Effect of donepezil hydrochloride & aerobic exercise training on learning and memory and its mechanism of action in an Alzheimer's disease rat model

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Abstract: This study was designed to explore the combined effect of donepezil hydrochloride and aerobic exercise training on learning and memory and to check its mechanism of action in an Alzheimer's disease (AD). Thirty rats were randomly divided into normal control group, model group and donepezil hydrochloride combined with aerobic exercise training group (n=10). Amyloid β-protein (Aβ1-40) was injected into rats to establish an elderly AD model. After 4 weeks of administration, changes in the spatial learning and memory of rats were tested in the Morris water maze. Choline acetyltransferase (ChAT) and acetylcholinesterase (AchE) in the hippocampus and cerebral cortex were measured by colorimetry. Compared with the normal control group, rats in the model group took more time to find the platform (P<0.001) and showed a longer swimming path (P<0.001). Rats in the intervention group took less time to find the platform (P<0.001) and showed a shorter swimming path (P<0.001) in comparison to the model group. Similarly, rats in the model group showed decreased ChAT activity (P<0.001) and increased AchE activity (P<0.001) in the hippocampus and cortex as compare to the normal control group. Compared with the model group, rats in the intervention group showed increased ChAT activity (P<0.001) and decreased AchE activity (P<0.001) in the hippocampus and cortex. The study concluded that Donepezil hydrochloride combined with aerobic exercise training improved the learning and memory function of rats with AD. The mechanism was also related to improved morphological structure of hippocampal neurons, reduced loss of neuronal cells, increased ChAT content and decreased AchE content.

Keywords: Donepezil hydrochloride, Aerobic exercise, Alzheimer's disease

INTRODUCTION

Senile dementia, also known as AD is a central nervous system disorder with insidious onset, slow and progressive course of disease and commonly seen in the early stage of aging (Chai *et al*., 2016; Harrison and Bookheimer, 2016). The main clinical manifestations of AD patients are significant declines in memory and cognition and the gradual loss of self-care ability. Severe cases are associated with mental and behavioural symptoms at the nervous level (Lacour *et al*., 2017; Warrier *et al*., 2016).The incidence of AD gradually increases with age and has become the fourth leading cause of death in the elderly following coronary heart disease, cancer, and stroke (Louwersheimer *et al*., 2016; Hu *et al*., 2016). Therefore, the study of AD is not only a major medical problem but also a social issue. Donepezil hydrochloride is an important clinical drug for the treatment of AD (Nakano *et al*., 2001). A study believed that donepezil hydrochloride can effectively improve the clinical symptoms of AD (Mehta *et al*., 2012). The importance of physical activity to maintain healthy psychological functions is widely known. Prior studies have suggested that aerobic training can improve cognitive performance of elderly patients with senile dementia of Alzheimer type (Palleschi *et al*., 1996). However, there are few studies about the effect of donepezil hydrochloride combined with aerobic exercise training on learning and memory and its mechanism of action in the AD rat model. Thus the present study was conducted to find out the joint effect of donepezil hydrochloride and aerobic exercise training over learning and memory and its mechanism of action in an AD rat model.

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MATERIALS AND METHODS

Research animals

The research was conducted from November 2016 to December 2017. Thirty healthy male Wistar rats were selected as the subjects of the study. The weight of the rats ranged between 220~260g and the average weight was (236.82±11.53g). The rats were randomly divided into a normal control group, a model group and an intervention group. Each group contained 10 rats. After grouping, the rats were given 3 days to adapt. Amyloid β-protein ($A\beta_{1-40}$) was injected into the brain of these rats to establish an elderly AD model.

The specific modelling process

This method was adopted from Lu *et al*, 2015. Briefly described here as, $A\beta_{1-40}$ was dissolved in sterile normal

saline (1g/L) and was incubated at 37°C before use. After anaesthesia with 10% chloral hydrate (3mL/kg/ip), the anesthetized rat was fixed on a stereotaxic apparatus and the hair on the top of the head was cut off. After sterilization, the scalp was cut along the midline of the head's top (sagittal incision). Then the subcutaneous tissues were separated to fully expose the anterior fontanelle and the surrounding skull. Finally, the skull was drilled through and the dura mater was opened with the injection needle. The dentate granule cell layer of the right hippocampus was selected as the injection area. The coordinates were 3.3mm behind the anterior fontanelle, cutting a 2.0mm incision toward the right with a depth of 3.0mm. The gap between the incisors was 2.4mm lower than the line between the ears horizontally. $A\beta_{1-40}$ 1μL was slowly injected through a micro injector within five minutes. After the injection, the needle was retained for five minutes. Then the micro injector was slowly pulled out and the wound was sealed with root canal sealers. Finally, the scalp was sutured and the rat was put back to the rearing cage. Penicillin 200,000U/rat was given after the operation for total three days at 1time /day in order to prevent infection. The normal control group was free from treatment.

After three days of modelling, normal saline and donepezil hydrochloride were intragastrically administered. The donepezil hydrochloride drug was prepared with sterile double-distilled water. Rats in the intervention group were intragastrically administered with donepezil hydrochloride 10mL/kg each time once a day along with aerobic training. The specific method of aerobic training was as follows: On the third day after $A\beta_{25-35}$ injection, when Aβ toxicity was strongest, aerobic training (non-loaded swimming) was performed. Swimming training was started since the day of the experiment. Time for each exercise was increased 10min per day from the first 10min/day up to 60min/day which was continued for a total of four weeks of training. The water temperature was 30±1°C and the swimming training pool was 180cm × 60cm × 80cm with smooth inner wall. The normal control group and the model group were intragastrically administered with a corresponding volume of double-distilled water each day.

After 4 weeks of administration, the learning and memory of each group of rats were tested 2 times a day for 4 days in total. During each exercise, the rat entered the pool through the quadrant opposite or adjacent to the quadrant with the platform. The rat was put into the water at the intersection of the wall of the pool and the midline of the quadrant with the head of the rat towards the wall. If the rat stood on the platform and didn't slide off within 3seconds, then the task was suspended. The time the rat reached the platform plus the length of the path the rat travelled, were recorded and the rat were allowed to stay on the platform for 10seconds. Rats who failed to find the platform within 90seconds were led to the platform and placed on the platform for 10seconds to guide them to learn and remember. The average time that rats took to find the platform and the average length of swimming paths on the 4th day were used to assess the learning and memory of these rats.

One hour after the Morris test, the animals were sacrificed by decapitation. The brains were quickly took out and placed on an ice box. The cerebrum was taken out to separate the left hippocampus and cortex. The brain tissues were weighed and normal saline was added to achieve the weight to volume ratio of 1:10.

ChAT activity

Centrifugation was carried out for 10min at 4°C with 3500 RPM and the supernatant was taken to measure immediately. Colorimetry was used to determine ChAT and AchE activity in rat hippocampus and cerebral cortex. Acetyl coenzyme A and choline were used as the substrates to measure the ChAT activity. Under the action of ChAT, the products of the reaction and the chromogenic reagent combined and the absorbance was measured at 324nm to calculate the ChAT activity. AchE hydrolysed acetylcholine into choline and acetic acid. Choline reacted with sulfhydryl chromogenic reagent and produced a yellow compound INB (1,3,5-trinitrobenzene). This compound was calorimetrically quantified according to the shades of the colour. The amount of choline in the hydrolysate could reflect the AchE activity.

Pathological observation of hippocampus

After the rats were decapitated, the brain was quickly placed on the frozen operating table. The hippocampus tissue was removed from one side and fixed with 40g/L paraformaldehyde for 2 to 5 days. Conventional paraffin sections about 5μm thick were HE stained to observe the morphological changes of neurons in the hippocampal CA1 area.

Ethical approval

Ethical approval was taken by Weiman Central Hospital through License No. 2016010302.

STATISTICAL ANALYSIS

SPSS 23.0 statistical software package was used for data analysis. Measurement data were represented by $\bar{x} \pm s$. One-way analysis of variance was used for comparison among groups and the SNK test was used for comparison between groups. $P<0.05$ was considered statistically significant.

RESULTS

Observation results of neuronal cells in the hippocampal CA1 region of rats with AD in each group

Fig. 1 showed the structure of the hippocampal CA1

region of each group observed under light microscope: in the control group, the pyramidal cells were arranged closely with clear cell structure and the size was consistent. Compared with the control group, the pyramidal cells in the model group were disorderly arranged, the structure was not clear and the cell loss was obvious. Compared with the model group, the morphological structure of the pyramidal cells in the intervention group was basically normal with less cell loss.

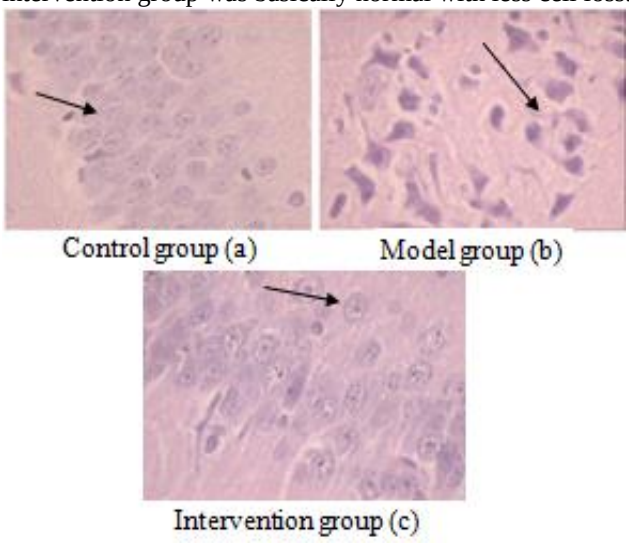

Fig. 1: Morphological changes of neurons in hippocampal CA1 area of each group of rats (HE staining×400)

Comparison of spatial learning and memory of each group of rats in the morris task

After 4 weeks of administration, there were significant differences among the three groups in the time rats took to find the platform and the length of swimming paths (both P<0.001). Compared with the normal control group, rats in the model group took more time to find the platform (P<0.001) and showed a longer swimming path (P<0.001). Compared with the model group, rats in the intervention group took less time to find the platform (P<0.001) and showed a shorter swimming path (P<0.001) as shown in table 1.

Comparison of ChAT Activity in the Hippocampus and Cortex of Rats in Each Group

After 4weeks of administration, there were significant differences in the hippocampus and cortex ChAT activity among the three groups (both P<0.001). Compared with the normal control group, rats in the model group showed decreased ChAT activity in the hippocampus and cortex (both P<0.001). Compared with the model group, rats in the intervention group showed increased ChAT activity in the hippocampus and cortex (both P<0.001) mentioned in table 2.

Comparison of AchE Activity in the Hippocampus and Cortex of Rats in Each Group

After 4weeks of administration, there were significant differences in the hippocampus and cortex AchE activity among the three groups (both P<0.001). Compared with the normal control group, rats in the model group showed increased AchE activity in the hippocampus and cortex (both P<0.001). Compared with the model group, rats in the intervention group showed decreased AchE activity in the hippocampus and cortex (both P<0.001) as given in Table 3.

DISCUSSION

AD starts insidiously and develops gradually. It is a chronic progressive disease that is mainly based on intellectual impairment. The exact cause and pathogenesis of AD are not clear yet (Burns, 2000). Most researchers believe that acetylcholine deficiency is an important cause of dementia. Cholinesterase inhibitors can improve the brain cholinergic system functions in patients with dementia and effectively improve their cognitive impairment (Ceravolo *et al.*, 2006; Sobów and Kłoszewska, 2007). Donepezil hydrochloride is a highly selective AchE inhibitor whose efficacy and adverse reactions have been described several times in the literature but there are few reports on its mechanism of action (Sampson *et al*., 2007; Greene *et al*., 2000). In this study, Aβ1-40 intracerebral injection was used to establish an elderly AD model. The results of the study showed that spatial memory and intracerebral ChAT activity of model animals were both significantly decreased which was very similar to the actual situation of clinical patients. It suggested that the neurotoxicity of Aβ1-40 damaged cholinergic neurons that results in a dysfunction of the central cholinergic system, a decrease in ChAT activity, a decrease in the synthesis of acetylcholine in the brain and a decrease in metabolic conversion rate thereby causing a decline in learning and memory (Blokland and Jolles, 1993; Provost *et al*., 1999).

Loss of cholinergic neurons in the brain is one of the most important pathological features of AD (Zheng *et al*., 2002). The loss of cholinergic neurons leads to the decline of acetylcholine, a neurotransmitter closely related to learning and memory in the brain. It is an important mechanism of learning and memory impairment in AD (Lapchak *et al*., 1991). ChAT is a synthetase of ACh (acetylcholine) and can promote the production of ACh by catalysing the reaction of acetyl-CoA and choline. Changes in ChAT activity can indirectly reflect changes in the synthetic rate of ACh in the brain (Gnahn *et al*., 1983). AchE is a hydrolase of ACh and can promote the production of choline and acetic acid by catalysing the decomposition of ACh. Changes in AchE activity can indirectly reflect changes in the decomposition rate of ACh in the brain (Neves *et al*., 2017). It can be said that the activity of ChAT and AchE is closely related to the content of ACh in the brain. They jointly maintain the dynamic balance of ACh content (Thome *et al*., 1997).

Table 1: Comparison of spatial learning and memory of each group of rats in the morris task (n=10)

Groups	Time to find the platform (s)		Length of swimming paths (cm)	
	Mean ±SD	p-value	Mean ±SD	p-value
Normal control group	8.04±1.27	<0.001	102.73±25.56	<0.001
Model group	14.15±3.63		170.14±46.09	
Intervention group	9.48±2.21		115.62±30.45	

Table 2: Comparison of chat activity in the hippocampus and cortex of rats in each group (n=10)

Groups	ChAT activity in the hippocampus (U/mg)		ChAT activity in the cortex (U/mg)	
	Mean ±SD	p-value	Mean ±SD	p-value
Normal control group	304.83±46.25	<0.001	291.31±41.27	<0.001
Model group	235.76±31.13		223.78±32.05	
Intervention group	271.14±27.28		264.95±23.01	

Table 3: Comparison of ache activity in the hippocampus and cortex of rats in each group (n=10)

Groups	AchE activity in the hippocampus (U/mg)		AchE activity in the cortex (U/mg)	
	Mean ±SD	p-value	Mean ±SD	p-value
Normal control group	0.445±0.037	<0.001	0.403±0.028	<0.001
Model group	0.614±0.052		0.597±0.034	
Intervention group	0.483±0.041		0.451±0.016	

Studies have shown that learning and memory decline is related to hippocampal mass loss, neuron number reduction and neuronal synaptic plasticity change. The lesions in the hippocampus of patients with AD tended to be earlier than those in the cerebral cortex and got worse as the disease progressed. The results of the present research showed that the hippocampus of the model group was blurred, the cell boundary was unclear and the number of neurons was decreased. The hippocampus structure in the intervention group was significantly improved with clear outline, the morphological structure of the pyramidal cells returned to normal and the cell loss was reduced. It suggested that donepezil hydrochloride and aerobic exercise training have protective effects on injured hippocampal neurons and can treat AD by reducing apoptosis of neuronal cells.

The research showed that after donepezil hydrochloride combined with aerobic exercise training treatment, the time that the AD rats took to find the platform and the length of swimming paths were both shortened. It suggested that donepezil hydrochloride combined with aerobic exercise training could significantly improve the learning and memory of AD rats.

The results of this study showed that donepezil hydrochloride combined with aerobic exercise training increased ChAT activity in the hippocampus and cortex of AD model rats, and inhibited AchE activity, thereby increasing the content of acetylcholine in the hippocampus and cortex and playing a certain role in the improvement of learning and memory in AD rats.

CONCLUSION

In summary, donepezil hydrochloride combined with aerobic exercise training improved the learning and memory function of rats with AD. The mechanism improved morphological structure of hippocampal neurons, reduced loss of neuronal cells, increased ChAT content and decreased AchE content in the AD model rats.

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