

Effect of Donepezil Hydrochloride on Cognitive Function Recovery of Rats With Alzheimer's Disease

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ABSTRACT

Objective: To explore the effect of donepezil hydrochloride on cognitive function recovery of rat with Alzheimer's disease and the positive expression of nitric oxide synthase and acetylcholinesterase (NOS and AchE) in the brain tissues of rats with Alzheimer's disease (AD).

Methods: Forty SD (Sprague Dawley) rats were randomly classified into a model group, a treatment group, a control group, and a sham operation group. Each group had 10 rats. AD rat models were prepared with Aβ1-40. Donepezil hydrochloride was orally administered to rats in the treatment group two weeks after modeling. Rat recognition function was assessed. The positive expression of NOS and AchE in rat brain tissues was observed via immunohistochemistry after administration of 28 days.

Results: Rat escape latency was significantly longer after modeling ($p < 0.010$). Compared to the model group, treatment group escape latency was significantly shortened and the difference was significant ($p < 0.05$). NOS and AchE positive expression mainly reflects in hippocampus region tissue. Compared with the control, and sham, operation groups, NOS positive expression decreased and AchE positive expression increased in the model group. The difference was statistically significant ($p < 0.01$). Compared with the model group, NOS expression in the treatment group increased and AchE expression decreased. The difference was significant ($p < 0.01$).

Conclusion: Donepezil hydrochloride can promote AD rat cognitive

function recovery. Its effects are also associated with increased NOS protein expression in hippocampus neuron cells in addition to specifically inhibiting the AchE activities.

Key Words: Donepezil hydrochloride; Rats; Alzheimer's disease

1 INTRODUCTION

Alzheimer's disease (AD) is a degenerative neurosis characterized mainly by cognitive and memorial dysfunctions. It is a common dementia among the elderly. AD is now the fourth most common elderly disease that causes death after cancer, heart and cerebrovascular diseases. It seriously reduces patient quality of life and is a heavy burden families and society. The association of learning and memory impairment of AD patients with the brain cholinergic dysfunction is known. Donepezil hydrochloride is a selective inhibitor of cholinesterase.

A large number of clinical studies proved that Donepezil can significantly improve AD patient cognitive and memory functions and it was considered an effective drug for treat AD patients^[1-2]. Therefore, it is of an important significance to positively explore the possible functional mechanism of donepezil hydrochloride except to inhibit the cholinesterase.

In this study, AD model rats were the research subject to study the effect of donepezil hydrochloride on NOS protein expression in hippocampal neurons and its effect on positive AchE expression in AD model rat brain tissue. The results are reported as follows.

2 MATERIALS

2.1 Experimental Animals

40 SD male rats, weight 200 ± 20 g from the Hunan SJA Laboratory Animal Co., Ltd., holding license numbered: SCXK (Hunan) 2009-0004.

2.2 Experimental Drug

Donepezil hydrochloride tablets (Shaanxi Ark Pharmaceutical Co., Ltd., National medicine permission number: H20030583). Daily dosage (5 mg) for 60 kg adult, the equivalent dosage for rats

was 0.5 mg/kg. It was prepared as a suspension of 0.05 mg/mL with the distilled water.

2.3 Reagents and Instrument

Amyloid β -protein 1-40 (A β 1-40), NOS, and AchE polyclonal antibodies purchased from Beijing Bioss Biotechnology Co., Ltd; PV9000 kit and DAB chromogenic agent were purchased from Beijing ZSGB Biotechnology Co., Ltd.; Morris water maze and brain stereotaxic apparatus (Shenzhen RWD Life Science Co., Ltd); Finesse 325 paraffin slicing machine (British Shando Corporation); B x 51 optical microscope and IPP6.0 image analysis system (Japanese Olympus Corporation).

3 METHODS

3.1 Animal Grouping and Administration

All rats were fed for one week. They were then divided into a control group, sham operation group, model group, and a treatment group (model + Donepezil hydrochloride). There were 10 rats in each group. The treatment group was administered orally with a donepezil hydrochloride suspension of 0.5 mg/kg two weeks after the modeling. The sham operation, and model, groups were administered normal saline. The control group was fed normally. They were administered once a day, for 28 continuous days.

3.2 Model Preparation (Modeling)

AD rat models were prepared with A β 1-40 in References^[3]. It was anesthetized with 10 % chloral hydrate (3 mL/kg) and fixed on the stereotaxic apparatus. A scalpel cut an incision of 1.5cm long along the anterior median line of the disinfected area. The bregma was located and adjusted and orient it with the stereotaxic apparatus to ensure the syringe needle would be aligned 2 mm on the right side of the bregma and 3.5 mm behind the bregma. This is the drill point. Drill and open the skull, slowly push the syringe needle at a speed of 0.3 mm/min to 2.7 mm into the skull at precisely the hippocampus. A β 1-40 was diluted into 5 μ g/ μ L with sterile normal saline and cultivated at 37°C to aggregate it. A β 1-40 of 5 μ g/ μ L was slowly injected to the rat with a microinjector of 1 μ L at the injection speed of approximately 0.1 μ L/min. Upon completing the injection, the needle was removed at a speed of 0.3 mm/min and the skull sealed with dental mud and

skin suturing. After completing the operation, rat was subject to a conventional feeding and intramuscular injection of penicillin against infection. 1uL of normal saline was injected into the hippocampus of rat in the sham operation group and the operation method was identical to that in model group.

3.3 Evaluation of Rat Learning and Memory

A Morris water maze was used to test learning and memory abilities. For one week after modeling and three weeks after drug administration, rats were subject to a water maze exercise of for 7 days, once in the morning and the afternoon. On day 7, a place of entry was selected to put the rats into the water facing the pond wall to observe and record the time required for rats to find and climb onto the platform, namely, escape latency.

3.4 NOS and AchE Immunohistochemistry testing

After completing administration, all were anesthetized with chloral hydrate and brain matter was harvested. Tissue was fixed in 4 % paraformaldehyde for 24 hours. Brain tissues were dehydrated with gradient alcohol, hyalinized with xylene, infiltrated and embedded by paraffin, and sliced into 5 um thick coronal sections. Sections were incubated in 3 % hydrogen peroxide for 10min, and washed for three times with 0.0 M PBS. They were then heated to boiling for 30s. Tissue was washed three times with 0.01 M PBS. Brain tissue from all groups was dropped with 50 uL of diluted rabbit anti-rat NOS polyclonal antibody (1:100) and rabbit anti-rat AchE polyclonal antibody (1:100), respectively. The primary antibody in rats in negative control was replaced by 0.01M PBS. They were incubated 2h at 37°C and washed three times with 0.01 M PBS. They were dropped with the goat anti-rabbit of PV 9000 kit biotin labeled goat anti-rabbit, incubated for 30min at 37°C and washed with three times with 0.01M PBS. They were dropped with PV 9000 kit horseradish

peroxidase labeled streptavidin, incubated for 20min at 37°C and washed three times with 0.01 M PBS. They were dropped with DAB developer to develop the color for 10min at a room temperature and washed with distilled water to terminate the reaction. The sections were then stained with hematoxylin stain for 10min and washed in running water for 5min. They were then subjected to color separation for 10s in 0.5 % hydrochloric acid alcohol separation solution and washed in running water for 2min. The sections were hyalinized with xylene and gradient alcohol and sealed with neutral resin. Three sections (slices) were taken from each brain tissue for each indicator. Each section was placed under the microscope 400 times to randomly select five views without repeated positive expressions. Positive cells of the same unit area were counted using the IPP 6.0 image processing system.

3.5 Statistical Analysis

SPSS19.0 statistical software was used for data analysis. All data are expressed by $\bar{x} \pm s$, and the single factor analysis of variance was used for comparison among groups. LSD method was used for homogeneity of variance, and Tamhane's T2 method was used for heterogeneity of variance. $p < 0.05$ indicates the statistically significant difference.

4 RESULTS

4.1 Learning and memory by group

The tests of escape latency via the Morris water maze show a significant extension of escape latency in the model, and treatment, groups before administration. The difference is statistically significant compared with the results of the control, and sham operation, groups ($p < 0.01$). Compared to the model group, treatment group latency is significantly shortened after administration ($p < 0.05$). See Table 1.

Table 1. Group Escape Latency before, and after, administration: a comparison ($\bar{x} \pm s$, s)

Group	n	Before Administration	After Administration
Control	10	38.26 ± 6.33	37.18 ± 5.92
Sham Operation	10	37.57 ± 7.02	36.50 ± 6.09
Model	10	92.65 ± 16.431 ¹⁾	65.29 ± 13.711 ¹⁾
Treatment	10	90.72 ± 17.801 ¹⁾	44.04 ± 10.222 ²⁾

Note: 1. Compared with control group and sham operation group, $p < 0.01$; 2. Compared with model group, $p < 0.05$.

4.2 NOS and AchE Positive Expression by Groups

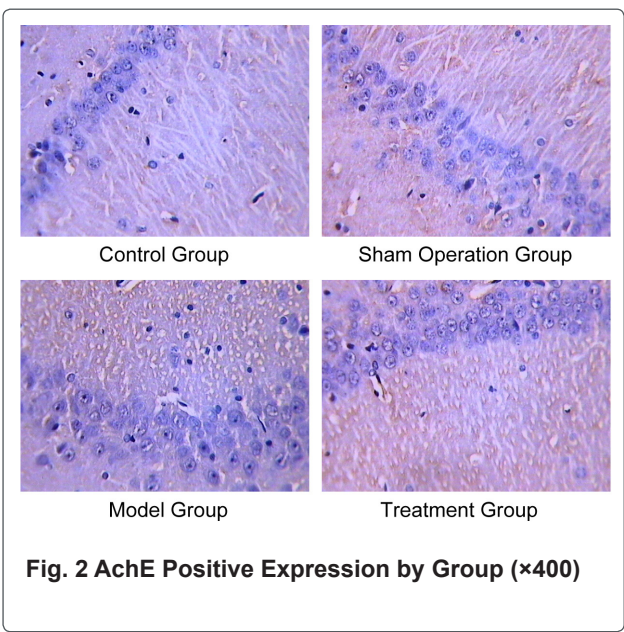
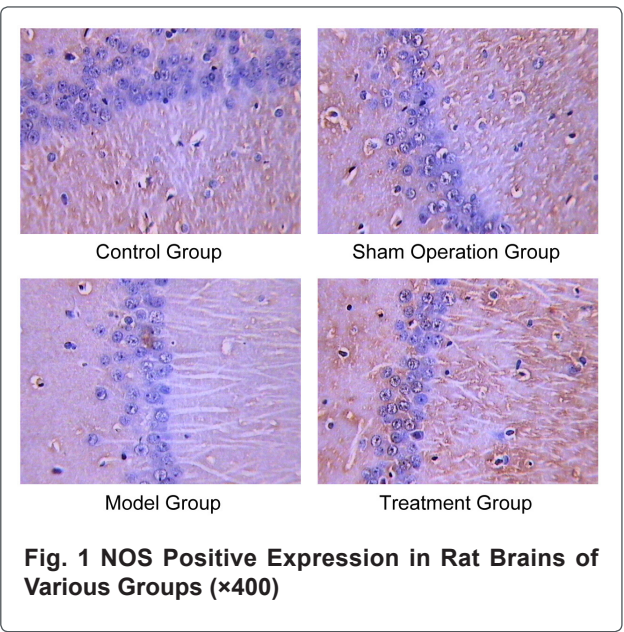
NOS and AchE positive expression by group was detected using immunohistochemistry. A chocolate brown, or brownish yellow, stain in neuronal cell cytoplasm, or protrusions, are positive. The main expression area is in hippocampus. NOS expression in the model group decreased while

AchE expression increased, indicating a statistically significant difference compared with the control, and sham operation, groups ($p < 0.01$). After drug administration, NOS expression in the treatment group increased and AchE expression decreased, which indicates a statistically significant difference compared with the model group ($p < 0.01$). (Table 2 and Fig. 1 and 2).

Table 2. NOS and AchE Positive Expression by Group ($\bar{x} \pm s$, piece/mm²)

Group	n	NOS	AchE
Control	10	116.85 \pm 21.431 ¹⁾	96.36 \pm 19.271 ¹⁾
Sham Operation	10	107.83 \pm 19.611 ¹⁾	103.52 \pm 24.851 ¹⁾
Model	10	59.52 \pm 13.09	177.50 \pm 35.43
Treatment	10	95.73 \pm 17.301 ¹⁾	112.02 \pm 22.351 ¹⁾

Notes: Compared with model group, $p < 0.01$;



5 DISCUSSION

Currently AD pathogenesis is unclear. There are many theories concerning its etiology, including: genetics theory, β -amyloid protein ($A\beta$) deposition theory, cholinergic theory, inflammatory theory, excitatory amino acid theory, free radical damage theory, and aluminum poisoning theory. AD pathology is characterized by cerebral cortical atrophy,

accompanied by $A\beta$ deposition, neurofibrillary tangles, large decreases in the number of memory neurons, and senile plaques formation. At present, $A\beta$ is generally accepted as the main factor inducing AD. The key for AD formation and development of the neuropathological process is that $A\beta$ continuously injures brain cells to lead to a progressive mental

decline and causing senile plaque formation. The A β deposition can decrease the choline acetyltransferase activities and cause the reduction of cholinergic neurons around A β deposition. This leads to memory impairment. It has been reported that some medications can result in a lessening of Leydig cells and regressive and / or degenerative changes in the epididymis, seminal vesicle, ventral prostate, and coagulating gland in male rats^[4]. This experiment used the currently accepted method of acute injection of A β in the rat brain. It can cause the functional loss of cholinergic neurons and learning and memory abilities dysfunction, and result in A β deposition in the brain. Therefore, it is a better animal model. In this experiment, model group escape latency persisted for two weeks after modeling ($p < 0.01$), indicating the decline of learning and memory ability of rats after modeling and a successful modeling.

The current accepted theory attributes AD cognitive symptoms to a shortage of cholinergic neurotransmitters. Acetylcholine (ACh) is an important neurotransmitter in the cholinergic pathway of the central nervous system. It is has widely involved in the cognitive process of in-vivo learning and memory. Learning and memory ability decrease with as ACh levels decrease. Donepezil hydrochloride is a hexaazopyridine oxide and the generation-II specific reversible central AChE inhibitor. It can increase ACh concentration by inhibiting ACh hydrolysis to improve the cognitive function of AD patients^[5]. However, ACh is not stable in the body and it hydrolyzes into choline and acetic acid. AChE activity levels can indirectly reflect the ACh level. Some animal studies reported that donepezil hydrochloride had treatment effects on A β -induced AD rats^[6]. It could significantly improve AD model rat learning and memory impairment and reduce the injury of free radicals in hippocampus neurons^[7]. The results of this study showed that donepezil hydrochloride could improve AD rat learning and memory function ($p < 0.05$), indicating that it could inhibit AChE activities in rat brains and AChE positive expression of hippocampal neurons in AD rats was also significantly decreased ($p < 0.05$).

It is currently thought that long-term potentiation (LTP) is the main mechanism of synaptic plasticity, learning and memory. Nitric oxide (NO) can maintain the LTP effect of hippocampal synapses, and it can promote ACh release and other neurotransmitter releases in the form of super-nitrite in the central nervous system to affect learning and memory^[8]. NO is considered a key transmitter in learning and memory. NO is not stable in the body. NOS is the key enzyme, rate-limiting enzyme in NO synthesis. Change in this activity can reflect NO

level in the body. Studies show that the learning and memory processes of experimental rats may be associated with nNOS activity increases^[9]. The results of this study showed the increased AChE positive expression and the decreased NOS positive expression in rat hippocampal neuron cells in the model group ($p < 0.01$), indicates a decrease in learning and memory ability of model rats. After the treatment with donepezil hydrochloride, NOS expression in AD rat hippocampal neuron cells increased and the difference was significant compared with model group rats ($p < 0.01$). This suggests that donepezil hydrochloride can improve rat learning and memory abilities by promoting NOS expression and enhancing the level of NO. These studies show that donepezil hydrochloride can improve AD rat learning and memory impairment, and it can also associate with the enhancement of NOS activity in the brain hippocampus and the adjustment of the metabolism of messenger molecular NO in the central nervous system in addition to the inhibition of the AChE activities.

CONFLICT OF INTEREST STATEMENT

No conflict of interests and financial disclosure is present. Qiliang Zhou contributes the manuscript, Siping Nie provides the technical guide and Zhihuo Liu provides the language editing service.

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