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Effect of Donepezil, an Acetylcholinesterase Inhibitor, on Spatial Learning and Memory in Mice

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Memory impairments may occur due to various reasons. The most often ones include neurodegenerative disorders, for instance Alzheimer's disease (AD). Nowadays, proper pharmacotherapy of this illness is not known, it is only possible to attenuate some symptoms of this disease by enhancing cholinergic neurotransmission. Donepezil is an example of acetylcholinesterase (AChE) inhibitors used in patients suffering from AD. In the present work using two spatial memory tasks, the Morris water maze and the two-day radial-arm water maze, we examined its anti-amnesic efficacy in the scopolamine-induced mouse model of memory impairments. Donepezil at a dose of 10 mg/kg b.w. only slightly improved cognition in both the assays. Hence, we conclude that the enhancement of cholinergic neurotransmission due to selective AChE inhibition may be insufficient to improve cognition in this model, and dual acetylcholinesterase/butyrylcholinesterase inhibitors seem to be a better option for the attenuation of memory impairments.

Key words: donepezil, acetylocholinesterase, spatial memory, Morris water maze, radial-arm water maze

INTRODUCTION

Alzheimer's Disease (AD) is the most common cause of dementia in humans (60-70%). Furthermore, an increase in prevalence and treatment costs of AD are reported nowadays; in 2013 there were about 44.4 million of patients worldwide. The currently used guidelines, according to the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10, updated in 2015), Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V, 2013) and The NINCDS-ADRDA Alzheimer's Criteria (1984, updated in 2007) (Dubois et al., 2007) distinguish similar diagnostic criteria and symptoms of AD, and report impairments in cognitive functions and memory as the most frequent ones. Over the years researchers

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have formulated several hypotheses of AD but its real mechanisms have never been fully understood. Current treatments provide only a reduction in leading symptoms and are based on the oldest cholinergic hypothesis according to which patient's cognitive dysfunction is due to deterioration in the cholinergic system action (Giacobini, 1990; Giacobini, 2003; Holzgrabe et al., 2007; Rahim et al., 2015). In the late 1980s and 1990s scientists proved the effectiveness of acetylcholinesterase inhibitors in several clinical trials (Davis et al., 1992; Farlow et al., 1992; Knapp et al., 1994). To date, the U.S. Food and Drugs Administration has approved five drugs for the therapy of AD. Four of them are acetylcholinesterase (AChE) inhibitors: tacrine (withdrawn from the market due to its hepatotoxicity), rivastigmine, galantamine and donepezil.

Acetylcholinesterase (EC 3.1.1.7) is an enzyme involved in acetylcholine (ACh) degradation. It belongs to hydrolases and is located in the postsynaptic membrane. There are also other enzymes, for instance butyrylcholinesterase (BChE, EC 3.1.1.8), that metabolize ACh. BChE is present not only in the central nervous system (Maurice et al., 2015) but also in plasma and liver. The gathered data suggest a beneficial effect of BChE inhibitors on cognitive functions, which is a novel approach to the treatment of memory impairments (Furukawa-Hibi et al., 2011; Hong et al., 2014; Huang et al., 2014; Reid and Darvesh, 2015). Thus, to assess the role of AChE in cognitive deficits in experimental animals, the present study is focused on the effect of donepezil, an AChE inhibitor, on spatial memory and learning processes in mice with scopolamine-induced cognitive deficits. Scopolamine is regarded as a "gold standard" for inducing memory impairments in mice (Klinkenberg and Blokland, 2011).

MATERIALS AND METHODS

Animals

Male C57BL/6 mice weighing 16-22 g were provided by the Animal Breeding Farm of the University Children's Hospital of Cracow. The animals were kept under standard laboratory conditions: light/dark cycle (12:12), at room temperature 22±2°C, in groups of 10 animals per cage. Food and water were available *ad libitum*. All the procedures applied in *in vivo* experiments were approved by the 1st Local Ethics Committee of the Jagiellonian University in Cracow (ZI/862/2013).

Chemicals and behavioural testing protocol

Donepezil was purchased from Sigma Aldrich (Poland). Before the experiments, it was dissolved in 1% solution of Tween 80 (Polskie Odczynniki Chemiczne, Poland) and administered intraperitoneally (*ip*) at a dose of 10 mg/kg b.w. Scopolamine hydrobromide was provided by Sigma Aldrich (Poland). To induce memory impairments it was dissolved in 0.9% solution of sodium chloride (Polpharma, Poland) and administered *ip* at a dose of 1 mg/kg b.w.

Donepezil was injected once daily, 1 h before the first training session. 0.5 h before the first swimming trial scopolamine hydrobromide solution was administered. Two control groups were used, i.e. mice receiving either (1) 0.9% saline 1 h before the first training or (2) 0.9% saline and scopolamine hydrobromide 1 h and 0.5 h before the training, respectively.

Morris water maze task

The Morris water maze task (MWM) was performed according to the method described previously (Patil et al., 2009). The apparatus for the MWM (Panlab-Harvard Apparatus, Spain) is a round pool (diameter: 120 cm, height: 60 cm), filled with water (temperature maintained at 23±1°C) to such a level that the round platform (diameter: 12 cm) is covered with a 0.5 cm layer of water. The whole experiment was recorded using a computer program (Smart v. 3.0, Panlab-Harvard Apparatus, Spain); the camera was attached to the room wall in such a manner that its lens was located above the central point of the pool. The pool was virtually divided into four equal quadrants, named after compass locations (NW, NE, SE and SW).

The test lasted 7 consecutive days and was divided into two parts: the acquisition phase (days 1–6) and the retention phase (day 7; drug-off trial). During the first 6 days of the MWM, each mouse swam 4 times daily (the maximum

time of swimming: 60 s), starting in the NE quadrant; the underwater platform was constantly located in NW. In this phase the following parameters were measured and then analyzed: the escape latency time to find the hidden platform and the travelled distance to the platform.

The retention phase of the MWM was performed on day 7, starting 24 h after the last swimming session on the previous day. During the retention phase the platform was removed from the pool and each mouse was subjected to only one swimming trial. No drugs were administered on that day and the following parameters were measured: the time spent in the target NW quadrant and virtual target crossings (i.e., crossings over the place where the platform was previously located).

Radial-arm water maze (RAWM)

The two-day RAWM paradigm was introduced (Alamed et al., 2006). An eight-arm maze was inserted into the round pool, the same as that used in the MWM task. The platform (diameter: 12 cm, height: 30 cm) was located in one of the maze arms.

On the first day of the test (the acquisition phase) each mouse swam 15 times (the maximum time of a single swimming trial: 60 s) starting each time from the constant arm of the maze. From session 1 to session 12 the height-adjustable platform was conversely visible (located slightly above the water surface) or hidden (slightly below the water surface), starting from the visible mode. During the last three sessions (13–15) the platform remained constantly hidden.

On the second day of the RAWM (the retention phase) the platform was hidden during all swimming trials. Mice started swimming from an arm which was distinct from that they started from on day 1; the starting arms varied for each mouse during the consecutive training sessions. If a mouse did not find the platform during the swimming trial, it was gently guided towards it and left there for 15 s, then cautiously dried and transferred back to the home cage.

The measured parameter in the RAWM was the number of errors made by the animal (entries into an arm containing no platform). This parameter is considered to be a more sensitive indicator of procognitive effect than the escape latency time (Alamed et al., 2006).

Statistical analysis

All the data were analyzed using Graphpad Prism software (v. 5, USA). Two-way repeated measures analysis of variance (ANOVA) and the Bonferroni multiple comparison test were performed. Additionally, unpaired Student's t-test was carried out. In every case $P<0.05$ was considered significant.

RESULTS

MWM – effect on memory acquisition

In all studied groups a gradual decrease in the latency time to reach the hidden platform during 6 consecutive training days was observed (Fig. 1). Vehicle-treated mice had the best performance in this test. Statistically significant differences in the escape latency time were not found between the scopolamine plus donepezil-treated group and the scopolamine-treated control group.

Also a reduction in the distance travelled to reach the platform was observed during the consecutive days of the acquisition phase. Mice in the vehicle-treated group achieved better results than scopolamine-treated control animals as they covered a significantly shorter distance to find the platform. No statistically significant differences were found between scopolamine plus donepezil-treated mice and scopolamine-treated control mice (Fig. 2).

Morris water maze – effect on memory retention (probe trial on day 7)

Analyzing the time spent in the target NW quadrant on day 7, no significant differences between the scopolamine plus donepezil-treated group and the scopolamine-treated control group were found (Fig. 3).

In contrast to the above findings, both vehicle-treated mice and scopolamine plus donepezil-treated mice made significantly ($P<0.05$) more virtual target crossings during the retention phase of the MWM as compared with the scopolamine-treated control (Fig. 4).

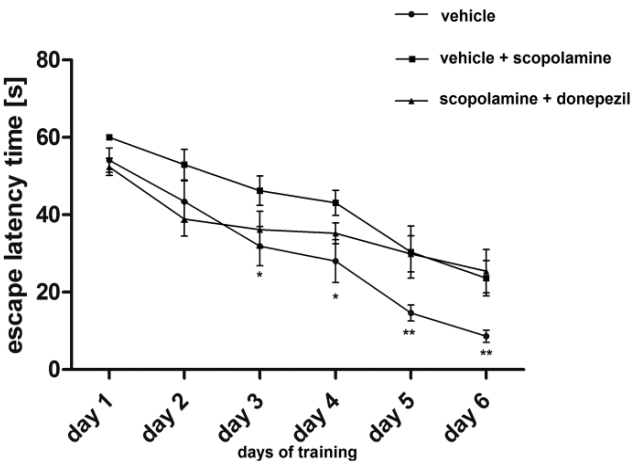

Fig. 1. Effect of donepezil on the escape latency time to reach the platform during the acquisition phase in the MWM. Statistical analysis: two-way repeated measures ANOVA, followed by the Bonferroni multiple comparison test: * P<0.05; ** P<0.01 (vs. the scopolamine-treated control).

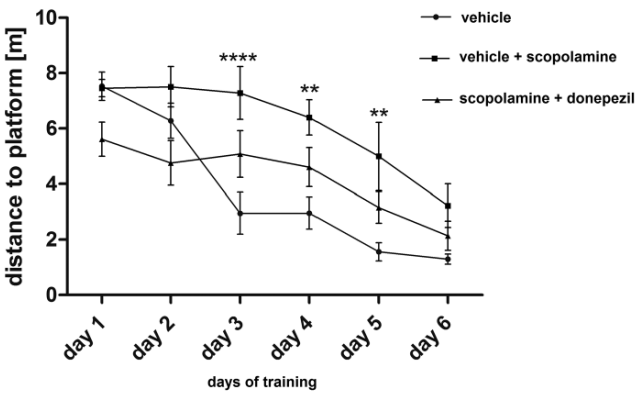

Fig. 2. Effect of donepezil on the distance travelled to reach the platform during the acquisition phase of the MWM. Statistical analysis: two-way repeated measures ANOVA followed by the Bonferroni multiple comparison test. Statistical significance: ** P<0.01, **** P<0.0001 (vs. the vehicle-treated group).

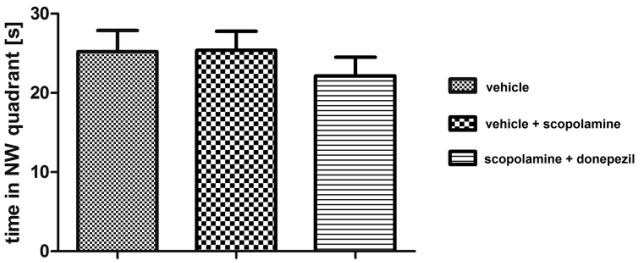

Fig. 3. Effect of donepezil on the time spent in the NW quadrant on day 7 of the MWM. Statistical analysis: Student's t-test; P>0.05.

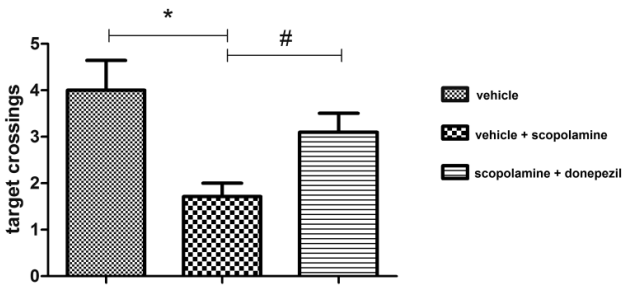

Fig. 4. Effect of donepezil on the number of virtual target crossings in the MWM task. Statistical analysis: Student's t-test: * P<0.05 (vs. the vehicle-treated control group) and # P<0.05 (vs. the scopolamine-treated control group).

Radial-arm water maze – effect on memory acquisition (day 1)

During the acquisition phase of the RAWM significant differences between the scopolamine plus donepezil-treated group and the scopolamine-treated control group were found during the first 3 swimming sessions. Then, scopolamine plus donepezil-treated mice still made fewer errors, but this difference compared with the scopolamine-treated control group was not significant. Overall, a reduction in the number of errors made by the mice was noticed in the consecutive swimming sessions (Fig. 5).

Radial-arm water maze – effect on memory retention (day 2)

On the second day of the RAWM the results were more uniform in all three groups. No significant differences were noticed; the effect of donepezil on memory was found to be negligible (Fig. 6).

DISCUSSION

In the present study donepezil, an AChE inhibitor, has demonstrated a weak effect on spatial learning and memory in mice. Such results are

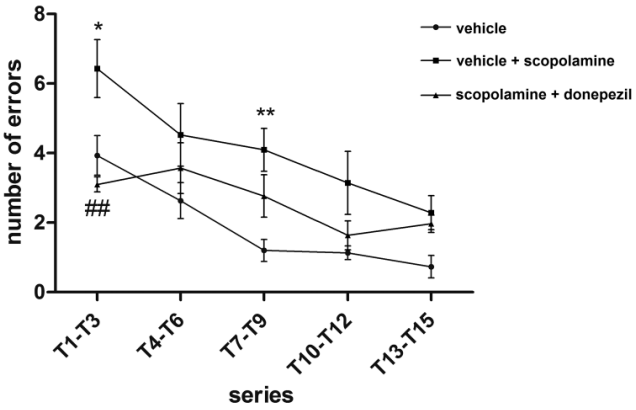

Fig. 5. Effect of donepezil on the number of errors made on day 1 of the RAWM (acquisition phase) during 15 consecutive trials divided into 5 trial blocks (T1-T3; T4-T6; T7-T9; T10-T12 and T13-T15). Statistical analysis: two-way repeated measures ANOVA, followed by the Bonferroni multiple comparison test. Statistical significance: * P<0.05, ** P<0.01 (vs. the vehicle-treated control group), ## P<0.01 (vs. the scopolamine-treated control group).

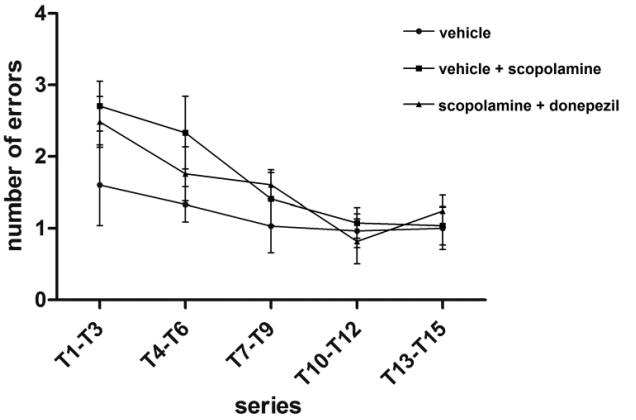

Fig. 6. Effect of donepezil on the number of errors made on day 2 of the RAWM (retention phase) during 15 consecutive trials divided into 5 trial blocks (T1-T3; T4-T6; T7-T9; T10-T12 and T13-T15). Statistical analysis: two-way repeated measures ANOVA followed by the Bonferroni multiple comparison test: P>0.05.

rather unexpected bearing in mind that donepezil has been widely used in humans suffering from AD (Tinklenberg et al., 2015). On the other hand, available literature data indicate that selective inhibition of AChE might be insufficient to achieve a satisfactory anti-amnesic effect, and the so-called "dual" AChE and BChE inhibitors are thought to be more effective in dementia-related disorders (Cheng et al., 2015). Rivastigmine is an example of these drugs, already approved for the treatment of AD in humans (Bono et al., 2015). The results of our unpublished studies using MWM and RAWM suggest that rivastigmine has a much better impact on cognition than donepezil. Other studies also point out a beneficial effect of rivastigmine on memory in rodent models (Bejar et al., 1999).

There are many studies concerning the activity of donepezil in tests assessing its impact on cognition in rodents. Guo et al. (2015) proved its beneficial impact (at lower doses than those used in the present work: 0.5–2 mg/kg b.w.) on memory in the MWM and novel object recognition tasks in mice; however, contrary to our work, chronic administration of donepezil was assessed and transgenic mice were used. Donepezil also positively modulated memory impairments in other learning and memory tests (Kwon et al., 2014). In a rat model of vascular dementia (bilateral common carotid arteries occlusion, BCCAo) chronic administration of donepezil caused a statistically significant prolongation of the time spent in the target quadrant compared with the BCCAo control group in the MWM. In another study (Nagakura et al., 2013) the activity of donepezil in Y-maze and MWM tests was compared with that of memantine. The AChE inhibitor demonstrated a weaker effect on working memory than the NMDA receptor antagonist in the transgenic mouse model of AD. In another study, donepezil improved performance in the MWM when administered concomitantly with selegiline, a monoamine oxidase-B inhibitor (Takahata et al., 2005). However, in one study oral administration of donepezil (5 mg/kg b.w.) resulted in a reduction in the escape latency time in mice in the MWM after intracerebral injection of streptozocin which is another model of memory impairments in rodents (Saxena et al., 2008). A similar effect was obtained after oral administration of tacrine (5 mg/kg b.w.). In another study in which transgenic mice with cognitive deficits were chronically treated with donepezil, rivastigmine or galantamine (Van Dam et al., 2005) there were not any statistically significant differences in MWM results for control and tested groups, except for

the travelled distance which was gradually being reduced in drug-treated mice. However, Van Dam et al. (2005) used much lower doses of donepezil (0.3 or 0.6 mg/kg b.w.) than we did. Donepezil did not attenuate scopolamine-induced special mapping impairment in rats in a study using acute treatment with this drug (2 or 3 mg/kg b.w.) in the MWM (Linder, 2006).

On the basis on the above data it can be concluded that donepezil influences spatial memory in rodent models of cognitive impairments but this effect strongly depends on the treatment protocol used (acute or chronic), as well as the dosage and the type of rodent model of dementia applied. It has to be strongly emphasized that the reason for this fluctuation still remains unknown.

As mentioned previously, there is no causal therapy for AD. Drugs like donepezil may only attenuate symptoms of this disease; hence there is a strong need for searching for new compounds acting in a different way. To date, there have been many promising results of studies assessing the activity of BChE inhibitors (Furukawa-Hibi et al., 2011; Huang et al., 2014). Some studies also proved that BChE-knockout mice showed good learning capacities and the deposition of amyloid-b was reduced (Maurice et al., 2015; Reid and Darvesh, 2015).

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