of p38 at any time point after intervention in the anesthetic group was increased with the extension of time, it was significantly higher than that in the control group

Table I. Comparisons of escape latency and length of swimming route of rats.

	Escape latency (s)				Length of swimming route (cm)			
Group	Before intervention	1 d	3 d	7 d	Before intervention	1 d	3 d	7 d
Control group	18.5±5.6	18.3±5.9	18.6±6.2	18.5±5.8	156.5±14.5	155.8±15.2	157.3±15.6	156.9±15.7
Anesthetic group	18.3±5.5	26.6±6.8	29.2±8.2	35.3±10.2	154.7±13.6	246.7±35.8	292.3±51.4	342.3±66.3
Adiponectin intervention group	18.4±5.8	23.2±6.2	24.5±6.6	25.6±7.4	155.9±12.8	172.6±25.7	195.8±32.2	222.6±39.8
p38-MAPK antagonist group	18.6±5.9	26.5±6.9	28.8±8.5	34.9±11.3	157.2±14.9	235.9±31.2	278.5±49.8	323.5±56.7

Note: The levels of AST, ALT and BUN in experimental group obviously decline. *$p<0.05$ *vs.* control group.

Table II. Comparisons of mRNA expression of p38 in hippocampus.

Group	1 d	3 d	7 d
Control group	0.0685±0.0059	0.0742±0.0054	0.0776±0.0062
Anesthetic group	0.3625±0.1234	0.3957±0.1265	0.4421±0.1352
Adiponectin intervention group	0.2526±0.0594	0.2674±0.0647	0.2489±0.0823
p38-MAPK antagonist group	0.0529±0.0052	0.0721±0.0034	0.0659±0.0058

($p<0.05$), and it was significantly lower in the adiponectin intervention group than that in the anesthetic group ($p<0.05$). However, there was no difference between the p38-MAPK antagonist group and the anesthetic group ($p>0.05$) (Table II).

Comparisons of Protein Expression of p38 in Hippocampus

The protein expression of p38 at any time point after intervention in the anesthetic group was increased with the extension of time, it was significantly higher than that in the control group ($p<0.05$), and it was significantly lower in the adiponectin intervention group than that in the anesthetic group ($p<0.05$). However, there was no difference between the p38-MAPK antagonist group and the anesthetic group ($p>0.05$) (Table III).

Discussion

The microscopic observations of the hippocampus tissues of the elderly rats with POCD[8] showed that the neurons in the CA1 region of hippocampus are disorderly arranged. The structure of mitochondrial crista is visible, the synapse is reduced in the quantity, and unevenly distributed. The synaptic cleft is enlarged, and the quantity of synaptic vesicles is also decreased. Wu et al[9] have shown that adiponectin is also one of the key markers in the injury of the central nerves and persistent inflammatory responses. Adiponectin, together with its receptor, widely exists in the pituitary and hippocampus tissues of brain and its secretion level is affected by various inflammatory factors, active oxygen, transcription factors, and hormones. For example, the secretion and activation of adiponectin can be inhibited by ROS, TNF-α, and IL-6[10,11]. Accordingly, Kamogawa et al[12] found that an increase in the adiponectin level in the serum of males can decrease the incidence and development of cognitive dysfunction.

In the current study, it was found that the escape latency and length of swimming route at any time point after the intervention in the anesthetic group were significantly longer than those in the control group, and they were significantly shorter in the adiponectin intervention group than those in the anesthetic group. However, there were no differences between the p38-MAPK antagonist and the anesthetic groups. The mRNA and protein expressions of p38 at any time point after intervention in the anesthetic group were higher than those in the control group, and they were significantly lower in the adiponectin intervention group than those in the anesthetic group. However, there were no differences between the p38-MAPK antagonist group and the anesthetic group. The above results suggest that exogenous adiponectin can improve the POCD of elderly rats after anesthesia using isoflurane, which may inhibit the p38-MAPK signal pathway in hippocampus tissues. However, it was also found that simply inhibiting the p38-MAPK signal pathway in hippocampus tissues could not decrease the incidence of POCD. This data indicated that p38-

Table III. Comparisons of protein expression of p38 in hippocampus.

Group	1 d	3 d	7 d
Control group	0.09±0.03	0.08±0.02	0.07±0.02
Anesthetic group	0.38±0.13	0.42±0.15	0.45±0.18
Adiponectin intervention group	0.24±0.12	0.23±0.11	0.22±0.09
p38-MAPK antagonist group	0.06±0.01	0.07±0.02	0.08±0.02

MAPK, instead of being the terminal process, is only one of the most important mechanisms, through which insulin can exert its protective effect on nerves. p38-MAPK, a kind of serine/threonine protein kinase widely existing in the cells of eukaryote, can participate in the transmembrane signal transduction of extracellular signals, and plays important roles in various biological behaviors, such as cell proliferation, differentiation, apoptosis, inflammation, and immune response[13,14]. After the p38-MAPK signal pathway is activated, its important functions can thus be exerted in the injury and repair of neurons through the expressions of various downstream target genes and proteins[15].

Abnormal metabolism of neurons and glial cells are considered to be associated with variations in cognitive functions. Li et al[16] have proved that a decrease in adiponectin is closely related to senile dementia caused by some diseases such as Alzheimer's disease. In addition, the low level of adiponectin is also negatively correlated with the occurrence of long-term injury of cognitive function in type-2 diabetes mellitus patients[17]. The supplementation of exogenous adiponectin can exert a positive protective effect on improving the POCD of elderly rats[18].

Conclusions

The present study indicated that adiponectin also shows a positive effect on the intervention in POCD induced by anesthesia. Nevertheless, further studies should be carried out to discover the specific signal pathway through which the adiponectin can exert its protective effect on the nerves, and the search of a more significant target is of great values for improving the application efficiency of adiponectin and its promotion in clinical practices.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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