Title: Chronic Administration of Donepezil on Inhibitory and Spatial Learning and Memory in Male Rats

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Abstract

Cholinergic dysfunction involves in age related cognitive deficits and Alzheimer disease. Donepezil is a reversible acetylcholinesterase (AChE) inhibitor. The present experiments examined the ability of chronic supplementation with donepezil on the cognition in the healthy young rats.

Twenty young male Wistar rats (140-160 g) were divided into control and donepezil groups. The rats received oral administration of saline or 0.3 mg/kg of donepezil for 30 consecutive days. Then they were trained and tested in the inhibitory avoidance (IA) and eight radial arm maze (RM) tasks.

The results indicate that there was neither significant difference in the number of trials to acquisition in the IA nor number of baited food arms in RM tasks between experimental groups. In the IA retrieval test, the time spent in the dark compartment in the donepezil treated group was significantly less than saline treated group. Also, in the RM retrieval test, the number of total memory and working memory errors of donepezil treated rats was significantly less than saline treated one.

It can be concluded that chronic administration of donepezil (0.3 mg/kg) had no significant effects on the learning process but it can improve memory performance in normal rat.

Keywords: Donepezil, learning, memory, Rat
Introduction

Acetylcholine (ACh) is a very widely distributed neurotransmitter in the central nervous system. It has a more modulatory function in the brain, being involved in the learning and memory (1). The blockage of acetylcholine receptors with cholinergic drugs causes learning impairment (2-4). Acetylcholinesterase is one of the most fundamental enzymes in cholinergic synapses (5).

Alzheimer’s disease is progressive disorder with a lack of acetylcholine in the brain (6). To date, approved treatments for Alzheimer’s disease are prevented against the reduction of acetylcholine in the brain (7, 8) using the acetylcholinesterase inhibitors such as donepezil hydrochloride (9). Donepezil (Aricept) is an acetylcholinesterase inhibitor has been approved for the treatment of Alzheimer disease allowing once-daily dosing (9, 10). The clinical efficacy of donepezil is based on acetylcholinesterase inhibition, resulting to an elevated of acetylcholine level at synapses, leading improvement of memory and learning impairments (11).

Donepezil in middle to high doses improved memory impairment in some task (12, 13). The acetylcholinesterase inhibitor, enhances the survival of neurons in the hippocampus (14) and prevent the amyloid beta peptide accumulation associated neurotoxicity (15, 16) and neuroprotective effects in transgenic mice (17, 18).

The high doses of donepezil have been used for treatment of patients with Alzheimer's disease (19). Using a low dose of donepezil reduce side effects of drug such as gastrointestinal side effect such as nausea, vomiting, diarrhea, and anorexia (9). However, the chronic high doses of donepezil modulated the cognitive behavior in treatment rats (20, 21), prolonged exposure of donepezil at low dose has not been clarified in normal rat. Therefore,
the purpose of this study was to evaluate the effect of chronic administration of donepezil on learning and memory in normal intact young rat.

Materials and Methods

Animals

Young male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 140–160 g were used. Animals had free access to food and water, and were housed four to a cage at 24±2°C under 12/12 hours' light: dark cycle (lights on at 7:00 am). All experiments were carried out during the light phase between 9:00 and 15:00. Two experimental groups consisted of ten animals and were chosen randomly from different cages. All procedures for the treatment of animals were approved by the research committee of the Hamadan University of Medical Sciences.

Experimental Design and Drugs

The animals were divided into control and treatment groups (n = 10 per each group). Donepezil (0.3 mg/kg, Sigma;) were administered orally using gavages needle to treatment groups for 30 consecutive days. The control rats received saline in same volume during one month.

Inhibitory avoidance (IA) test

The IA apparatus and procedure was basically the same as our previous studies (22-24). Briefly, the apparatus consisted of two boxes of the same size (20 × 20 × 30 cm). There was a guillotine door in the middle of a dividing wall. The lighted illuminated chamber separated to
a dark chamber with a guillotine door. The floors of both chambers were made of stainless steel rods (3 mm diameter, 10 mm apart) spaced 1 cm apart. The floor of the dark compartment could be delivered an electrified shock generator.

In the training phase, all animals were allowed to habituate in the apparatus. Each rat was gently placed in the white compartment and 5 secs later the guillotine door was raised and the animal was allowed to enter the dark module. Upon entering the dark compartment, the door was closed and after 30 s the rat was removed from the dark compartment into its home cage. This habituation trial was repeated 30 min later.

Then, the first acquisition trial started 30 min after the second habituation trial. The latency to enter the dark compartment (step-through latency during acquisition, STLa) was recorded when the animal had placed all 4 paws in the dark compartment. During acquisition the guillotine door was closed and electric shocks (50 Hz, 0.6 mA intensity) were delivered to the grid floor of the dark compartment for 3 secs immediately after the animal had entered the dark compartment (23). The rat was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the animal was retested in the same way as in the previous trials. Then two min later the procedure was repeated. The rat was received the same shock each time it reentered in the dark compartment with 4 paws in this chamber. Training was ended when the rat remained in the lighted chamber for 120 consecutive seconds. The number of trials to acquisition (entries into the dark chamber) was recorded.

Retention test was done 24 hours following the training phase. The rat was placed in the illuminated chamber, the guillotine door was raised 5 secs later and the latency of entering the dark compartment, step-through latency (STLr) and the percentage of time spent in dark compartment (%TDS) were recorded in the absence of electric foot shocks during 5 min.

Radial Arm Maze (RAM)
The rats were tested in an 8-arms radial maze. Apparatus and procedure was basically the same as our previous research (25). The radial arm maze made of wooden and clear Plexiglas elevated 10 cm from the floor with a central platform arena (20 cm in diameter). The apparatus consisted of one central compartment and eight arms radiated from it in equal intervals. The center arena surrounded by high clear Plexiglas guillotine doors and extends to the eight arms (50 cm long, 15 cm high, 10 cm wide). Plexiglas walls are at the sides and on the top of each arm. There was a cup at the end of each arm filled with food pellets. During the acquisition, four arms of the maze (1, 4, 5 and 7) were baited randomly with the food. In radial maze, to improve exploratory behavior required to obtain diets, the animals were food-restricted to reduce and maintain their weight at 85% of baseline weight.

In each trial, the rat was placed into central compartment (start position) closed off by door. After 15 seconds to navigate central compartment, guillotine doors were raised and were allowed to find the food pellets in arms until all 4 pellets arms had been eaten or 5 minutes was elapsed. All rats were trained 4 trials per day for 18 consecutive days. The interval between trials was 5 minutes. In acquisition phase trial termination time and number of baited food arms were recorded. One week after learning trial, the retention test was performed. The numbers of memory errors were calculated. The number of entries into never baited arms was regarded as reference memory errors, while the number of re-entries into the arms where the pellet had already been eaten was regarded as working memory errors.

Statistical Analysis

The statistical analysis of data was performed by Student t-test. The value of \( p < 0.05 \) was considered significant. The data were expressed as mean ± S.E.M.
Results

Inhibitory avoidance test

In the acquisition trial, there is no difference between the control and donepezil groups in the step through latency (STLa), and the number of trials to acquisition. Both of data for STLa and the number of trials were not shown.

Effect of 30 days treatment of donepezil on the percentage of time spent (%TDC) in the dark compartment after first stepping in the retention trial which was carried out 24 h after acquisition of inhibitory avoidance learning task in the control and treated groups. The Student t-test showed that there are significant differences in the percentage of time spent in dark compartment (%TDC) after first stepping between groups (T (18) = 51.53, P < 0.05; Fig.1.B). In the retention test (24 h after training), the step through latency in reacquisition (STLr) of donepezil treatment group was similar to the control rats (Fig. 1.A).

Radial Arm Maze

There are statistically significant differences between donepezil and control groups in acquisition trials. On the first day of training, the donepezil receiving rats show greater ability compared to the control group in the number of baited food arms (T (18) = 15.78, P < 0.05; Fig. 2). During the next days, there were not observed significant differences between the two groups in the mention variable.

In test day, the statistical analysis revealed that, there were no significant differences in the number of baited food arms and trial termination times between the groups of rats. Student t-test shown that, donepezil caused a significant decrease in the number of total memory errors in test trial (T (18) = 3.72, P < 0.05; Fig. 3.A). On the other hands, the number of working memory errors in the treated group of rat, were significantly fewer than the control group (T
(18) = 6.92, P < 0.001; Fig. 3.B). However, there are no statistically significant differences between donepezil and control animals in the number of references memory errors in test day.

Discussion

The present findings demonstrated that donepezil (0.3 mg/kg) potentiated avoidance memory after 30 days' treatment. The donepezil potentiated learning at the first day and did not change in the next days. Donepezil potentiated memory via decrease the errors working memory and decrease the errors reference memory.

The current result is associated to previous studies that reported memory potentiation effect of donepezil may be due to the inhibition of acetylcholinesterase which increasing the amount of acetylcholine released in the cholinergic terminals during learning memory. Acetylcholinesterase inhibitors enhance the Acetylcholine level in neural structures like hippocampus (12, 13, 26). Chronic administration of donepezil ameliorated memory functions explorative strategies in healthy young rats (21). This study confirms that daily consumption of donepezil reduced recognition impairment, improved verbal encoding and facilitates dependent memory consolidation in healthy young and older individuals vulnerable (27-29). Acute effect of donepezil reinforced spatial memory in young, healthy volunteers (30).

This result showed that donepezil did not affect learning in healthy young rat. The evidence indicated that donepezil improves learning and memory in memory deficit conditions. Donepezil ameliorated scopolamine-induced memory deficits in young and aged mice (13, 31), and prevented isoflurane-induced spatial memory impairment (26). It is well demonstrated that cholinergic system plays an important role in the memory function. Donepezil has a protective effect of against neuronal death (32-35). It is stimulated the acetyl
choline receptors regulation (12), enhanced hippocampal neurogenesis and choline acetyltransferase (14). The neuroprotective effects of donepezil have shown in wild type animals (15, 16) and transgenic mice (17, 18).

The donepezil did not affect learning in intact animals following chronic treatment. Three-week administration of donepezil (10 mg/kg) had no effect learning in Morris water maze task in healthy animals (36). Other study shows that the healthy older subjects exhibited negative cognitive and neurophysiological responses to donepezil treatment (20). The contradictory study indicated that donepezil injection accelerated acquisition and memory functions in intact adult rats following 7-week treatment (21).

Each of learning and memory have different and complex mechanisms. Many protein, neurotransmitters and receptor involved in learning and memory. One of the mains system is NMDA (N-Methyl-D-aspartate) receptor, which enhance learning and memory (37). The anticholinesterase activity of donepezil is specific for acetylcholinesterase enzyme at cholinergic system and did not impress NMDA and other receptors in the brain (38). Donepezil, has been used in combination with NMDA receptor antagonist for the treatment of Alzheimer disease patient (39). That is the reason for the question why donepezil improved memory without any restoring effects on learning in present study.

Conclusion

In conclusion, present study demonstrate that chronic treatment of donepezil reinforced avoidance and working memory and did not change reference memory in healthy rat. Further researches are necessary to evaluation the role of donepezil in learning processing in healthy animals.
Acknowledgements

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References


Fig. 1. Effects of chronic supplementation of donepezil administration on the IAL. The step through latency in the retention test (STLr) (A), and the time spent in the dark compartment in the retention test percentage time spent in the dark compartment after first stepping (%TDC) in the retention test (B). *P < 0.05 compare to control group. Each column and bar represents mean±S.E.M. n =10 per group.
Fig. 2. The effects of donepezil administration on the number of baited food arms. *P < 0.05 compare to control group. Each column and bar represents mean±S.E.M. n =10 per group.
Fig. 3. Effects of chronic supplementation of donepezil administration on the RM. The number of total memory errors (A), the number of working memory errors (B). *P < 0.05, ***P < 0.001 compared to control group. Each column and bar represents mean±S.E.M. n = 10 per group.