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Characterization of cognitive deficits in a transgenic mouse model of Alzheimer's disease and effects of donepezil and memantine

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ABSTRACT

Alzheimer's disease is characterized by a progressive decline in cognitive function and involves β-amyloid (Aβ) in its pathogenesis. To characterize cognitive deficits associated with Aβ accumulation, we analyzed PS1/APP mice overexpressing mutant presenilin-1 (PS1, M146L; line 6.2) and amyloid precursor protein (APP, K670N/M671L; line Tg2576), a mouse model of Alzheimer's disease with accelerated Aβ production. Age-dependent changes in working and spatial memory behaviors were investigated using Y-maze and Morris water maze tasks, respectively, in female PS1/APP mice at ages of 2, 4, 6, and 12 months. Significant deficits in working and spatial memory were observed from 4 and 6 months of age, respectively. Acute single-dose administrations of memantine, a low-to-moderate-affinity *N*-methyl-D-aspartate (NMDA) antagonist, showed improvements in working memory deficits at 4 months of age, whereas donepezil, an acetylcholinesterase (AChE) inhibitor, did not. However, both drugs improved spatial memory dysfunction at 6 months of age at therapeutically relevant doses. No age-related dramatic changes were observed in expression levels of several proteins relating to memory dysfunction and also the mechanisms of donepezil and memantine in the cerebral cortex of PS1/APP mice until 6 months of age. Taken together, these results suggest dysfunctions in cholinergic and/or glutamatergic transmissions may be involved in the cognitive deficits associated with Aβ toxicity. Since donepezil and memantine have been widely used for treating patients of Alzheimer's disease, these results also suggest that cognitive deficits in PS1/APP mice assessed in the Y-maze and Morris water maze tasks are a useful animal model for evaluating novel Alzheimer's disease therapeutics.

1. Introduction

Alzheimer's disease, the most common form of dementia, is a neurodegenerative disorder clinically characterized by a progressive loss of cognitive and behavioral function (Sadowski and Wisniewski, 2007). Its underlying pathogenesis involves neuronal loss relating to accumulation of abnormal extracellular β-amyloid (Aβ) peptides, either as oligomers or as neuritic plaques, as well as an intraneuronal aggregation of hyperphosphorylated tau in the form of neurofibrillary tangles (Blennow et al., 2006). Animal models provide one approach for investigating the pathophysiological processes underlying Alzheimer's disease and the effects of drug therapies. Considerable efforts have been made to date to generate transgenic mice using mutant amyloid precursor protein (APP) and tau to model Alzheimer's disease in the lights of face, constructive and predictive validity.

One such example is the PS1/APP mouse, a transgenic mouse model of accelerated Alzheimer's disease resulting from double overexpression of the human mutant presenilin 1 (PS1, M146L; line 6.2) and amyloid precursor protein (APP, K670N/M671L; line Tg2576) (Holcomb et al., 1998). This mouse shows enhanced production of Aβ, and amyloid burden in this model is first evident in several regions at the age of 4 months, thereafter growing in size and number of Aβ plaques as the animal ages in the hippocampus and cerebral cortex, eventually peaking at 12 months of age, as previously shown using chase-contrast X-ray imaging (Noda-Saita et al., 2006). This rate of Aβ plaque development is faster than in other transgenic mouse models such as Tg2576, PDAPP, APP/PS1 (M146L), and APP/PS1 (line 6.2) mice (Arendash et al., 2001; Dineley et al., 2002; Games et al., 1995; Takeuchi et al., 2000). Besides brain pathological changes of the transgenic mice, functional deficits have also been investigated. PS1/APP mice are shown to have age-dependent memory impairments and a correlation between Aβ levels and synaptic and cognitive deficits has been observed (Arendash et al., 2001; Trinchese et al., 2004). These pre-clinical findings no doubt validate the utility of the transgenic animals and have facilitated the efforts to explore novel therapeutics for Alzheimer's disease, although it warrants further investigations to fully understand the value and limitation of the model and also to further carefully

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compare the phenotypes and outcomes of any pharmacological manipulations with clinical findings.

At present, four drugs have been approved by the Food and Drug Administration (FDA) for the symptomatic treatment of Alzheimer's disease. The three drugs, donepezil, galantamine, and revastigmine as acetylcholinesterase (AChE) inhibitors, were developed because of the observation that cholinergic neurons are lost in the basal forebrain, resulting in a subsequent reduction in cholinergic transmission to the cerebral cortex of brains with Alzheimer's disease (Coyle et al., 1983; Davies and Maloney, 1976). Specially, donepezil acts as a brain-selective, reversible, competitive AChE inhibitor that can stimulate cholinergic transmission and has been shown to improve performance in several pharmacological models of impaired learning and memory (Ogura et al., 2000). Large-scale clinical studies have proven symptomatic efficacy of donepezil treatment in mild, moderate and even severe stages of Alzheimer's disease based on cognitive function, daily activities, and behavior (Birks and Harvey, 2006). The fourth, non-AChE inhibitor drug is memantine, a low-to-moderate-affinity antagonist for *N*-methyl-D-aspartate (NMDA) receptors. Recently, soluble Aβ oligomers have been reported to induce memory impairment and synapse loss by activating NMDA receptors in Alzheimer's disease pathophysiology (Lacor et al., 2007; Lesné et al., 2006, Shankar et al., 2007). Memantine can improve performance in aged rats with impaired baseline memory function (Barnes et al., 1996) and in patients with moderate-to-severe Alzheimer's disease (Reisberg et al., 2003).

While neuropathology and cognitive deficits of these transgenic mice have been well investigated as stated above and have helped to evaluate pharmacological interventions like active and passive immunizations of amyloid and more recently inhibitors of amyloid biosynthesis (e.g. γ-secretase and β-secretase inhibitors) (Chang et al., 2011; Comery et al., 2005), the response to clinically available symptomatic drugs such as donepezil or memantine has not really been investigated in PS1/APP transgenic mice. Therefore, we thought it important to understand the acute response of symptomatic drugs on cognitive deficits of transgenic animals and also investigate pharmacokinetic and pharmacodynamic relationship comparing the data with clinical findings with the drugs in the patient with Alzheimer's disease. To achieve the goal, we firstly investigated age-related impairment of learning and memory functions using behavioral tasks such as Y-maze and Morris water maze tasks in PS1/APP transgenic mice, and also evaluated donepezil and memantine on age-related behavioral changes in this mice. We also investigated the plasma and brain concentration of these drugs at cognitively effective doses in PS1/APP mice to compare with previous clinical observations. The study was also expanded to investigate changes in brain neurochemistry in animals focusing the possible alterations of protein levels of molecules that are related to mechanisms of donepezil and memantine and also to basis of learning and memory.

2. Material and methods

2.1. Transgenic mouse model

Female hemizygous double transgenic PS1/APP mice and their wild-type littermates were obtained by cross-breeding hemizygous transgenic mice overexpress human mutant APP (K670N/M671L; line Tg2576) (Hsiao et al., 1996) licensed from the Mayo Foundation for Medical Education and Research (Rochester, MN, USA) and homozygous transgenic mice overexpressing human mutant PS1 (M146L; line 6.2) (Holcomb et al., 1998). We used no male PS1/APP mice because of their aggressive fighting behavior. The mice were genotyped by PCR using DNA from their tails. Breeding and maintenance of the PS1/APP mice were conducted in Charles River Japan (Atsugi, Japan).

Mixed genotype animals were housed in standard mouse cages under conventional laboratory conditions with a 12:12 light-dark cycle (lights on at 7:30 AM, off at 7:30 PM) at a constant temperature (22 ± 2 °C) and humidity level (55 ± 5%) with food and water available ad libitum. All facilities were approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC), and all experiments were conducted in accordance with the Astellas Pharma Inc. guidelines for the care and use of animals and under approved protocols from the Institutional Animal Care and Use Committee of Astellas Pharma Inc.

2.2. Y-maze task

Working memory performance was examined by recording spontaneous alternation behavior during the Y-maze task (Maurice and Privat, 1997). The maze apparatus was constructed of gray vinyl chloride. Each arm was $40 \times 13 \times 3/10$ cm (length $\times$ height $\times$ width at the bottom/top) and converged at equal angles. Mice were acclimated in the experimental room for at least 1 h. Each mouse was placed at the end of one arm, and the numbers of arm entries and alternations were counted for 8 min. Alternations were defined as entries into all three arms on consecutive occasions. The alternation rate (%) was calculated as follows:

$$\text{Alternation rate (\%)} = (\text{alternations}/\text{ total entry} - 2) \times 100$$

2.3. Morris water maze task

Spatial memory was tested with the Morris water maze task, which consisted of three parts: visible platform training (Day 1), hidden platform training (Days 2–4), and a probe trial (Day 4) with some modification (Trinchese et al., 2004). A circular pool (100 cm in diameter) was filled with opaque water at a temperature of 21 ± 1 °C. During the visible platform training, a clear acrylic platform (9 cm in diameter) was located at the center of one of the four quadrants of the pool, and indicated by a marker that emerged above the water. In the first part of the test (visible platform training), the ability of the mice to locate the visible platform was tested, with differences in vision and motivation excluded by conducting 4 trials. In the second part of the test (hidden platform training), mice were trained to locate the hidden platform during two daily sessions (60 s each, 4 h apart), each consisting of three consecutive trials (designated H1–H6). In the hidden platform training, the escape platform was moved to opposite quadrant, and submerged 1.5 cm below the surface of the water. It was kept in a constant position at the center of one of the four quadrants of the pool during hidden platform training periods. When the mice found the platform, they were allowed to remain on it for 30 s. If the mice did not find the platform within 60 s, they were removed from the water and then placed on the platform for 30 s. In the third part of the test (probe), we examined whether or not the mice spent more time searching in the quadrant that contained the platform (target quadrant) than in the other quadrants of the pool. This test was performed immediately after H6 and gave a putative measure for the retention of spatial memory. Escape latency (time to find the hidden platform) and time spent swimming in the target quadrant were recorded for each trial. The cumulative latencies as sum of 18th hidden platform trials were calculated for each treatment group.

2.4. Drug administration

Donepezil hydrochloride (Aricept® purchased from Eisai Inc., Tokyo, Japan and extracted and purified at Astellas Pharma Inc.)