Original Article

Comparison of Donepezil and Riluzole in Improving Spatial Memory of Male Wistar Rats

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Abstract

Purpose: Alzheimer's disease (AD) is a degenerative brain disorder and the major cause of dementia and cognitive deficits in the elderly. Riluzole modulates glutamate concentration and improves memory performance in aged rats and may be of benefit in AD. Donepezil is a cholinesterase inhibitor that is used for the treatment of mild-to-moderate AD. In this study, we compared their effects on attenuation of learning and memory deficits in a rat model of AD. **Materials and Methods:** Scopolamine injection for 14 consecutive days induced memory impairment. Effect of riluzole on this impaired memory was evaluated by Morris water maze protocols: accusation phase and probe trial test. Adult male Wistar rats (250–300 g) were trained for 4 consecutive days, 24 hours after last scopolamine injection. Spatial memory and learning index (%) were measured depending on the time taken to find the platform and the time utilized in the target quadrant (Q_2). The time/distance was measured by the computer. Results were analyzed by one-way analysis of variance and Tukey *post hoc*. **Results:** Riluzole was effective in the treatment of memory impairment of scopolamine-injected group. The riluzole-treated group, on test day, showed better spatial memory rather than scopolamine-treated group. Besides, learning index (%) improvement was significantly higher in the riluzole-treated group, rather than scopolamine-injected group. **Conclusion:** It can be concluded that riluzole administration at the same time with scopolamine injection or after it causes marked improvements in learning index during training days and the spatial memory on the test day. Therefore, this study strengthens the hypothesis that acute riluzole treatment is capable of treatment of diseases related to memory impairment such as AD.

Keywords: *Alzheimer's disease, donepezil, learning, riluzole, spatial memory*

Key messages:

- Riluzole strongly improves learning index and spatial memory.
- Riluzole would have therapeutic potential for Alzheimer's disease.
- Coadministration of donepezil and riluzole has synergic effect on memory and learning impairment more than that of monotherapy.

Introduction

Alzheimer's disease (AD) is one of the most prevalent forms of dementia with the rising world-wide health concerns and medical care, and by 2050 the prevalence of AD is expected to approach nearly 107 million people.[1] AD is defined as a progressive senile neurodegenerative disorder and as the most common type of dementia.[2] The major underlying mechanism is accumulation of extracellular amyloid beta (Aβ) plaques and hyperphosphorylated intracellular tau protein in neurofibrillary tangles, nerve and synapse loss, and possibly neurogenesis deficit. Toxic Aβ oligomers interact with the *N*-methyl-D-aspartate (NMDA) receptors and dysregulate glutamate hemostasis.[3] AD causes memory loss, progressive cognitive decline, intellectual function impairment, and behavioral changes. In addition, patients with AD show visuospatial processing deficits.[4]

Scopolamine was used as a nonselective, anticholinergic drug for creating a pharmacological model of AD based on a cholinergic hypothesis, which states acetylcholine deficiency among other neurotransmission pathway impairment may be thought to be responsible for AD.[5] Scopolamine could induce short-term amnesia in young healthy human subjects, which was similar to untreated old human subjects' amnesia,[6] and could be used as a pharmacological model for human AD.[7]

Donepezil as a reversible acetylcholine esterase inhibitor affects the central cholinergic system and increases cortical acetylcholine

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concentration. Donepezil administration is beneficial in pharmacological memory-impaired rats at certain doses.

According to the glutamatergic hypothesis, glutamate dysfunction as an acidic amino acid is toxic for neural cells. Glutamate affects memory and learning.[8]

Riluzole (2-amino-6-(trifluoromethoxy)benzothiazole) as an anti-glutamate agent has an anti-excitotoxic and a neuroprotective effect, and is the only FDA-approved drug for the treatment of amyotrophic lateral sclerosis.[9] Besides, riluzole protects neurons against non-excitotoxic oxidative stress by inhibiting protein kinase C.[10] Unfortunately, the precise mechanism of action of riluzole remains unclear. It seems that riluzole inhibits voltage-gated Na^+ channels and modulate presynaptic glutamate release via NMDA receptors and elevated astrocytic uptake of extracellular glutamate.[11] Riluzole can prevent neural degeneration of pyramid cells of CA1 in the hippocampus of ischemic gerbils.[12] Therefore, riluzole could prevent memory loss. It has been confirmed that riluzole has shown antioxidant properties, maybe due to lowering lipid peroxidation and inhibiting cytosolic PLA 2.[13]

In this study, we aimed to evaluate the effects of riluzole on amelioration of learning and memory deficits as a neuroprotective agent in comparison to donepezil as a reference drug on scopolamine-induced dementia in male Wistar rats.

Materials and Methods

Animals

Mature male Wistar rats, weighing 250–300 g, measured at the beginning of the experiment, were housed in transparent plastic cages (8 rats per cage) with food and water *ad libitum* except during testing. Animals were maintained in a temperature-controlled room at 23 ± 2°C under a 12/12 light to dark cycle.[14]

Ethics

All animals were used only once then omitted according to animal handling ethical codes. All the procedures were approved by the Medical Ethics Committee of Zanjan University of Medical Sciences, Zanjan, Iran (protocol no: ZUMS.REC.1394.106).

Drugs

Scopolamine and donepezil were purchased from Sigma-Aldrich (Germany). Riluzole was purchased from Jalinus (Tehran, Iran). Scopolamine and donepezil were mixed in 0.9% NaCl (saline) and were administered by intraperitoneal (i.p.) injection. Riluzole was dissolved in 0.5% carboxymethyl cellulose and administered by per oral (p.o) route.

Experimental design

Animals (n = 56) were randomly divided into the following seven groups of eight rats: (1) Control, received saline for 14 consecutive days via i.p. injection; (2) scopolamine-treated group: rats injected scopolamine (Sigma) at a dose of 1 mg/kg (i.p.) for 14 days (SCO); (3) donepezil group: rats gavaged an aquatic suspension at 1 mg/kg per os (p.o.) and scopolamine at 1 mg/kg for 14 days (DON); (4) riluzole group: rats were injected riluzole (i.p.) at 8 mg/kg and scopolamine at 1 mg/kg for 14 days (RIL); (5) and (6) intact groups received donepezil at 1 mg/kg and riluzole at 8 mg/kg, respectively, for 14 days; and (7) the last received riluzole and donepezil after scopolamine injection.

Administration of scopolamine and drugs in experimental groups was carried out 24 hours before the start of behavioral testing and during behavioral testing.

Morris water maze tank

The Morris water maze (MWM; Pishro Andishe Sanat, Tabriz, Iran), a round black cylinder (round tank) of 150-cm diameter, and 60-cm depth, was filled with water to reach half of its height. The square platform was placed in the second quarter center, northwest of the round tank. The platform was submerged 2 cm below the surface. The maze was fixed in the middle of a room with enough surrounding visual clues. The clues were not rearranged during tests as animal reference points for locating where the platform was placed. Infrared camera was used as a tracking system to record path and latency, and swim red light was used to minimize light reflection from the water as tracking software was sensitive to light. Water temperature was maintained at 25 ± 2°C in all test days.

Spatial reference memory

Each animal was placed into the water, facing the wall of the tank, at the beginning of each trial. Animals were not dropped. Start positions were selected, among four fixed starting points, randomly by a computer program. Acquisition phase started 24 hours after the last injection. The acquisition involved one training trial per day for 4 consecutive days. Each training session involved 60 seconds of swimming in MWM. Platform location was not changed over all the trials. The trial was completed after 60 seconds or until the animal located and climbed up onto the platform. Animals reaching for the platform were allowed to rear up for 5 seconds and then pulled out of the experiment. Animals could not reach the platform within 60 seconds were gently guided by experimenter toward the platform and were allowed to rear up for 20 seconds to identify their spatial location. Then the experiment was terminated and animals were returned to their cages.[15] Learning index, defined as time utilized by each rat to find the target platform and the time spent in the target quadrant (Q_2) on trial day, was noted down.[16]

Probe trial test

One day after training was completed, the platform was removed from the pool. Animals were allowed to swim for 60 seconds to find platform location. They were placed gently, by chance into the water. Starting points were chosen accidentally by a computer system. Path pattern and time spent in different quadrants, especially target quadrant, were counted as criteria for spatial memory assessment.[17]

Statistics

SPSS 16.0 was used for data analysis. To analyze test results, one-way analysis of variance (ANOVA) was carried out to compare pair groups. Tukey test was performed. $p < 0.05$ was considered significant.

Results

Success determination of memory and learning impairment (scopolamine attenuates spatial memory)

Scopolamine-HCl (1 mg/kg i.p.) was injected for 14 consecutive days before 4 days of rat trials in the MWM. Figure 1 illustrates the effect of scopolamine on time spent in target quadrant in contrast to normal saline in the test day. Spatial memory is determined by time length spent in the target quadrant (Q_2).

Learning index (%) difference between scopolamine-injected group (SCO) and control group (NS) was significant. Learning index (%) was calculated by the determination of time length spent on target quarter and total navigation time. The learning index for scopolamine-injected rats (SCO) was significantly less than that of the control ($p < 0.001$) [Figure 1].

Donepezil ameliorates learning and spatial memory impairment in scopolamine-injected rats

Donepezil (1 mg/kg i.p.) was administrated for 14 consecutive days after scopolamine treatment. We used MWM to demonstrate whether donepezil improves spatial memory after scopolamine injection. The time spent in target quadrant for scopolamine-treated rats (SCO) was shorter than that of the control ($p < 0.05$). Although this shorter time was significantly increased after donepezil injection, the target quadrant time measured for the control group (NS) was still longer. The difference was significant ($p < 0.001$) [Figure 1].

Learning index difference between scopolamine-injected rats and donepezil-treated rats is shown in Figure 2. Learning index was calculated by dividing time spent in the target quadrant (Q_2) by total exploration time spent in MWM. It was observed that the scopolamine-induced rats showed a decreased learning index, which increased significantly after donepezil treatment. The increase in learning index in scopolamine-treated donepezil-injected rats was the same as that in the control group ($p < 0.05$). Donepezil treatment in scopolamine-treated ones improved their learning index. Donepezil administration in rats, without injection of scopolamine, caused learning index increase ($p < 0.05$) [Figure 2].

Riluzole improves memory and learning deficits in scopolamine-treated rats

Riluzole (8 mg/kg p.o.) administration during 14 days after scopolamine injection significantly increased the time spent in Q_2 and improved spatial memory as compared with untreated scopolamine-injected rats. As data represent spatial memory difference between riluzole-treated scopolamine-injected rats and control group was not significant. Rats that were treated only with riluzole showed better spatial memory compared with the control group but the difference was not significant, too. Data are shown in Figure 3.

Learning index for scopolamine-injected rats increased after riluzole was given. Learning index in riluzole-treated rats without scopolamine treatment was growing the same as control group but riluzole injection alone without scopolamine treatment made them perform better than the control group ($p < 0.001$) [Figure 4].

Coadministration of donepezil and riluzole

One-way ANOVA represented that coadministration of donepezil (1 mg/kg i.p.) and riluzole (8 mg/kg p.o.) for 14 consecutive days in scopolamine-injected rats demonstrated more increase in spatial memory improvement and learning

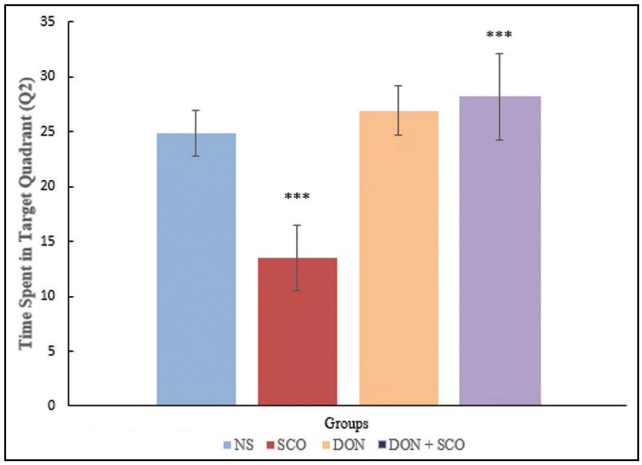

Figure 1: Effect of donepezil on the time spent in target quadrant in scopolamine-injected rats during the Morris water maze. Every column expresses the mean time ± SEM of a rat group ($n = 8$ per group). ***$p < 0.001$ vs. SCO group of rats. SCO = scopolamine, DON + SCO = donepezil + scopolamine, NS = normal saline

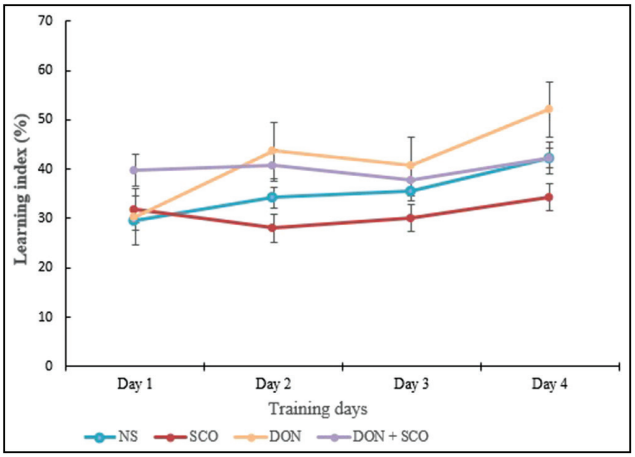

Figure 2: Effect of donepezil on scopolamine-induced learning impairment. Training days were performed for 4 consecutive days. Rats were treated with scopolamine (1 mg/kg i.p.) 1 day before trial initiation. Data show learning percentage ± SEM ($n = 8$ per group). SCO = scopolamine, DON + SCO = donepezil and scopolamine, NS = normal saline, DON = donepezil

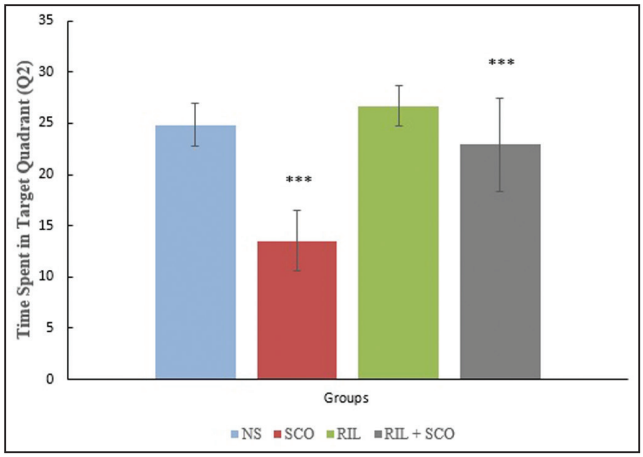

Figure 3: Effect of riluzole on spatial memory. Spatial memory difference was significant between SCO + RIL group and SCO group. $^{***}p < 0.001$ vs. SCO (n = 8 per group). Every column shows data ± SEM. $^{***}p < 0.001$. SCO = scopolamine, RIL = riluzole, RIL + SCO = riluzole and scopolamine, NS = normal saline

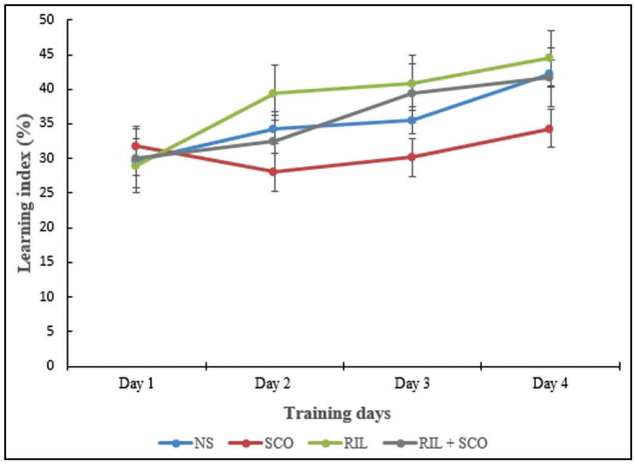

Figure 4: Effect of riluzole on learning index after scopolamine injections. Every column represents data ± SEM (n = 8 per group). $^{**}p < 0.01$. NS = normal saline, SCO = scopolamine, RIL + SCO = riluzole and scopolamine, RIL = riluzole

index ($p < 0.01$). Donepezil and riluzole were administered after scopolamine injection. Data are shown in Figure 5.

Discussion

The cholinergic and glutamatergic neurotransmitter systems alongside the GABAergic system are the major common systems associated with memory formation process. In this study, we assessed the effect of riluzole on learning and memory deficits in the animal model of AD and determined the possible connections between the cholinergic receptors and glutamatergic system. Our behavioral experiments demonstrated that riluzole attenuated spatial memory in the MWM and conditioned learning, and it is not depended on cholinergic function in the brain. The possible role of neuronal destruction in memory impairment was examined as central cholinergic neurons are selectively destructed.[18] Previous studies have shown that 14 days of i.p. injection of scopolamine

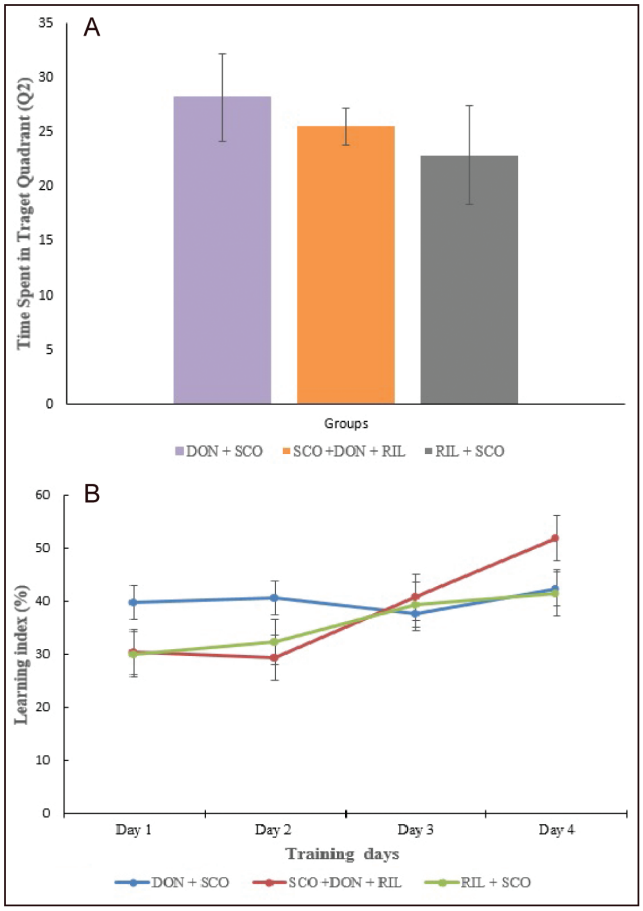

Figure 5: The influence of coadministration of donepezil and riluzole on (a) spatial memory and (b) learning index (%). Donepezil and riluzole were administered at the same time for 14 days, after scopolamine injection. Administration of donepezil or riluzole alone, after scopolamine treatment, is less effective than coadministration of both. Data represent mean ± SEM (n = 8 per group). $^{*}p < 0.05$, $^{**}p < 0.01$. SCO = scopolamine, DON + SCO = donepezil and scopolamine, RIL + SCO = riluzole and scopolamine, SCO + DON + RIL = scopolamine, donepezil, and riluzole

hydrochloride significantly decreases memory.[19] Scopolamine was used because it impairs cholinergic system as a cholinergic antagonist and causes spatial memory and learning impairment in rat according to the cholinergic hypothesis of AD.[5,20,21]

This study showed that scopolamine administration causes memory and learning impairment as compared with the control group. Scopolamine group (SCO) spent less time in target quadrant rather than the control group, and their learning was impaired during training days. Donepezil pretreatment prevents spatial memory impairment by increasing acetylcholine in the brain.[22] Donepezil treatment in scopolamine-injected rats increased the time spent in target quadrant and it was capable of improving spatial memory as a result of acetylcholine elevation in the brain. Spatial learning improvement was observed in memory-impaired rats treated with donepezil, but their learning index was improving the same as a normal saline group. Donepezil administration, alone, ameliorated learning index.

Glutamate role in AD pathogenesis has been demonstrated as glutamine–glutamate cycle changes.[23] Deng *et al*.[24] showed

that pretreatment with riluzole as a glutamate-release blocker inhibits methylmercury neurotoxicity through downregulating the interaction between the oxidative damage and excitotoxic signaling pathways that are related to glutamate transmitters, glutamate–glutamine cycle, and NMDA receptor expression and calcium. Protective effect of riluzole has been reported previously.[25]

Neuronal destruction role in AD could be due to apoptosis, necrosis, or abnormal association of phosphorylated microtubules called tau.[2]

Riluzole as a sodium channel inhibitor decreases presynaptic glutamate concentration. In addition, riluzole regulates tau microtubules and is a neuroprotective drug.[26]

Riluzole administration for 14 consecutive days, after scopolamine injection, significantly improved spatial memory and learning index in comparison to scopolamine-injected rats, but their difference with the control group was not significant. Riluzole administration, alone, ameliorated spatial memory, but their difference with the control group was not significant, either.

Learning index in riluzole-treated scopolamine-injected rats was more than that in a scopolamine-injected group but was growing the same as control. Riluzole administration, only, in healthy rats, improved learning index.

Combination of donepezil with riluzole enhanced memory and learning deficits more than that of treatment with only one drug.

Conclusion

It is concluded that riluzole showed a neuroprotective effect against memory deterioration induced by scopolamine injection. Riluzole decreases glutamate release, tau proteins, and pathological phosphorylation of tau proteins. Further studies are required to find the possible mechanism of riluzole memory restoration in AD.

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Conflicts of interest

There are no conflicts of interest.

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