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ORIGINAL RESEARCH

Hyperbaric Oxygen Improves Long-Term Learning and Memory Impairment by Attenuating Neuronal Apoptosis in aMCI Rats

Yonggui Zhang[1], Xianzhong Lin[2], Chen Ye[2], Peiling Zhang[3]

[1]Department of Anesthesiology, The Second Hospital of Longyan, Fujian, People's Republic of China; [2]Department of Anesthesiology, The First Affiliated Hospital of Fujian Medical University, Fuzhou, People's Republic of China; [3]Department of Anesthesiology, The Second Affiliated Hospital of Fujian Medical University, Quanzhou, People's Republic of China

Correspondence: Xianzhong Lin, Department of Anesthesiology, the First Affiliated Hospital of Fujian Medical University, 20 Chazhong Road, Fuzhou, People's Republic of China, Email lxzyjs@fjmu.edu.cn

Background: With the aging of the population and the increasing incidence of neurological diseases, amnestic mild cognitive impairment (aMCI) has attracted attention. Hyperbaric oxygen (HBO) has gradually shown the potential in the treatment of aMCI as an emerging treatment method in recent times. This study is to observe the effect of HBO on the long-term learning memory of aMCI rats, and investigate the associated mechanisms.

Methods: Seventy-two male rats (4-month-old) were randomly divided into control (CON) group, aMCI group, HBO group, 24 rats in each group. Each group was randomly divided into CON_1, CON_7, CON_{28}; $aMCI_1$, $aMCI_7$, $aMCI_{28}$; HBO_1, HBO_7, HBO_{28}, 8 rats in each group. The aMCI model rats were established in aMCI and HBO groups. HBO group was treated with HBO for 7 days. The ethological and cytopathology which include Morris water maze (MWM) test, HE staining, TUNEL staining and the expression of Fas/FasL on neuron membrane were conducted to evaluate the effects of HBO on day 1, day 7 and day 28 after HBO treatment.

Results: MWM test showed that the spatial learning and memory ability of the rats decreased in aMCI group, and recovered in HBO group; Compared with aMCI group, the pathological damage of hippocampal nerve cells was alleviated, the number of apoptotic cells was significantly reduced ($P < 0.05$), and the expression of Fas/FasL on the surface of nerve cell membrane was significantly weakened in HBO group ($P < 0.05$). There were no significant changes in the spatial learning and memory ability, pathological damage of hippocampal neurons, the number of apoptotic cells, and the changes of Fas/FasL on the surface of hippocampal neurons in HBO_1, HBO_7, and HBO_{28} groups ($P > 0.05$). However, in $aMCI_1$, $aMCI_7$, and $aMCI_{28}$ groups gradually aggravated ($P < 0.05$).

Conclusion: 1. HBO can improve the long-term learning and memory impairment by attenuating neuronal apoptosis in aMCI rats. 2. Fas/FasL mediated cell receptor death pathway is involved in the apoptosis of hippocampal neurons.

Keywords: amnesic mild cognitive impairment, hyperbaric oxygen, apoptosis, learning and memory impairment

Introduction

With the aggravation of population aging, the number of people suffering from Alzheimer's disease (AD) has increased significantly. It is expected that by 2050, the number of people suffering from dementia will reach 150 million,[1] which has become a public health problem of global concern. aMCI is an intermediate state between normal aging and dementia, which is mainly characterized by mild memory loss, cognitive decline and other symptoms.[2] It accounts for 30% of the constituent ratio of MCI.[3] About 15–20% of aMCI patients will transform into AD every year, and half of aMCI patients may develop into clinical AD within three years.[4] Patients with aMCI have a higher risk of dementia.[5]

At present, the treatment of aMCI mainly focuses on drug intervention, physical therapy, lifestyle adjustment and other aspects, mainly including moderate physical exercise, life behavior intervention, cognitive training, nerve stimulation and some intellectual activities.[6–9] Some medications can reduce some AD-related symptoms, including thinking problems, cognitive dysfunction and motor skills, but the effect is not very satisfactory. There is still no drug that can

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cure AD.[10] It is particularly important to seek therapeutic strategies to treat aMCI or slow the development of aMCI to AD. Therefore, it is of great significance to explore the pathogenesis of aMCI and find effective intervention methods.

In recent years, the role of neuronal apoptosis in the pathogenesis of aMCI has attracted increasing attention. Neuronal apoptosis is a programmed cell death process involving the activation of multiple signaling pathways. Among them, the death receptor pathway is one of the important pathways of neuronal apoptosis. This pathway is mainly composed of death receptors (such as Fas, TNFR, etc.) and corresponding ligands (such as FasL, TNF, etc.). When ligands bind to receptors, they trigger a cascade of intracellular apoptotic signals and eventually lead to neuronal apoptosis.

As an emerging treatment method, HBO has shown unique advantages in the treatment of a variety of diseases. By increasing the oxygen pressure in the environment, increasing the oxygen content of the tissue cells, reducing oxidative stress, inflammation and nerve cell apoptosis, promotes the metabolism and repair of cells, and regulates the gene expression associated with aging.[11,12] In recent years, more and more studies have shown that HBO can inhibit neuronal apoptosis and improve hippocampal function and cognitive function,[13,14] which provides new ideas for the treatment of neurodegenerative diseases.

HBO can improve cognitive impairment, but how long the therapeutic effect lasts and whether the condition recurs after treatment have not been determined. HBO reduces oxidative damage through the antioxidant system and reduces neuronal apoptosis by reducing phosphorylation, and reduces brain damage by inhibiting PKC pathway.[15] However, the mechanism by which HBO inhibits the expression of pro-apoptotic proteins and reduces neuronal apoptosis is not clear. The hippocampus is a brain region closely related to spatial learning and memory. Therefore, the purpose of this study was to evaluate the behavior of aMCI rats after HBO intervention, hippocampal histopathology, immunohistochemical changes, Fas and FasL expression in hippocampal neurons of aMCI rats. To explore the apoptotic mechanism of HBO in the treatment of cognitive dysfunction and the persistence of the curative effect of HBO in the treatment of aMCI, and to provide a theoretical basis for clinical and experimental application.

Materials and Methods

Animal

The animal experiment was authorized by the Experimental Animal Ethics Committee of Fujian Medical University. Experiments were carried out under the project license (No.: 2018–321) granted by Fujian experimental animal association. All experiments were conducted in accordance with EU Directive 2010/63/EU for animal experiments.

A total of 72 clean healthy male SD rats, aged 4 months, weighing 280–310g, were provided by Shanghai Slack Laboratory Animal Co, LTD, License No:SCXK (Shanghai) 2017–0005.

Main Reagents and Drugs

D-galactose (D-gal) from AMRESCO, USA, Tunel kit from Roche, Rabbit Anti-CD95/Fas Antibody, Rabbit Anti-Fas Ligand antibody is derived from Servicebio, Wuhan.

Main Instruments and Equipment

Morris Water Maze from Shanghai Xinsoft Information Co, LTD, Small animal hyperbaric oxygen chamber from Yantai ice wheel hyperbaric oxygen chamber.

Experimental Methods

Experimental Grouping

There were 72 healthy male rats. The rats were fed and watered freely under standard conditions, fed adaptively for 1 week, and excluded from swimming and visual disorders. According to the random number table method, they were randomly divided into 3 groups: CON group, aMCI group and HBO group, with 24 animals in each group. Each group was randomly divided into CON_1, CON_7, CON_{28}; $aMCI_1$, $aMCI_7$, $aMCI_{28}$;HBO_1, HBO_7, HBO_{28} group according to the 1st, 7th and 28th day after the completion of HBO intervention, with 8 animals in each group.

Establishment of aMCI rat model

In order to build the amci model rat, rats in the aMCI group and HBO group were subcutaneously injected with D-gal 1000mg·kg^{-1}·d^{-1} for 7 days to establish aMCI model.[16] The control group was injected with a corresponding volume of normal saline. The skin bulge on the back of the neck after injection is a sign of success. During the modeling period, the diet, water intake and body weight of the rats were measured and recorded every day.

HBO Therapy

HBO group was treated with HBO on the first day after modeling. Treatment regimen: Rats were placed in animal hyperbaric oxygen chamber and oxygenated with 100% O_2 at a flow rate of 3.0L.min^{-1} to reduce air content and rapidly increase oxygen concentration. Then, the pressure was slowly increased to 0.2 MPa absolute pressure at 100 kPa.min^{-1} for about 15 minutes. The absolute pressure of 0.2MPa was maintained for 60 min, and then slowly decompressed for 15min to atmospheric pressure, once a day for 7 days. The start time of hyperbaric oxygen treatment was fixed at 9 am every day. The CON group and the MCI group are placed in the same environment without pressure.

Behavioral Analysis

Morris water maze test (MWM) was performed to evaluate spatial learning and memory ability on the 1st, 7th and 28th day after HBO treatment. MWM, a test of spatial learning ability in rodents, has been shown to be robust and reliable and significantly correlated with hippocampal synaptic plasticity and NMDA receptor function.[17] The main device is a 160 cm diameter circular water tank. Water (23±2°C) was injected into the tank. The sink walls and bottom are black. The escape platform was a 10 cm diameter transparent disc placed 2 cm below the water surface. The swimming path, time and distance of the rats were accurately tracked and identified by the software.

(1) Positioning navigation experiment: the tank was divided into four quadrants, the escape platform was set in the middle of the fourth quadrant, and the fourth quadrant was selected as the starting position. The rats were placed into the water facing the tank wall. The time from entering the water to finding the escape platform was recorded, which called Escape Latency. If it exceeded 90s, the rat was guided to the platform and stayed for 15s, the escape latency was recorded as 90s. Each of the four quadrants was required to serve as the starting position for each rat for daily training for 4 days.

(2) Spatial probe test: The escape platform was removed on the day after positioning navigation test. The rats were placed in water from the contralateral quadrant of the original platform quadrant for a 90s. The number of times rats entered the virtual platform and the time of target quadrant activity were recorded.

Pathological Examination

In order to observe the pathological changes of the hippocampus, the rats in each group were were anesthetized by an intraperitoneal injection of 0.3% pentobarbital sodium (50mg·kg^{-1}) on the day after the MWM test, and the chest was cut to expose the heart and aorta. The needle was inserted into the left ventricle from the apex to the aorta. The right atrial appendage was cut open, and 150mL of 0. 9% sodium chloride at 37°C was slowly injected into the left ventricle until the blood became clear, then 150mL of 4% paraformaldehyde was infused. The brain was taken out and fixed with 4% paraformaldehyde at 4°C for 24 hours. Paraffin sections were stained with HE and Tunel after deparaffinization and hydration. The slices were sealed with neutral gum and observed under a light microscope.

The expression of Fas/FasL was detected by immunohistochemical staining. For immunohistochemical areal density analysis, at least 3 slices in each group were selected and photographed at 200 × field of view. Image-Pro Plus 6.0 software was used to select the same brown color as the unified standard for judging the positivity of all photos, and each photo was analyzed to obtain the cumulative optical density (IOD) value of positivity and the pixel AREA of tissue. The areal density was calculated, and the areal density=IOD/AREA. The higher the value of areal density, the higher the positive expression level.

Statistical Methods

SPSS 20.0 statistical software was used for statistical analysis. Measurement data conforming to normal distribution were represented by ($\overline{x} \pm s$). Food intake, body weight, target quadrant activity time of water maze crossing platform times and

immunohistochemical Fas/FasL surface density values of rats in each group were analyzed by One-way ANOVA. Analysis of variance was used for comparison among multiple groups, LSD-*t* test was used for pair comparison between groups, and repeated measure analysis of variance was used for comparison of escape latency in rats. $P < 0.05$ was considered statistically significant.

Result

General Information

Before modeling, the mental state of each group was good, and there was no obvious difference in appearance and reflexes, and no visual disturbance. There was no significant difference in body weight among the 1-day group ($F = 2.367$, $P = 0.122$), the 7-day group ($F = 1.852$, $P = 0.182$) and the 28-day group ($F = 1.441$, $P = 0.263$) (Figure 1).

Behavioral Test Results

Through MWM experiment, HBO group was compared with aMCI group and CON group in day 1 group, day 7 group and day 28 group. The behavioral changes of rats in each intervention group were observed on day 1, day 7 and day 28 after HBO treatment.

Comparison Between Different Time Groups

1-day group: CON_1 group $aMCI_1$ group HBO_1 group
7-day group: CON_7 group $aMCI_7$ group HBO_7 group
28-day group: CON_{28} group $aMCI_{28}$ group HBO_{28} group

Rats in the 1-Day Group

Escape latency: there was no statistical difference between HBO_1 group and CON_1 group ($P = 0.73$). HBO_1 group was significantly shorter than aMCI1 group ($P < 0.001$).

The number of platform crossing: there was no statistical difference between HBO_1 group and CON_1 group ($P = 0.84$); Compared with aMCI1, the number of crossing platforms in HBO_1 group was significantly increased ($P < 0.05$).

Activity time in the fourth quadrant: there was no significant difference between HBO_1 group and CON_1 group ($P = 0.82$). Compared with $aMCI_1$, the activity time in the fourth quadrant in HBO_1 group increased significantly ($P < 0.05$) (Figure 2).

Rats in the 7-Day Group

Escape latency: there was no statistical difference between HBO_7 group and CON_7 group ($P = 0.704$). HBO_7 group was significantly shorter than aMCI7 group ($P < 0.001$).

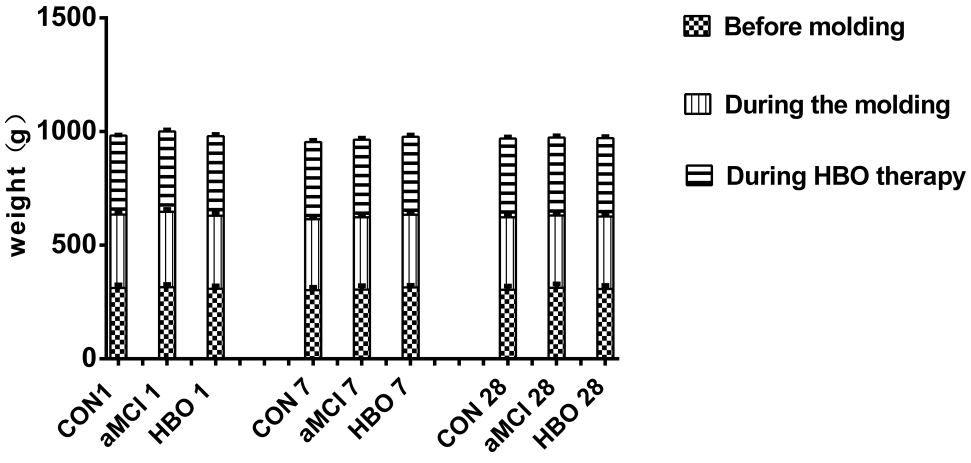

Figure 1 Comparison of body weight in each group ($\overline{X} \pm S$, n=8).

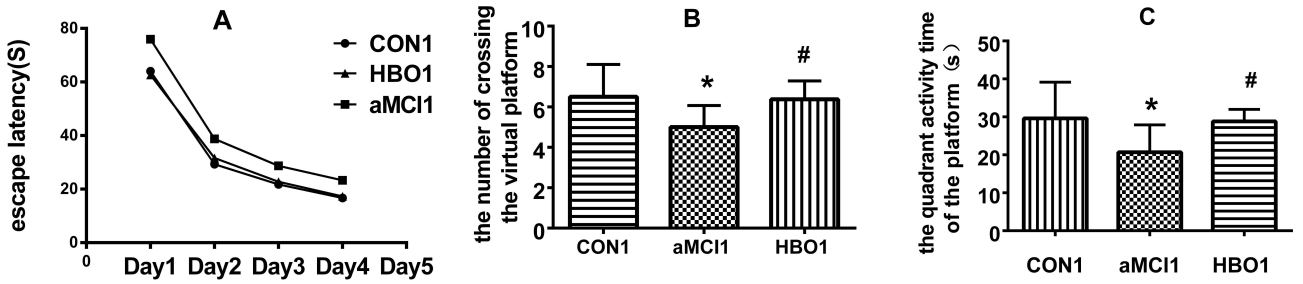

Figure 2 HBO treatment alleviated learning and memory impairment in the 1-day groups. (**A**) Escape latency:Compared with CON1 group, ▪P <0.05; Compared with aMCI1, ▲P <0.05. (**B**)The number of platform crossing: Compared with CON1 group, *P<0.05; Compared with aMCI1, #P<0.05 (**C**) Activity time in the fourth quadrant: Compared with CON1 group, *P<0.05; Compared with aMCI1, #P<0.05.

The number of platform crossing: there was no statistical difference between the HBO_7 group and the CON_7 group (P = 0.635); Compared with $aMCI_7$, the number of crossing the platform in HBO_7 group was significantly increased (P < 0.05).

Activity time in the fourth quadrant: there was no significant difference between HBO_7 group and CON_7 group (P = 0.598); Compared with $aMCI_7$, the activity time in HBO_7 group was significantly extended in the fourth quadrant (P<0.05) (Figure 3).

Rats in the 28-Day Group

Escape latency: there was no statistical difference between HBO_{28} group and CON_{28} group (P = 0.627). HBO_{28} group was significantly shorter than aMCI28 (P < 0.001), and the difference was statistically significant.

The number of crossing the platform: there was no statistical difference between the HBO_{28} group and the CON_{28} group (P = 0.859); Compared with $aMCI_{28}$, the number of crossing the platform in HBO28 group was significantly increased (P < 0.05).

(3) Activity time in the fourth quadrant: there was no significant difference between HBO_{28} group and CON_{28} group (P = 0.775). Compared with $aMCI_{28}$, the activity time of HBO group was significantly extended in the fourth quadrant (P < 0.05) (Figure 4).

Comparison Between Intervention Groups

CON groups: CON_1 CON_7 CON_{28}
aMCI groups: $aMCI_1$ aMC_{I7} $aMCI_{28}$
HBO groups: HBO_1 HBO_7 HBO_{28}

Rats in CON groups:

The escape latency: there was no significant difference between CON_1 group and CON_7 group (P = 0.760). There was no significant difference between CON_7 group and CON_{28} group (P = 0.963).

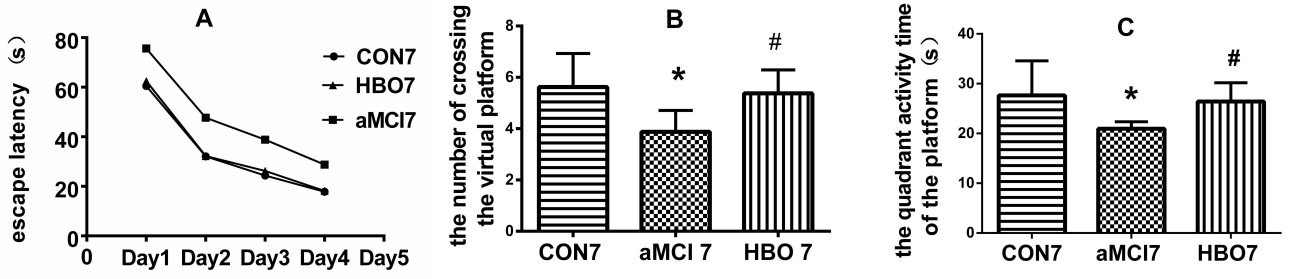

Figure 3 HBO treatment alleviated learning and memory impairment in the 7-day groups. (**A**) Escape latency: Compared with CON_7 group,▪P <0.05; Compared with $aMCI_7$, ▲P <0.05. (**B**) The number of platform crossing: Compared with CON_7 group, *P<0.05; Compared with $aMCI_1$, #P<0.05. (**C**) Activity time in the fourth quadrant: Compared with CON_7 group, *P<0.05; Compared with $aMCI_7$, #P<0.05.

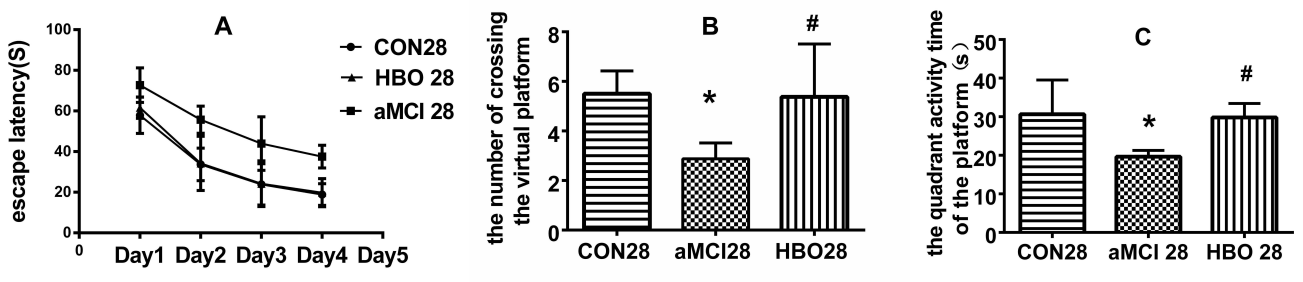

Figure 4 HBO treatment alleviated learning and memory impairment in the 28-day groups. (**A**) Escape latency: Compared with CON_{28} group, ▪P <0.05; Compared with $aMCI_{28}$, ▲P <0.05. (**B**) The number of platform crossing: Compared with CON_{28}group, *P<0.05; Compared with $aMCI_{28}$, #P<0.05. (**C**) Activity time in the fourth quadrant: Compared with CON_{28} group, *P<0.05; Compared with $aMCI_{28}$, #P<0.05.

The number of crossing the platform: there was no statistically significant difference between CON_1 group and CON_7 group (P = 0.195). There was no significant difference between CON_7 group and CON_{28} group (P = 0.85).

Fourth quadrant activity time: there was no significant difference between CON_1 group and CON_7 group (P = 0.651). There was no significant difference between CON_7 group and CON_{28} group (P = 0.494) (Figure 5).

Rats in aMCI Groups

The escape latency: compared with $aMCI_7$ group, $aMCI_1$ group was significant shorter than $aMCI_7$ group, (P < 0.05). Compared with $aMCI_{28}$ group, $aMCI_7$ group was significant shorter than $aMCI_{28}$ group (P < 0.05).

The number of crossing the platform: $aMCI_1$ group was significantly higher than $aMCI_7$ group (P < 0.05). Compared with the $aMCI_{28}$ group, $aMCI_7$ group was significantly higher than $aMCI_{28}$ group (P < 0.05).

The fourth quadrant activity time: compared with the aMC_{I7} group, $aMCI_1$ group was longer than that $aMCI_7$ group, but the difference was not statistically significant (P = 0.896). Compared with $aMCI_{28}$ group, $aMCI_7$ group was longer than $aMCI_{28}$ group, but the difference was not statistically significant (P = 0.549) (Figure 6).

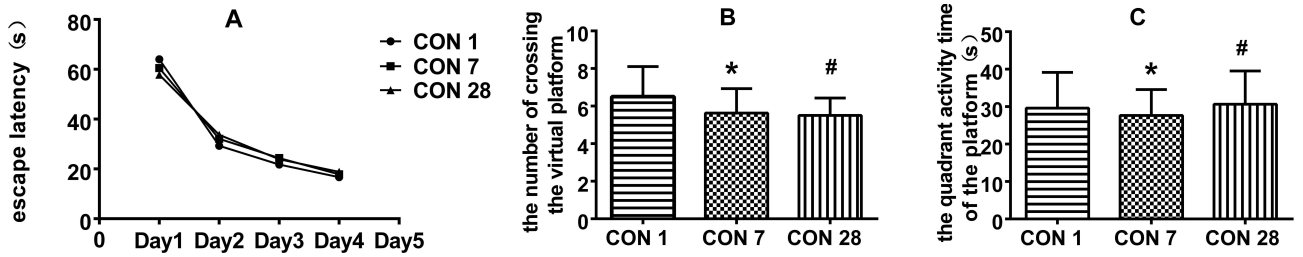

Figure 5 CON group rats: (**A**) Escape latency: Compared with CON_1 group, ▪P >0.05; Compared with CON_7, ▲P >0.05. (**B**) The number of platform crossing: Compared with CON_1group, *P>0.05; Compared with CON_7, #P>0.05. (**C**) Activity time in the fourth quadrant: Compared with CON_1 group, *P>0.05; Compared with CON_7, #P>0.05.

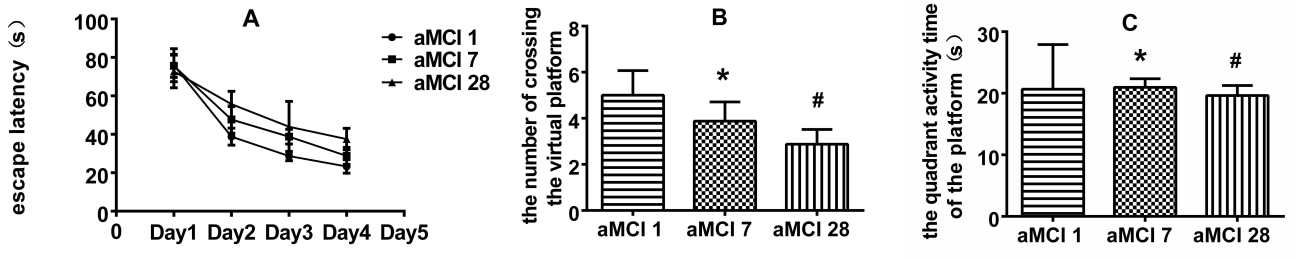

Figure 6 aMCI group rats: (**A**) Escape latency: Compared with $aMCI_1$ group, ▪P <0.050.05; Compared with $aMCI_7$, ▲P <0.05. (**B**) The number of platform crossing: Compared with $aMCI_1$group, *P<0.05; Compared with $aMCI_1$, #P<0.05. (**C**) Activity time in the fourth quadrant: Compared with $aMCI_1$ group, *P>0.05; Compared with $aMCI_7$, #P>0.05.

Rats in HBO Groups

The escape latency: There was no significant difference in escape latency between HBO_1 group and HBO_7 group (P = 0.714). There was no significant difference between HBO7 group and HBO28 group (P = 0.959).

The number of crossing the platform: there was no significant difference between HBO_1 group and HBO_7 group (P = 0.18). There was no significant difference between HBO_7 group and HBO_{28} group (P = 1.0).

The fourth quadrant activity time: there was no significant difference between HBO_1 group and HBO_7 group (P = 0.191). There was no significant difference between HBO_7 group and HBO_{28} group (P = 0.69) (Figure 7).

Pathology and Immunohistochemistry of Rat Hippocampus

HE Staining results of Hippocampal CA1 Region of Rats in Each Group

There were more pyramidal cells in the hippocampal CA1 region of CON group, which were arranged neatly and well. The cell size was uniform, the shape was regular, the structure was clear, the cytoplasm was clear, the nucleus was round and regular, and the nucleolus was clear. In aMCI group, the pyramidal cells in hippocampal CA1 region were less, arranged disorderly, the cell volume was different in size, the shape was extremely irregular, the structure was disorderly, the cytoplasm was concentrated and dark stained or even vacuolated, and the nucleolus was fuzzy. Compared with the aMCI group, the number of pyramidal cells in the hippocampal CA1 region of the HBO group increased, the arrangement was more orderly, the morphology and structure were significantly improved, and the nucleus tended to be normal.

With the passage of time, the number of pyramidal cells in the hippocampal CA1 region of the aMCI groups was gradually reduced, the arrangement was disordered, the cell volume was different, the shape was extremely irregular, and the structure was more disordered. On the 7th day, the cell swelling was obvious, the cytoplasm was concentrated and deformed, and the nucleoli were blurred. On the 28th day, most of the nuclei were reduced, fragmented, and some even disappeared. The cell volume in the hippocampal CA1 region of the HBO groups was relatively uniform, and the cytoplasm of a small number of pyramidal cells was concentrated, dark and deformed, and the nucleus was eccentric distributed, which did not show a trend of aggravation over time. There was no significant change in cell morphology in the CA1 region of hippocampus in CON groups (Figure 8).

Detection of Apoptosis by TUNEL Staining

There was no obvious apoptosis in the hippocampal CA1 region of the CON group, but apoptotic cells were observed in the aMCI group, and a small number of apoptotic cells were scattered in the hippocampal CA1 region of the HBO group (Figure 9).

Immunohistochemical Detection of Nerve Cell Membrane Molecules Fas/FasL Expression Results

There were significant differences between the aMCI groups and the CON and HBO groups on the 1st, 7th and 28th day. At 1 day (P < 0.001), 7 days (P < 0.001) and 28 days (P < 0.001), the aMCI group had more positive cells than the CON group and the HBO group (Figure 10).

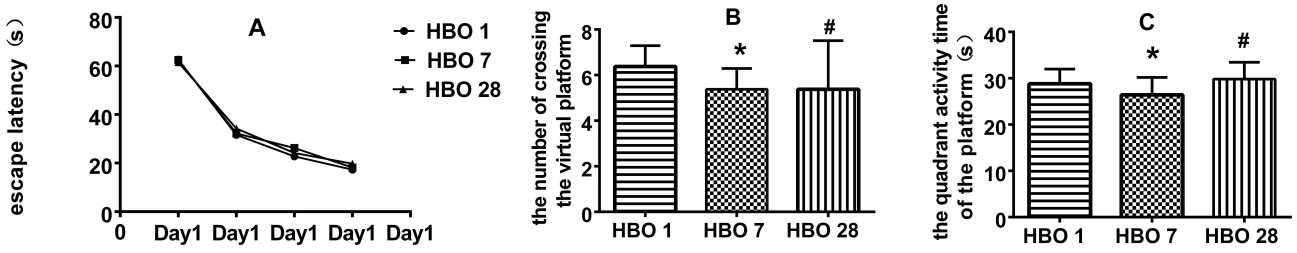

Figure 7 HBO group rats: (**A**) Escape latency: Compared with HBO_1 group, ▪P >0.05; Compared with HBO_7, ▲P >0.05. (**B**) The number of platform crossing: Compared with HBO 1 group, *P>0.05; Compared with HBO_7, #P>0.05. (**C**) Activity time in the fourth quadrant: Compared with HBO_1 group, *P>0.05; Compared with HBO_7, #P>0.05.

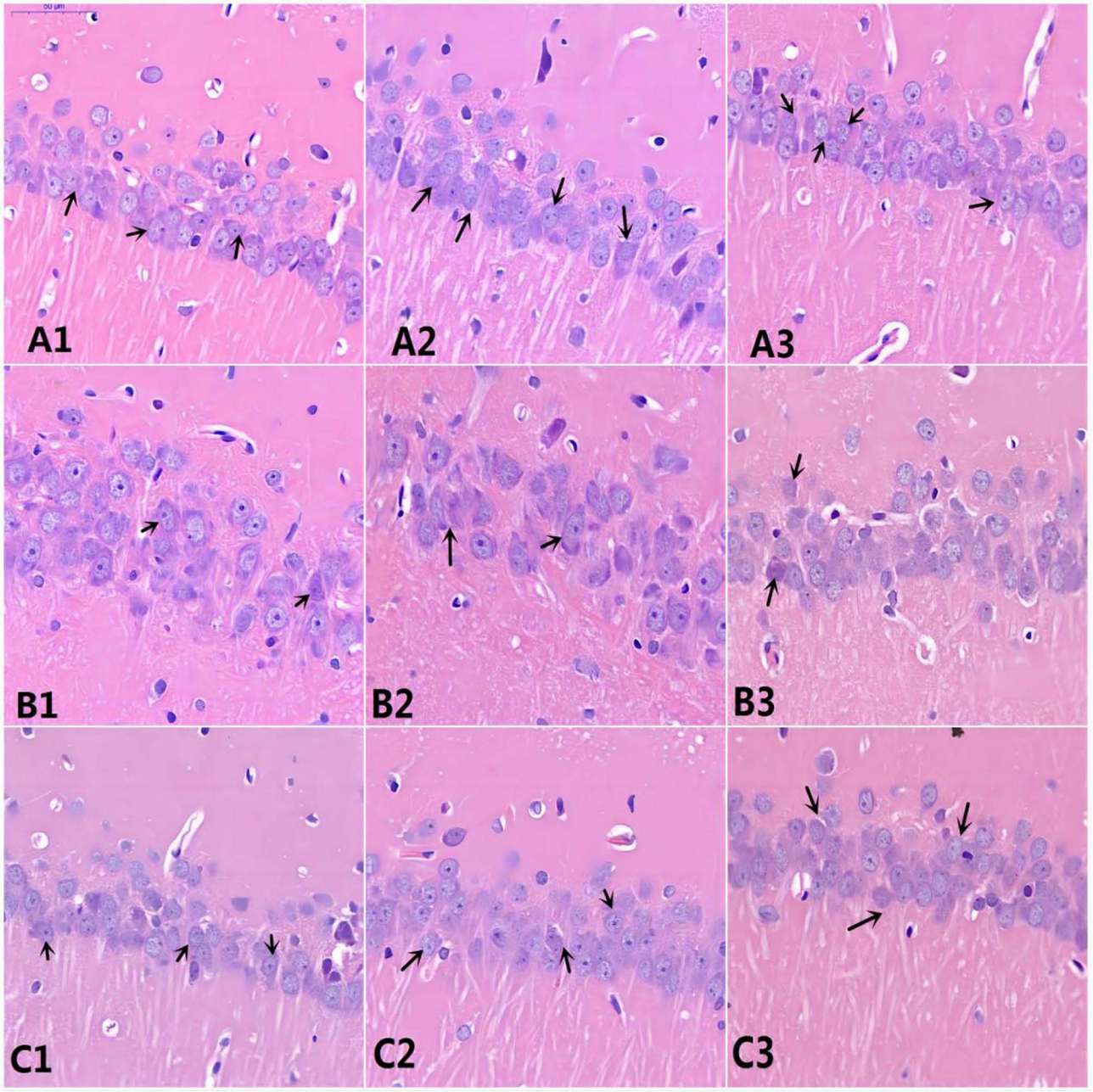

Figure 8 Results of HE staining in hippocampus CA1 region of rats in each group (HE staining x 400) ↑: pyramidal cells. A1:CON1, A2: CON7, A3:CON_{28}, normal pathological appearance. B1:$aMCI_1$, B2: $aMCI_7$, B3: $aMCI_{28}$, pathological damage was obvious and had a trend of aggravation. C1:HBO_1, C2 HBO_7, C3: HBO_{28}, pathological damage was reduced.

There was no significant difference in the surface density of Fas and FasL among the CON group and the HBO group ($P > 0.05$). The expression of Fas/FasL on the surface of the nerve cells in the aMCI group increased gradually over time, and the difference was statistically significant ($P < 0.05$) (Figure 11).

Discussion

This study demonstrated that the learning and memory function of aMCI rats was significantly improved after HBO treatment, as indicated by behavioral experiments, hippocampal pathological changes, and immunohistochemical changes. The behavioral and pathological damages of aMC model rats was significantly recovered after HBO treatment,

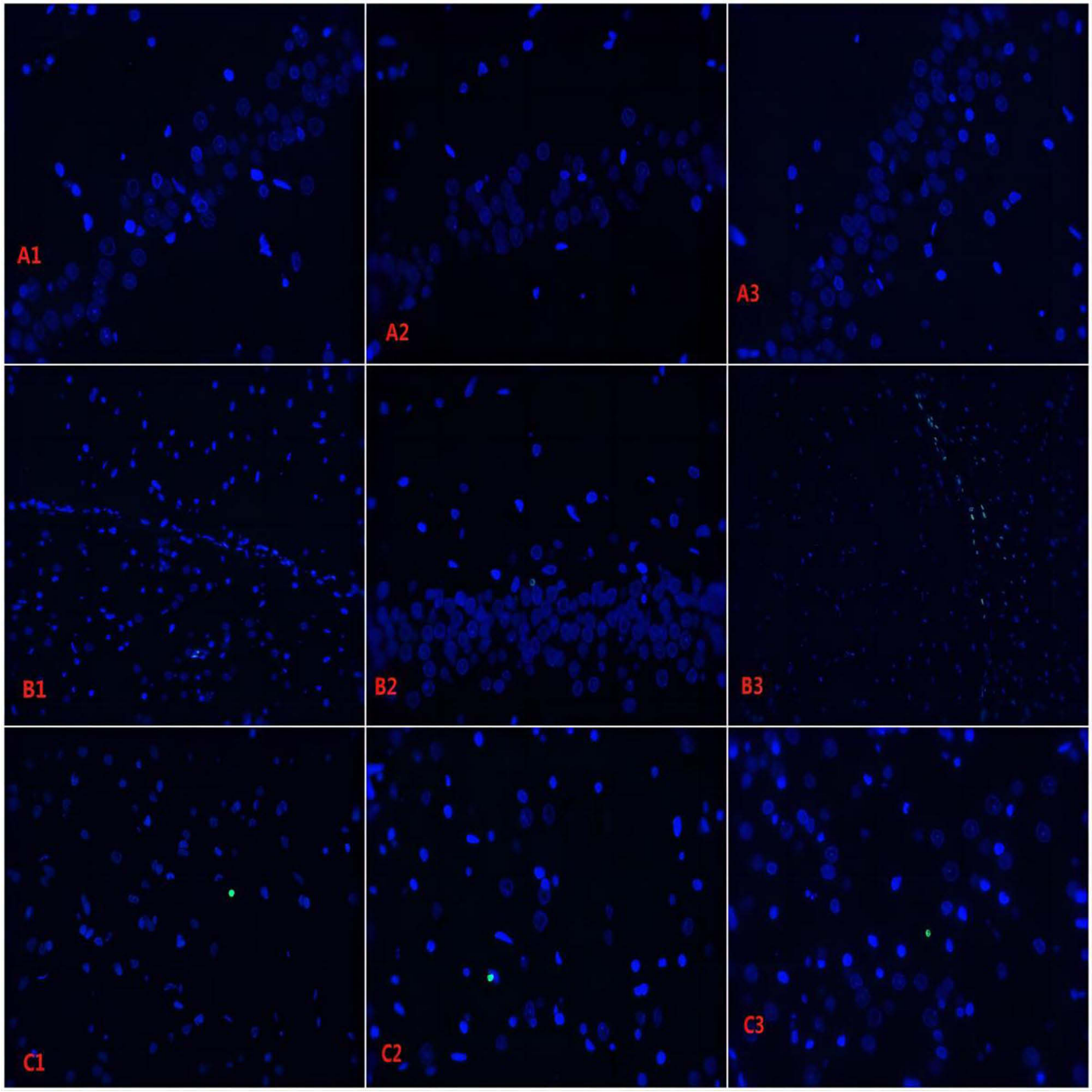

Figure 9 Comparison of TUNEL detection in the hippocampus of rats in each group (TUNEL staining, x 400). A1:CON_1, A2: CON_7, A3:CON_{28}, no obvious apoptosis was observed. B1:$aMCI_1$, B2: $aMCI_7$, B3: $aMCI_{28}$, a large number of apoptotic cells were observed. C1:HBO_1, C2: HBO_7, C3: HBO_{28}, a small number of apoptotic cells were scattered.

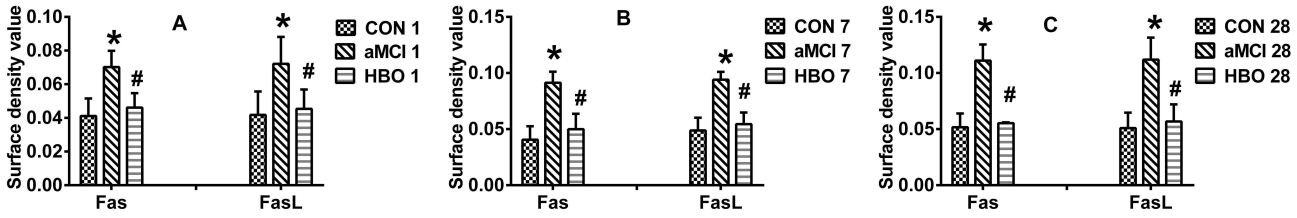

Figure 10 Fas/FasL expression in each group: (**A**) 1 day group: Compared with CON_1group, *P <0.05; Compared with $aMCI_1$, #P <0.05. (**B**) 7day group: Compared with CON_7group, *P<0.05; Compared with $aMCI_7$, #P<0.05. (**C**) 28day group: Compared with CON_{28}group, *P<0.05; Compared with $aMCI_{28}$, #P<0.05.

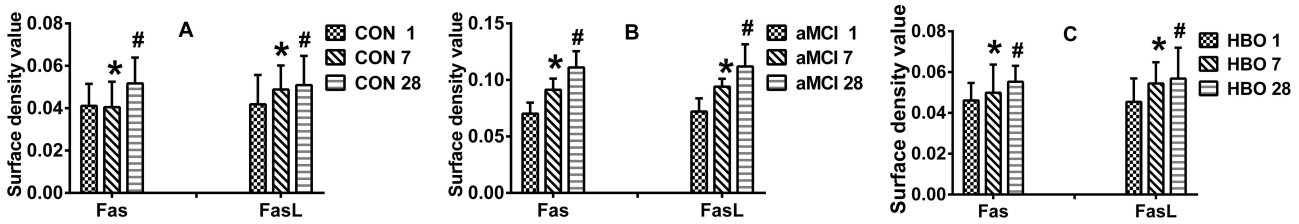

Figure 11 Fas/FasL expression in each group: (**A**) CON group: Compared with CON_1group, *P >0.05; Compared with CON_7, #P <0.05. (**B**) aMCI group: Compared with $aMCI_1$ group, *P<0.05; Compared with $aMCI_7$, #P<0.05. (**C**) Compared with HBO_1group, *P>0.05; Compared with HBO_7, #P>0.05.

and the improvement state could be maintained for a long time. The expression of Fas/FasL on the membrane of nerve cells in the HBO group was significantly lower than that in the aMC group, suggesting that Fas/FasL mediated cell receptor death pathway is involved in the apoptosis of hippocampal nerve cells in aMCI rats. HBO played a protective role in aMCI rats by regulating the neuronal apoptosis via the cell receptor death pathway.

Efficacy Analysis of HBO in the Treatment of aMCI

HBO has neuroprotective effects. It is widely used in the treatment of spinal cord injury, traumatic brain injury sequelae, carbon monoxide poisoning and other nerve injury diseases.[18–20] Current studies mainly focus on the effectiveness of HBO by improving oxygen partial pressure, reducing brain edema, reducing inflammatory factors, clearing free radicals, anti-apoptosis and anti-cholinergic effects.[21] The effect and mechanism of HBO on cognitive decline and hippocampal dysfunction are not fully understood. This study found that the behavioral test, hippocampal pathological changes and immunohistochemical changes at each time point after treatment were significantly better in HBO group than in aMCI group. With the passage of time, the water maze test and hippocampal pathological changes in the aMCI group showed that the degree of cognitive dysfunction became more and more serious, while the HBO group did not change significantly. It is consistent with the conclusion of clinical study by Liu Yunan et al[22] that HBO can significantly improve the cognitive function of elderly patients with aMCI, and the curative effect lasts for a long time. HBO has definite long-term effects, and can significantly improve the learning and memory function of aMCI rats.

Investigation of the Pathogenesis of aMCI

The pathogenesis of aMCI is closely related to neuronal apoptosis caused by β-amyloid (Aβ) and tau protein. Aβ is the main component of senile plaques in the cerebral cortex, and Aβ is also formed in the hippocampus of aMCI rats. Free radicals and reactive oxygen species produced by Aβ damage membrane lipids and cause neuronal apoptosis. Excessive deposition of Aβ leads to neuronal apoptosis in the hippocampus and impairs the memory and learning ability of the rat model.[23] Aβ stimulates oxidative stress and inflammation, leading to the death of neurons in the hippocampus, thalamus, and cerebellum and forming cognitive and behavioral disorders.[24] Abnormal phosphorylation of tau protein can cause nerve fiber entanglement, and abnormally high phosphorylation of tau protein can lead to structural changes of nerve cells, loss of function and neuronal apoptosis.[25] Studies have shown that the levels of tau protein and Aβ42 protein in the cerebrospinal fluid of aMCI patients are changed, and these pathological changes can trigger A series of reactions to the nervous system, such as inflammatory response, oxidative stress and apoptosis.[26] The hippocampal CA1 region is closely related to spatial discrimination learning and memory function, and the damage of the hippocampal region can lead to background memory and spatial memory ability.[27] Studies related to cognitive dysfunction with the decline of learning and memory ability as the main symptom[28] have found that there are apoptotic neurons in the hippocampus, and the expression of Fas and FasL on the neurons is up-regulated. Fas/FasL mediated death receptor pathway is one of the most important pathways in cell apoptosis. Fas belongs to type I membrane protein, and its ligand FasL belongs to a type II membrane protein on the cell surface. FasL can bind to Fas, leading to the formation of activated trimer in the death region of Fas cells, and then recruit the adaptor protein Fas associated death domain (FADD), leading to the conformational change of FADD. In this case, FADD can bind to Caspase-8 with its own death effector domain (DED) in the manner of DED-DED and then activate Caspase-8 to form FasL-Fas-FAD-Caspase-8 death inducing signaling complex (DISC). After the formation of DISC, it further activates and initiates its downstream caspase-related protein cascade, which eventually leads to cell apoptosis.[29,30]

Mechanism of Apoptosis in aMCI Treated with HBO

Neuronal cell loss or dysfunction can lead to cognitive decline, and neuronal apoptosis is one of the important reasons. HBO can inhibit the apoptotic process of cells, regulate the apoptosis of nerve cells and protect mitochondrial function by inhibiting the expression of apoptosis-related genes, and improve cognitive and motor impairment, so as to protect nerve cells.[31,32] Studies on neuronal apoptosis damage have shown that HBO can significantly increase the expression of anti-apoptotic proteins Bcl-2 and glial fibrillary acidic protein, reduce the expression of pro-apoptotic protein Bax, and then reduce the formation of caspase-3, thereby reducing neuronal apoptosis.[33] It is verified that the mitochondrial apoptosis pathway is involved in the apoptosis of cognitive dysfunction treated by HBO, but whether the cell death receptor pathway is involved in the apoptosis mechanism of cognitive dysfunction treated by HBO is not clear. Cutting off the neuronal apoptosis pathway is also one of the key points to reduce nerve function damage. Reducing the expression of fas/fasL in nerve cells is one of the methods to block the cell death pathway. In the experiment, the expression of fas/fasL on the surface of hippocampal nerve cells in the aMCI group was significantly higher than that in the CON group and the HBO group, and the expression intensity of fas/fasL on the surface of hippocampal nerve cells was positively correlated with the degree of mild cognitive impairment. Some studies[34,35] have shown that HBO can reduce the expression of Fas/FasL on the surface of nerve cells, reduce cell apoptosis after spinal cord injury in rats, and facilitate the recovery of spinal cord function, which is consistent with the results of this study. It was confirmed that Fas/FasL mediated cell receptor death pathway was involved in the physiological and pathological process of hippocampal neuronal apoptosis. In addition, HBO may also reduce oxidative stress to nerve cells by increasing the activity of antioxidants, and further protect the cells from apoptosis.

Limitations and shortcomings of the experiment

1. The sample size of each group in this experiment is limited, and it may affect the results of the minor changes.
2. In the case of HBO treatment, the experiment was not conducted in the water maze before treatment, but compared with the control group, the model group and the treatment group only.
3. There are various mechanisms of cognitive dysfunction, which is only a single effect of the apoptosis mechanism on the cognitive function of the model rats, and fails to illustrate the impact of other mechanisms.
4. We only explored the apoptotic pathway preliminary, and did not use the agonist or inhibitor to further specify.

Conclusion

1. HBO can improve the learning and memory impairment in aMCI rats, and maintain for a long time.
2. HBO was used to Attenuate the apoptosis of the hippocampal nerve cells in the aMCI rats.
3. Fas/FasL mediated cell receptor death pathway was involved in the apoptosis of hippocampal neurons.

In conclusion, HBO had improved the learning and memory impairment of the aMCI model Rats, and maintained for a long time. Reducing the apoptosis of neuronal cells in the hippocampus was one of the mechanisms. The fas and fasl-mediated death receptor pathway was involved in the apoptosis process. HBO may improve the cognitive impairment of aMCI rats by inhibiting cell apoptosis. Future research can be carried out in several ways, such as the impact of HBO on the expression of the related genes of the apoptosis, and the effect on the mitochondrial function of nerve cells, and the use of an agonist or an inhibitor to further clarify the apoptotic pathway. At the same time, more large-scale randomized controlled trials are needed to assess the practical effect and application value of HBO in aMCI. Through these research, the treatment of HBO is expected to provide more effective methods and strategies.

Disclosure

The authors report no conflicts of interest in this work.

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