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DL0410 can reverse cognitive impairment, synaptic loss and reduce plaque load in APP/PS1 transgenic mice

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ABSTRACT

Cholinesterase inhibitors are first-line therapy for Alzheimer's disease (AD). DL0410 is an AChE/BuChE dual inhibitor with a novel new structural scaffold. It has been demonstrated that DL0410 could improve memory deficits in both $A\beta_{1-42}$-induced and scopolamine-induced amnesia in mice. In the present study, the therapeutic effect of DL0410 and its action mechanism were investigated in APP/PS1 transgenic mice. Six-month old APP/PS1 transgenic mice were orally administered with DL0410 (3, 10, 30 mg/kg/day). After 60 days, several behavioural tests, including the Morris water maze and step-down tests, were used to investigate the effects of DL0410 on mice behaviours. All the behavioural experimental results showed that DL0410 significantly ameliorated memory deficits. Meanwhile, DL0410 attenuated neural cell damage and reduced senile plaques significantly in the hippocampus of APP/PS1 transgenic mice. In addition, DL0410 significantly decreased Aβ plaques, while increasing the number of synapses and the thickness of PSD in the hippocampus. We also found DL0410 decreased the expression of APP, NMDAR1B and the phosphorylation level of NMDAR2B, and increased the phosphorylation level of CAMKII and the expression of PSD-95. In this study, the results of behavioural tests demonstrated for the first time that DL0410 could improve learning and memory dysfunction in APP/PS1 transgenic mice. The mechanism of its beneficial effects might be related to cholinesterase inhibition, Aβ plaques inhibition, improvement of synapse loss by regulating of expression of proteins related to synapses. As a result, DL0410 could be considered as a candidate drug for the therapy of AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease in which patients have declarative memory impairments and increasingly severe dementia. Numerous pathological changes have been found in the postmortem brains of AD patients, including plaques, tangles, inflammation, neuron loss and synapse loss. According to the American Alzheimer's Association, from 2000 to 2010, the proportion of deaths resulting from heart disease, stroke, and prostate cancer decreased 16%, 23%, and 8%, respectively, whereas the proportion resulting from AD increased 68% (Thies and Bleiler, 2013). AD has become an enormous negative factor impacting human health. There is currently no effective therapy for AD.

It has been shown that the amount of acetylcholine is decreased markedly in the brains of AD patients. DL0410 (1,1′-([1,1′-biphenyl]-4,4′-diyl)bis(3-(piperidin-1-yl) propan-1-one)dihydrochloride), with a novel new structural scaffold, can increase the choline content by inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Our previous study showed that DL0410 bounds to the active-site gorge of AChE similarly to donepezil, and its safety and efficacy were also confirmed by a series of experiments (Fang et al., 2013). It has been demonstrated that DL0410 could improve memory deficits in both $A\beta_{1-42}$-induced and scopolamine-induced amnesia in mice.

Some researchers have found that the soluble amyloid β (Aβ) oligomers in brain were highly correlated with learning and memory dysfunction in AD (Trinchese et al., 2004; Texido et al., 2011; Talantova et al., 2013). It would be interesting to observe if DL0410 can clear senile plaques produced by the Aβ oligomer. Mutations in amyloid precursor protein (APP) and presenilin-1 (PS1) genes can result in a relative increase in the production of Aβ and cause symptoms of AD (Puolivali

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; APP, amyloid precursor protein; PS1, presenilin-1; PBS, phosphate buffered saline; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; ASCh, acetylthiocholine iodide; BuSCh, S-butyrylthiocholine chloride; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); HRP, horseradish peroxidase; MWM, Morris water maze; IHC, immunohistochemistry; NMDA, N-methyl-D-aspartate; NMDAR, NMDA receptor; CAMKII, calcium/calmodulin-dependent protein kinase II; PSD-95, postsynaptic density-95.

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et al., 2002; Yan et al., 2009). In APP/PS1 transgenic mice, acceleration of Aβ pathological process leads to cognitive deficits, while treatment with Aβ-lowering agents could result in improvements in cognitive performance (Viayna et al., 2014). In the present study, an APP/PS1 double transgenic mouse model was used to investigate the therapeutic effect of DL0410 (Fig. 1) and the effect of DL0410 on Aβ plaques and the structure of synapses were also assessed.

2. Material and methods

2.1. Drugs and reagents

DL0410 (≧98% tested by HPLC) was synthesised by Institute of Materia Medica, Chinese Academy of Medical Sciences. Rivastigmine was purchased from Nantong Baihua Bio-pharmaceutical Company. Memantine was purchased from Shanghai Jingchun Chemical Industry Limited Company. Acetylthiocholine iodide (ASCh), S-butyrylthiocholine chloride (BuSCh), and 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich (St Louis, USA). The primary antibodies to amyloid precursor protein (APP), N-Methyl-D-aspartate receptor Type 2B (NMDAR2B), p-NMDAR2B, the NMDAR1 subunit of the NMDAR (NMDAR1B), calcium/calmodulin-dependent protein kinase II (CAMKII), p-CAMKII, postsynaptic density-95 (PSD-95) were purchased from Epitomics (CA, USA). The horseradish peroxidase (HRP)-conjugated secondary antibody, anti-GAPDH and anti-Aβ antibody were purchased from Millipore (MA, US).

2.2. Animals and treatments

All experiments were performed in accordance with the guidelines established by the National Institutes of Health for the care and use of laboratory animals, and were approved by the Animal Care Committee of the Peking Union Medical College and Chinese Academy of Medical Sciences. The B6.Tg(APPswe/PSEN1dE9)85Dbo double transgenic mice (referred to here as "APP/PS1", female:male = 3:1) were purchased from the National Model Animal Research Center of China. They were housed under a 12-h light/12-h dark cycle at constant temperature (23 ± 2 °C) with free access to food and water.

When mice were fed for 6 months, they were randomly divided into 6 groups, including AD group, 2 mg/kg rivastigmine group, 3 mg/kg memantine group and 3, 10, 30 mg/kg DL0410 groups. There were 8–9 female mice and 3 male mice in each group. The control group was composed of 13 wild-type C57 mice. There were 5 litters in each group, and 2–3 mice in each litter. Female and male mice were fed in different litters. Because our aim was to demonstrate the therapeutic effect rather than the preventive effect of DL0410 for AD, it was necessary to establish that our APP/PS1 mice showed evidence of cognitive deficits prior to the start of the drug trial. This was accomplished by finding memory deficits in both the step-down and step-through passive avoidance tests at 6 months of age, which led us to begin administration of DL0410, rivastigmine, memantine and isovolumetric water to the AD group and isovolumetric water to the control group by oral gavage for 60 days beginning at this age. All the drugs were dissolved in distilled water. During the experimental period, the animals' body weight and food consumption were monitored twice every week. There were no significant differences in chow consumption or weight gain among different groups of mice.

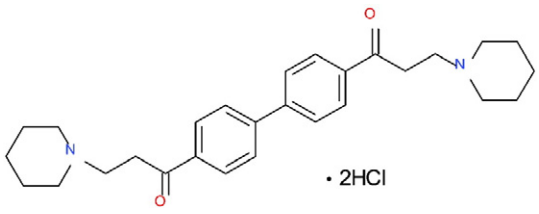

Fig. 1. The chemical structure of DL0410.

2.3. Morris water maze (MWM) test

Sixty days after the beginning of drug treatment, the MWM test was used to evaluate the effect of DL0410, rivastigmine, and memantine on the APP/PS1 transgenic mice. The test indexes included latency to platform and entry times into the platform. The maze was made of white opaque with a diameter of 120 cm and 40-cm-high walls, and was filled with water at 23 ± 1 °C to avoid hypothermia. A small escape platform (10 × 6.5 × 21.5 cm) was placed at a fixed position in the centre of one quadrant, 25 cm from the perimeter, and was hidden 1 cm beneath the water surface. The room contained a number of fixed visual cues on the walls.

2.3.1. Acquisition phase

The acquisition trial phase consisted of 5 training days (days 1–5) and two trials per day with a 15 min intertrial interval. The start location of first trial per day is the contralateral quadrant of platform, and that of the second trial per day is the adjacent quadrant. If an animal did not reach the platform within 60 s, it was guided to the platform, where it had to remain for 10 s before being returned to its home cage. Mice were kept dry between trials in a plastic holding cage filled with paper towels. The path length and escape latencies were recorded (Vorhees and Williams, 2006).

2.3.2. Probe trial

One day after finishing the acquisition task (day 6), a probe trial was performed to assess the spatial memory (after a 24 h delay). The platform was removed from the maze and animals were allowed to swim freely for 60 s. The time that mice spent swimming in the target quadrant (where the platform was located during the hidden platform training) was measured. For the probe trials, the number of times the mouse crossed over the platform site was recorded. All data were recorded with a computerised video system (Brandeis et al., 1989; D'Hooge and De Deyn, 2001).

2.4. Step-down test

The apparatus consisted of acrylic box (26 × 9 × 17 cm) with a stainless-steel grid floor. A platform (5 × 5 × 5 cm) was fixed at one end of the box. Electric shocks (36 V, 1.5 mA) were delivered to the grid floor with an isolated pulse stimulator. At the beginning of training, mice were placed on the platform to adapt for 1 min. When the mouse stepped down and placed its paws on the grid floor, it would jump to the platform when a shock was delivered. Step-down latency (duration of a mouse would remain on the platform) and the number of errors were recorded within 300 s and repeated 24 h after training. The apparatus were cleaned with 70% ethanol between trials to prevent the buildup of olfactory cues.

2.5. Step-through passive avoidance test

The step-through passive avoidance apparatus consisted of an illuminated chamber (11.5 × 9.5 × 11 cm) lit with a 100 W lamp, attached to a darkened chamber (23.5 × 9.5 × 11 cm) containing a metal floor that could deliver mild electric shocks (36 V, 1.5 mA). A guillotine door separated the two compartments. Each mouse was placed into the illuminated chamber, facing away from the door to the dark chamber and allowed to acclimatise for 1 min. As soon as the mouse entered the dark chamber the door was slid back into place, triggering an electric shock. The mouse was immediately removed from the chamber and returned to its cage. The retention test was