Effects of Sevoflurane at Different Concentrations on ApoE in Hippocampus of Aged Rats With Hypercholesterolemia

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Research Article

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Abstract

Objective: To observe the behavioral changes of aged rats with hypercholesterolemia after inhalation of 1.0 MAC and 1.3MAC sevoflurane , the levels of hippocampal ApoE3, ApoE4 and $A\beta_{1-42}$, as well as the changes of $A\beta_{1-42}$ levels of optical density in hippocampal CA3 and CA1 regions, and to investigate the effects of different concentrations of sevoflurane on cognitive function in aged rats with hypercholesterolemia.

Method: The 15-month-old male SD rats were fed a high-fat diet for 9 months. Rats successfully modeled (N=54) were randomly divided into three groups:control group (Con group, n=18), low concentration sevoflurane group (Sev1.0 group, n=18), high concentration sevoflurane group (Sev1.3 group, n=18). Rats in the three groups inhaled aero mixed gas(1L/min O2+1L/min Air), 1.0MAC sevoflurane and 1.3MAC sevoflurane for 2h respectively.1 day, 30 days and 90 days after sevoflurane treatment were defined as T_1, T_{30} and T_{90} experimental periods, respectively. In the three experiments, 6 rats were randomly selected from Con group, Sev1.0 group and Sev1.3 group to complete the behavior experiment in Morris water maze.Subsequently, the levels of ApoE3, ApoE4 and $A\beta_{1-42}$ in the left hippocampus were detected by Western Blot.Expression of $A\beta_{1-42}$ in the right hippocampal CA3 and CA1 regions of rats in each group was detected by frozen immunofluorescence assay.

Result: 1. No behavioral changes were found in T_1, T_{30} and T_{90} experiments.

2. At T_1 and T_{30}, ApoE4 and $A\beta_{1-42}$ in Sev1.3 group was significantly different from that in Con group. At T_{90}, there was no difference in the levels of ApoE4 and $A\beta_{1-42}$ between groups. ApoE3 expression was not statistically significant in the three experimental periods. 3. At T_1 and T_{30}, the average optical density of $A\beta_{1-42}$ in CA3 and CA1 region, Sev1.3 group was significantly different from that in Con group. At T_{90}, there was no significant difference between groups.

Conclusion: After inhalation of 1.3MAC sevoflurane in aged SD hypercholesterolemia rats, the levels of ApoE4 and $A\beta_{1-42}$ were increased in hippocampus at early and middle stage, and the average optical density of $A\beta_{1-42}$ was increased in CA3 and CA1 area. The change trend of the two ones was consistent, but not enough to cause behavioral changes.

Background

With the coming of aging society, the proportion of the elderly population is growing rapidly. According to the survey of the World Health Organization, the elderly population is expected to increase to 2 billion by 2050, accounting for 1/5 of the world's total population. The medical treatment for the elderly has gradually become a hot issue of social concern. With the introduction of the concept of enhanced recovery after surgery (ERAS), people not only attach importance to the evaluation of treatment effect, but also pay more attention to the quality of rehabilitation. Anomalies in the elderly postoperative mental state is an important content of cognitive dysfunction, its performance is nonsense, delirious, memory

and disorientation(1), which seriously affected the health of the elderly patients and the quality of survival, and patients with vascular risk factors, such as hyperlipidemia, hypertension, and diabetes mellitus, may have a higher incidence of postoperative cognitive dysfunction(2).

Hyperlipidemia is one of the most common underlying disorders in older people, including elevated cholesterol and/or triglycerides .Relevant studies have shown that the increase of blood lipids and abnormal lipid metabolism may affect the microvessels, increase the permeability of the blood-brain barrier, destroy the lipid balance in the brain, and increase the risk of neurodegeneration. Apolipoprotein E (ApoE) is the carrier of cholesterol in the brain. Its physiological function is redistribute lipid in the cell and maintain the balance of cholesterol in the brain environment. ApoE is a polymorphic protein with three common subtypes: ApoE2, ApoE3 and ApoE4. At present, the study of ApoE polymorphism is a hot topic in various disciplines. According to relevant studies, the incidence of alzheimer disease (AD) in ApoE4 gene carriers is increased and the onset age is earlier(3).Spatial learning and memory deficits in developing mice with ApoE gene deletion(4).ApoE4 expression up-regulation can indicate nerve injury(5).From these studies, the abnormal expression of ApoE may affect cognitive function.In addition, our previous studies have shown that ApoE and its genotype may play an important role in the clearance and deposition of Aβ.

Amyloid β-peptide (Aβ) is hydrolyzed from β-protein precursors and secreted by cells. It is highly neurotoxic after accumulation and precipitation of the cellular matrix(6).Aβ can be divided into $Aβ_{1-40}$ and $Aβ_{1-42}$ according to the site of proteolytic enzyme cleavage. $Aβ_{1-42}$ is more toxic and more likely to aggregate, thus forming the core of "senile plaques" precipitation and triggering neurotoxic effects. Related research at home and abroad show that inhaled anesthetics can induce beta amyloid (Aβ) gathered(7), lead to changes in mitochondrial shape function, inhibit the enzyme activity of electron transport chain, the lower levels of oxidative phosphorylation, the decrease of the ATP generation, can also promote the extracellular Ca^{2+} internal flow causes severe calcium overload and eventually lead to mitochondrial function and loss of structural integrity, that cause neural cell apoptosis(8, 9). From these studies, sevoflurane can affect cognitive function by affecting Aβ deposition, and ApoE and its genotype may play a key role in influencing Aβ aggregation.

Sevoflurane is an inhalation anesthetic commonly used in clinical work, which has the advantages of strong controllability and small hemodynamic fluctuation, and is often used in the anesthesia of elderly patients. In this special group of elderly patients with hypercholesterolemia, the expression of ApoE related to cholesterol metabolism in the brain is upregulated. After sevoflurane anesthesia, could it affect the expression of ApoE gene polymorphism and then the oligomer $Aβ_{1-42}$, thus affecting cognitive function?And how long does it last? Therefore, this experiment to the aged rats with hypercholesterolemia as the research object, through the observation of the aged rat hippocampal ApoE polymorphism and $Aβ_{1-42}$ level after different concentration of sevoflurane treatment for 1 day, 30 days, 90 days, and combining the behavioral test results, to investigate the changes of cognitive function and possible related molecular mechanisms and pathways in high-fat elderly rats after inhalation of

sevoflurane at different concentrations. The study may provide some research basis for rational use of general anesthesia in elderly patients with hypercholesteremia in clinical work.

Materials And Methods

Animal and the model of the aged rats with hypercholesterolemia

A total of 60 SPF healthy male adult SD rats (Sprague Dawley), weighing 300-400g, aged 15 months, were provided by the Animal Experimental Center of Daping Hospital of the former Third Military Medical University [License No. : SCXK (Chongqing) 2012-0005].Conventional cage feeding, 5 per cage.All the rats were fed high-fat diet(10) for 9 months, during which 6 rats died and the mortality rate was 10%. The cause of death may be the change of ambient temperature or the death by the bite of the same kind.The rat tail vein was sampled every 3 months during the feeding cycle to detect the serum cholesterol content, determine the effect and stability of the Hypercholesterolemia model, and establish the aged rats with hypercholesterolemia.The formulation of this high fat feed is the most economical and effective formula obtained by referring to relevant literature, which can establish the model of high cholesterol. Animal feeding and care follow the regulations of the China Laboratory Animal Protection and Ethics Committee.

Group

According to the experimental design, 0.1mL of tail venous blood was taken from SD high-fat elderly rats (N=54), and serum cholesterol content was detected by enzymatic method. The rats were randomly divided according to different serum cholesterol content according to the random number table. Control group (Con, N= 18), air oxygen mixture was inhaled for 2h; Sevoflurane 1.0MAC group (Sev1.0, n=18), 1.0MAC (2.4%) sevoflurane inhalation for 2h ; Sevoflurane 1.3MAC group (Sev1.3 , n=18), 1.3MAC(3.2%) sevoflurane inhalation for 2h.

Sevoflurane treatment

The size of the inhalation anesthesia box was 75×40×15cm3. The holes on both sides of the long axis of the box were connected with the inlet and outlet air pipes of the anesthesia machine respectively, and the side holes of the joints were connected with the gas monitor. The bottom of the box was evenly covered with calcium and lime with a thickness of about 3cm, and a thin gauze was laid on the calcium and lime to prevent the rats from being burned by calcium and lime during the anesthesia process. Rats in each group were respectively put into inhalation anesthesia box and treated with sevoflurane at the set anesthesia concentration for 2 hours, while the control group was inhaled air oxygen mixture for 2 hours.

Behavioral testing

1, 30 and 90 days after sevoflurane treatment were defined as T_1, T_{30} and T_{90}, respectively. The three groups of rats were tested on spatial learning and memory by Morris water maze experiment, which included orientation navigation experiment and spatial exploration experiment. The declarative memory

ability of rats was tested in the location-navigation experiment. The space exploration experiment tested the rats' ability to make spatial associations, recall and explore.

Positioning navigation experiment and Space exploration experiment

The experiment lasted for 5 days. Each rat was trained 4 times a day, once at the water entry point in each quadrant, for a total of 20 times.The rats were placed into the water facing the basin wall from the middle points of the four quadrants divided by the basin wall successively, and the time from entering the water to boarding the platform was recorded as the escape latency, with a time limit of 120s.If the rats did not climb the platform in 120s, the rats were led to the platform to rest for 10s, and the positioning navigation experiment was conducted again. If the rats failed to climb the platform for 3 times, they would be excluded.On the fifth day of the experiment, after the positioning navigation experiment, the platform in the quadrant of the original platform was removed, and the rats were placed at any entry point to swim freely for 120s. The acquisition and recording system recorded and analyzed the retention time percentage of the rats crossing the quadrant of the original platform, the movement distance percentage of the original quadrant of the original platform, and the entry times percentage of the original quadrant of the original platform.

Sampling and specimen preparation

Each experiment period last Morris water maze experiment done immediately, each group take 6 rats, intraperitoneal injection of 1% sodium pentobarbital 30 mg/kg anesthesia in rats, stripping complete brain tissue, ice salt water quickly clean tissue, brain tissue cutting on mesa around separated into two parts, the stripping on the left side of the hippocampus, frozen in - 80 ℃ refrigerator spare, specimen can hold 3 months;The right brain tissue was removed, soaked in 4% paraformaldehyde solution for 24 hours, then dehydrated to 30% sucrose until tissue precipitation was obtained.

The tissue blocks were embedded with OCT embedding agent and stored in a refrigerator at 4℃ for later use. Finally, the animal carcasses were uniformly collected to the animal center for treatment.

Western blotting

According to the experimental operation procedure, the first step is to extract the protein. To each group, left hippocampus of rats, full cut up in the centrifuge tube, then transferred to the glass in the mill, join the RAPI cracking liquid 250μl and PMSF 2.5μl, fully grinding, ice cracking 20 min. The grinding liquid was transferred to a 1.5mL centrifuge tube, centrifuged at 14000rPm at 4℃ for 5min, and the supernatant Was transferred to a 1.5mL centrifuge tube. The supernatant was centrifuged at 14000rPm at 4℃ for 20min. The supernatant was transferred to a new centrifuge tube, which was the protein sample, and stored at 4℃ for later use. The second step is to determine the protein concentration using a microplate analyzer. Thirdly, Western blot was performed to detect the levels of ApoE3, ApoE4 and $A\beta_{1-42}$ by preparing concentrated gel and separation gel, electrophoresis, gel cutting, membrane transfer, sealing, membrane washing, secondary antibody incubation, and exposure.

Frozen immunofluorescence

After embedding the parallel between groups on the right side of brain tissue in rats microtome coronal cut into 40 μm brain slice, grouping soaked in PBS solution. The sections of each group were sealed with 10% bovine serum Albumin (BSA) for 2h, the primary antibody was incubated overnight at 4℃, and the secondary antibody was incubated on the second day.Avoid light environment each slice with DAPI 10μl, cover glass sealing piece. Fluorescent inverted microscope observation: put in the chips after staining fluorescence under inverted microscope, 4 times observations looking for targets objective observation of hippocampal area, remove the view to target area after switching to 10 times and 20 times and transformation to the scanning imaging objective, observe slice scan and gathering pictures, use IPP software analysis of optical density, integral optical density divided by the area of the selected object to calculate the average optical density, use the average value of the each sample take five horizons. This experiment involves 270 fields of 54 samples, that is, the average value of 270 fields is calculated.

Statistic analysis

Using SPSS17.0 software, The measurement data was expressed as x±s. Repeated measurement ANOVA was used for the escape latency results of positioning navigation in Morris water maze experiment at different time points after anesthesia treatment.P>0.05, indicating that the experimental data accord with the spherical hypothesis, can be used for one-way ANOVA; P<0.05, does not conform to the spherical hypothesis, then it needs to conduct multivariate analysis of variance.

Results

Results of blood lipid sampling in aged hypercholesterolemia rats during modeling

Before establishing a hypercholesterolemia model in aged rats, and during the high-fat feed feeding cycle, serum cholesterol was detected in rats every 3 months (breeding to March, June, September). 0.1 mL of tail venous blood was randomly selected from the model rats to detect serum cholesterol, and compared with the rats fed on general diet during the same period. There was no statistical difference between the groups before modeling (P>0.05), and there was statistical difference between the groups at 3, 6 and 9 months after modeling (P<0.05) (Fig. 1), suggesting that the model of hypercholesterolemia aged rats was established successfully.

Behavioral changes

In the positioning navigation experiment and space exploration experiment, there were no differences in the escape latency at the three experiment periods of T_1, T_{30} and T_{90} (P>0.05) (Fig. 2). There were no differences in the percentage of retention time crossing the original platform quadrant, the percentage of movement distance of the original platform quadrant and the percentage of entry times of the original platform quadrant in the three experiment periods of T_1, T_{30} and T_{90} (P>0.05) (Fig. 3).

The levels of ApoE3、ApoE4 and $A\beta_{1-42}$ in hippocampal

(1) T_1: The expression of ApoE4 protein in Sev1.3 group was significantly different from that in Con group and Sev1.0 group. The level of $A\beta_{1-42}$ in Sev1.3 group was significantly different from that in Con group. (2) T_{30}: there was a statistically significant difference in the expression of ApoE4 protein between Sev1.3 group and Con group. The level of $A\beta_{1-42}$ protein in Sev1.3 group was statistically significant compared with Con group and Sev1.0 group. (3) T_{90}: there were no statistically significant differences in the levels of ApoE4 and $A\beta_{1-42}$ protein between the groups.(4) the expression level of ApoE3 protein in each group in the three experimental periods was not statistically significant. (Fig. 4)

(1)T_1 and T_{30}: In CA3 area, the average optical density of $A\beta_{1-42}$ in Sev1.3 group was significantly different from that of Con group and Sev1.0 group, while in CA1 area, the difference between the average optical density of $A\beta_{1-42}$ in Sev1.3 group and Con group was statistically significant. (2)T_{90}: there was no significant difference between the groups. (Fig. 5)

Discussion

Morris water maze experiment is an experiment in which rats and mice are forced to swim and learn to find hidden platforms in the water. It has been widely recognized in the industry and is the first choice of classic experiment in learning and memory research(11), including positioning navigation experiment and space exploration experiment. The orientation navigation experiment was used to test the declarative memory ability of rats, while the spatial exploration experiment was used to test the spatial association and recall exploration ability of rats. Navigation experiment is to let the rat in training for many times, learn to look for a fixed position hidden platform, forming stable space cognition, the spatial reference memory after entering consciousness system, its storage mechanism mainly involves the hippocampus and cerebral cortex, the brain areas, often accompanied by Hebb synaptic modification(12), belongs to the declarative memory. It only takes a few days for adult rats to establish a stable declarative memory. In this study, the escape latency of rats in each group was rapidly shortened on the second experimental day compared with the first experimental day, indicating that the hyperlipidemic elderly rats encoded and stored the information obtained by the brain after receiving external stimuli very quickly. After the second experiment day, the escape latency time gradually shortened, and reached stability on the fourth and fifth experiment days. This process is to gradually strengthen and store the same stimulus signals in the brain, and gradually transform short-term memory into long-term memory. The hippocampus is an important central nervous system for this process. When the declarative memory of rats is impaired, it can be manifested as a prolonged escape latency. In this study, there was no difference between the three experimental periods T_1, T_{30} and T_{90}, namely, the short-term, medium-term and long-term escape latency of rats, indicating that different concentrations of sevoflurane did not cause the decline of memory storage capacity in aged rats with hypercholesterolemia. The spatial exploration experiment is a process in which the rats read the spatial memory which has been formed and express the release. In the process of searching for the hidden platform, the percentage of retention time of the original platform quadrant,

the percentage of movement distance of the original platform and the percentage of entry times of the original platform quadrant were recorded, which could reflect the output function of the core brain region responsible for memory in rats. In this study, there was no difference in spatial exploration indexes of rats in the three experimental periods of T_1, T_{30} and T_{90}, indicating that the inhalation of sevoflurane at different concentrations did not affect the memory output in the core brain region. In conclusion, sevoflurane at different concentrations of 1.3MAC and 1.0MAC did not cause cognitive function changes in aged rats with hypercholesterolemia

Western blot results showed that the effect of sevoflurane on ApoE polymorphism mainly focused on the change of ApoE4 expression. There was no regular change of ApoE3 in the three experimental periods of this study, so ApoE3 was not recommended as a molecular mechanism for further study. In the T_1 and T_{30} experimental periods, the expression of ApoE4 in the aged rats with hypercholesterolemia was increased in a concentration-dependent manner after inhalation of 1.3MAC and 1.0MAC sevoflurane, and the difference between Sev1.3 group and Con group was statistically significant (P<0.05). During the T_{90} experiment period, the ApoE4 level of the Sev1.3 group decreased, and there was no difference between the Sev1.3 group and the Con group. ApoE in the central nervous system is mainly expressed and secreted by astrocytes and some microglia. Research evidence shows that neurons can induce the expression of ApoE under pathological conditions such as stress or injury and stimulation(13). In conclusion, 1.3MAC sevoflurane can induce the expression of ApoE4 until 30 days after anesthe.

In the T_1 and T_{30} periods, 1.3MAC sevoflurane increased the expression of $A\beta_{1-42}$ in the hippocampus of aged rats with hypercholesterolemia, while the levels of $A\beta_{1-42}$ were also increased. In the T_{90} period, the levels of $A\beta_{1-42}$ and ApoE4 were decreased, and the trends of the two were consistent. Related studies have shown that ApoE genotype affects the production and clearance of Aβ(14). In terms of Aβ production, ApoE4 protein can increase the endocytosis of APP and accelerate the hydrolysis of APP(15), and can improve the transcription of APP through non-classical mitogen-activated protein kinase signaling pathways such as ERK1/2, MKK7, DLK and so on, thereby increasing the production of Aβ(16). In the aspect of Aβ metabolism, the 200 - 299 region of the ApoE carboxylic terminal is the key site for binding to the 12 - 28 amino acid sequence (region) of the Aβ peptide, and the two interact and bind to form the stable ApoE/Aβ complex, which is associated with the low density lipoprotein receptor-related protein 1 (LRP 1), low density lipoproteinreceptor (LDLR), heparan sulfate proteoglycan (HSPG) and other surface receptors bind, transport to lysosomes to induce Aβ degradation, and finally excretion through intertissue fluid or blood brain barrier (BBB)(17). Compared with the ApoE2 / Aβ and ApoE3 / Aβ complexes, the structure of ApoE4 / Aβ complexes is more stable, which makes the oligomer Aβ more difficult to clear.

Meanwhile, frozen immunofluorescence also showed that 1.3MAC sevoflurane increased the average optical density of $A\beta_{1-42}$ in the CA3 and CA1 regions of the hippocampal signaling pathway during T_1 and T_{30} experiments, and decreased the average optical density of $A\beta_{1-42}$ during T_{90} experiments, which was consistent with the protein change trend detected in Western blot. Studies have shown that ApoE4

can affect the formation of hippocampal declarative memory by reducing the activity of post-synaptic cAMP response factor binding protein (CREB) and impair the late long-term enhancement of the hippocampus (L-LTP)(18). In addition, ApoE4 can affect the contact length and density of dendritic spines of nerve cells(19), damage the mitochondrial function of nerve cells(20), signal conduction disorder between nerve cells(21), aggravate neuroinflammation(22), etc. Some studies have also shown that Aβ deposition can form the core of "senile plaques", which together with nerve fiber tangles constitute "senile plaques", affect the conduction of hippocampal signaling pathway, and cause decreased learning ability and impaired memory.

However, the increase of ApoE4 and $A\beta_{1-42}$ levels during T_1 and T_{30} did not induce behavioral changes in the aged rats with hypercholesterolemia. It can be seen that 1.3MAC sevoflurane can cause the increase of ApoE4 level, but it is not enough to cause the change of cognitive function.$A\beta_{1-42}$ levels increased in the short and middle periods after sevoflurane treatment, but decreased again after 90 days, suggesting that sevoflurane can cause the increase of $A\beta_{1-42}$ levels, but does not form "senescence spots" in CA3 and CA1 zones. The brain is A complex central system. In addition to the increase of harmful proteins, related protective mechanisms are activated, such as the clearance of elevated ApoE4 and $A\beta_{1-42}$, and the restoration of the homeostasis of the brain environment. These mechanisms remain to be studied.

Therefore, this study proved the correlation between ApoE4 and $A\beta_{1-42}$ and sevoflurane concentration. Although it did not cause behavioral changes, will further increase sevoflurane concentration or prolong the action time of sevoflurane lead to an "inflection point"? What is the brain's protective mechanism for removing harmful proteins?This lays a foundation for the follow-up research, and also provides a theoretical basis for the rational selection of sevoflurane concentration in clinic. In patients with hypercholesterolemia, low concentration of sevoflurane is safer than high concentration of sevoflurane, causing lower expression of nosotoxic proteins ApoE4 and $A\beta_{1-42}$.

Conclusion

After inhalation of 1.3MAC sevoflurane in aged SD hypercholesterolemia rats, the levels of ApoE4 and $A\beta_{1-42}$ were increased in hippocampus at early and middle stage, and the average optical density of $A\beta_{1-42}$ was increased in CA3 and CA1 area. The change trend of the two ones was consistent, but not enough to cause behavioral changes.

Abbreviation

Concept of enhanced recovery after surgery (ERAS), Apolipoprotein E (ApoE), Alzheimer disease (AD), Amyloid β-peptide (Aβ), Bovine serum Albumin (BSA), Receptor-related protein 1 (LRP 1), Lipoproteinreceptor (LDLR), Heparan sulfate proteoglycan (HSPG), Blood brain barrier (BBB), Post-synaptic cAMP response factor binding protein (CREB), Hippocampus (L-LTP).

Declarations

Ethics approval and consent to participate

The animal study protocol was legally approved by the Animal Committee of Zunyi Medical University (Approval No:KLL-2021-025). All experiments conformed to the regulations of the China Laboratory Animal Protection and Ethics Committee. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publish

Not applicable.

Availability of data and material

The data that support the findings of this study are partly available from the corresponding author upon reasonable request. The data are not publicly available due to surveillance data share requests.

Competing Interest

There is no conflict of interest in this study.

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Authors' contributions

YH Z , LL and LZ performed major experiments such as animal model establishment and water maze detection, etc. ZY W, YY, WQ Y performed sampling of experimental animals and western blotting testing. YH Z , LL as major contributors in writing the manuscript. LZ, ZY W, YY and WQ Y analyzed the data. ZQ Z, XQ R and JF W made revisions and decisions. All authors read and approved the final manuscript.

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Figures

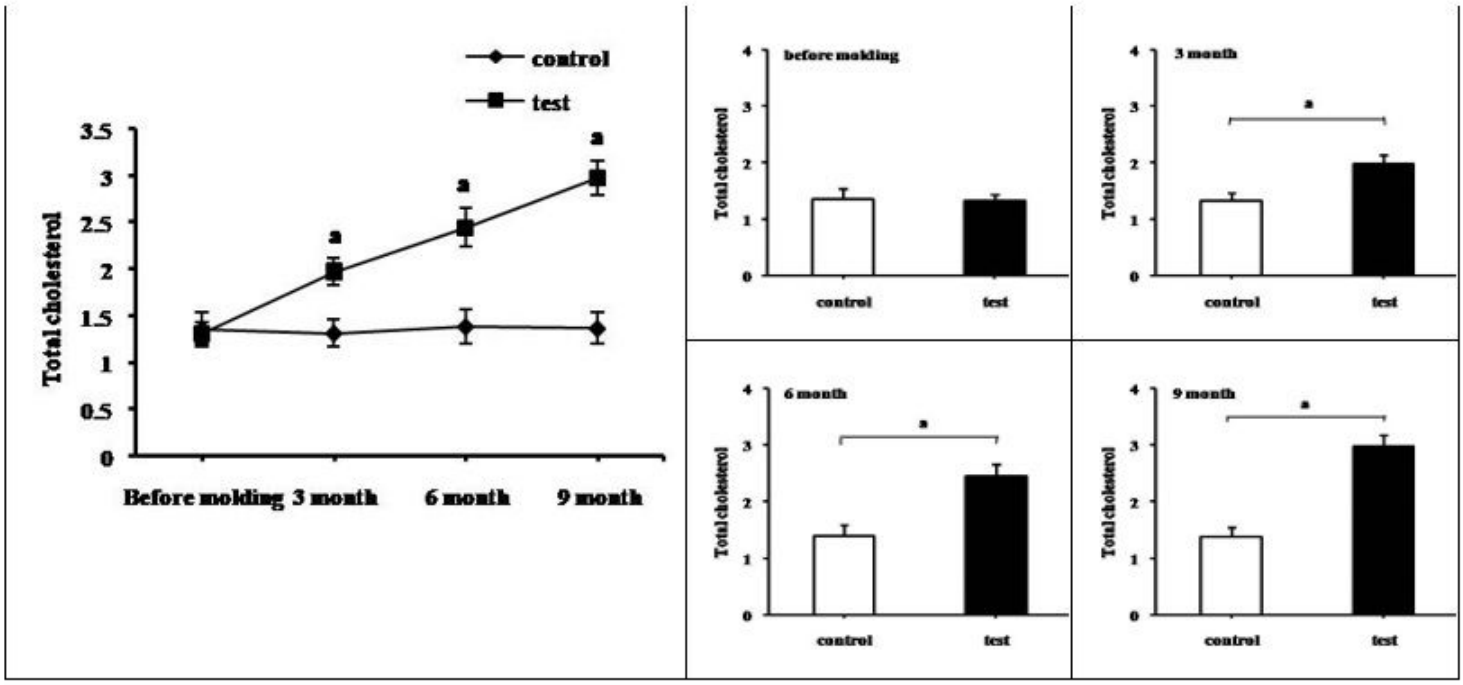

Figure 1

The change trend of serum cholesterol of experimental rats in each period. There was no difference in serum cholesterol level between the experimental group and the normal feed rats before modeling (P>0.05). At 3, 6 and 9 months after modeling, the serum cholesterol level of rats in the experimental group increased gradually, and acomparation with ontrol group, P < 0.05

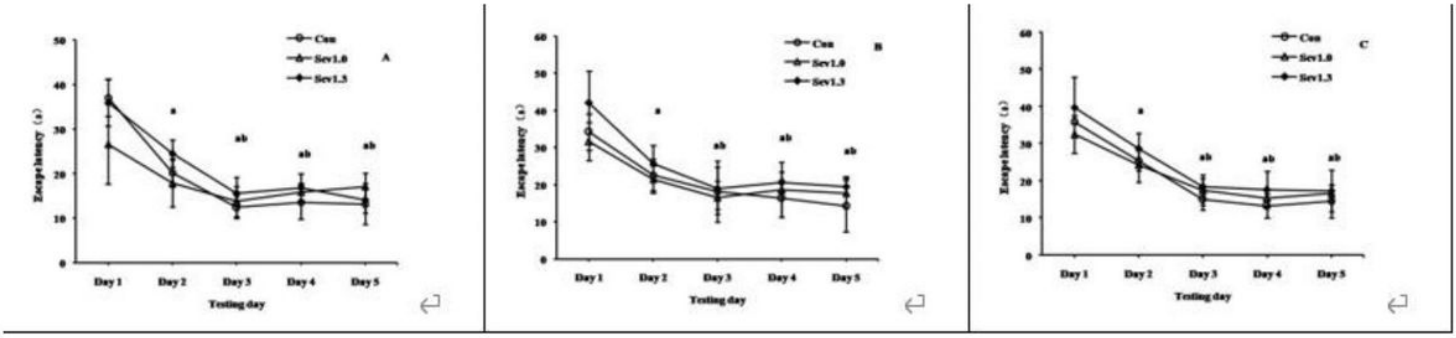

Figure 2

Escape latency: A□B□C represent the experimental periods of T1, T30 and T90. acomparation between the Day1 and other days, p<0.05; bcomparation between the Day2 and other days, p<0.05□but there was no difference between the groups

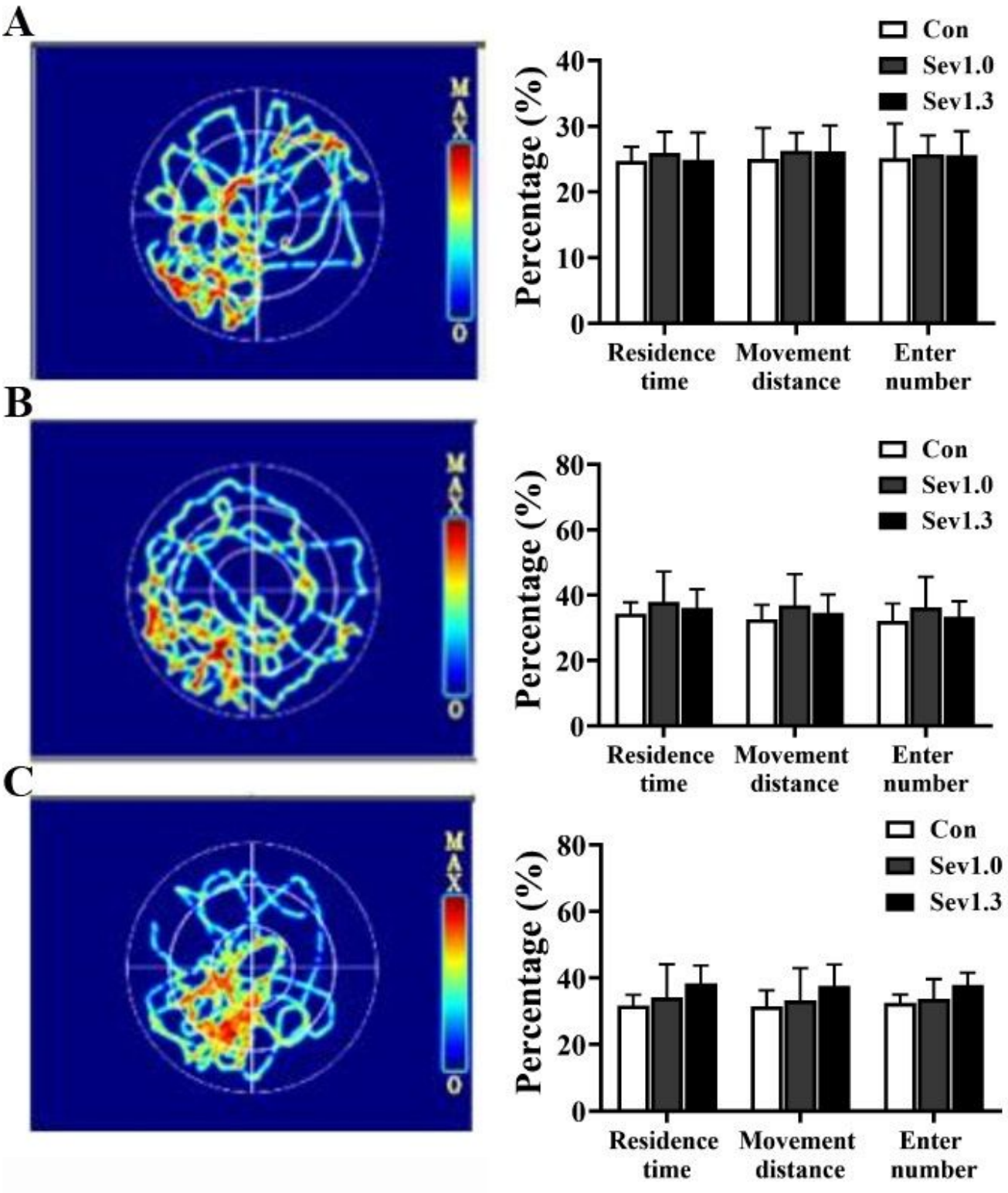

Figure 3

Space exploration experiment: A、B、C represent the experimental periods of T1, T30 and T90. The retention time percentage, the movement distance percentage and the entry times percentage of the original platform quadrant showed no difference among the groups

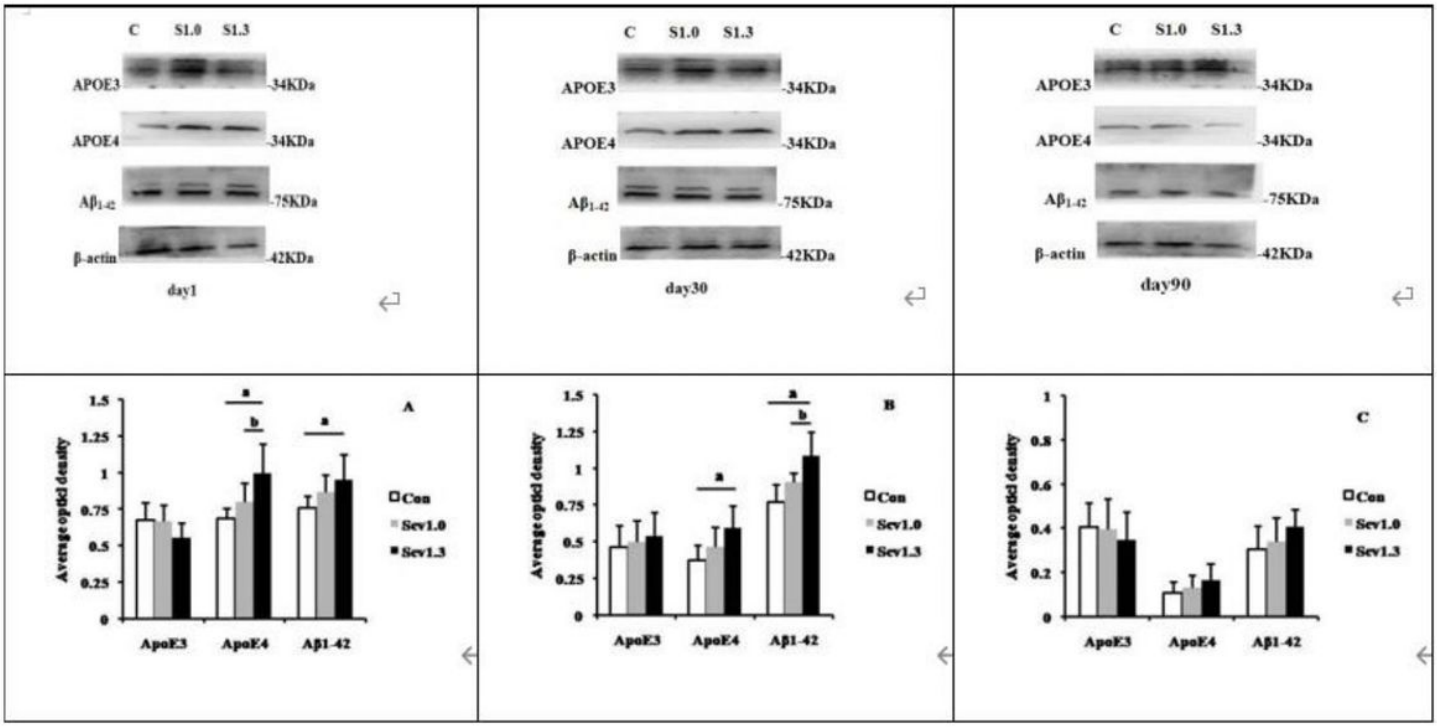

Figure 4

The levels of ApoE3, ApoE4 and Aβ1-42. A、B、C represent the experimental periods of T1, T30 and T90,acomparation between the Sev1.3 and Con, $p<0.05$, bcomparation between the Sev1.3 and Sev1.0, $p<0.05$

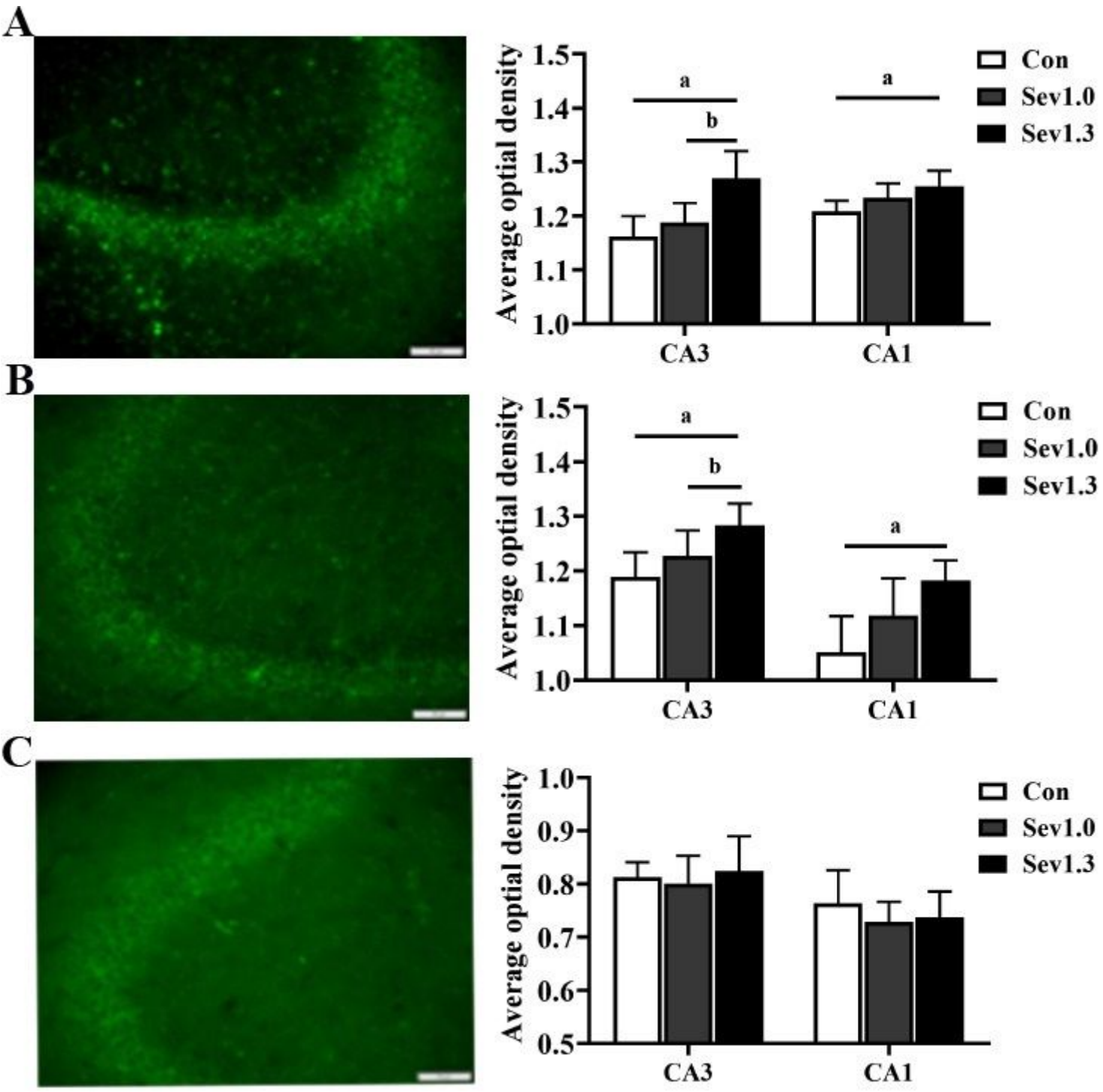

Figure 5

Immunofluorescence expression of Aβ1-42 in hippocampus CA3 and CA1. Under 20 x fluorescence microscope, Aβ1-42 was expressed in cytoplasm ; A□B□C represent the experimental periods of T1, T30 and T90; acomparation between the Sev1.3 and Con, $p<0.05$; bcomparation between the Sev1.3 and Sev1.0, $p<0.05$

Supplementary Files

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