BEHAVIORAL AND PATHOLOGICAL CHANGES IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER’S DISEASE

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ABSTRACT

Alzheimer’s disease (AD), a neurodegenerative brain disorder, is the most common cause of dementia, characterized by amyloid-β plaque accumulation, intracellular tangles and neuronal loss in selective brain regions. The frontal cortex, important for executive functioning, is one of the regions affected. In this communication, it has been investigated that learning and memory function and pathological changes of APP<sub>swe</sub>/PS1<sub>E9</sub> (APP/PS1) double transgenic mouse. Ten male APP/PS1 double transgenic mice and 10 male C57BL/6J mice were used in this study. The learning and memory functions were studied by Morris water maze, the pathological changes in brain tissue by modified Bielschowsky and Nissl’s staining and the distribution of Aβ plaques in the APP/PS1 mouse brain by immunohistochemistry using avidin-biotinylated complex staining. We found that the APP/PS1 mice provided novel insights into the regional selective vulnerability of the frontal cortex to Alzheimer’s disease and therefore these mice may prove as useful animal models for understanding the pathogenesis of Alzheimer’s disease in people.

Key words: Alzheimer’s disease, APP<sub>swe</sub>/PS1<sub>E9</sub>, Morris water maze, Pathological changes, Transgenic mouse.

INTRODUCTION

Alzheimer’s disease is a severe, progressive brain disorder that affects a significant portion of the aged human population. The neuropathological hallmarks of the disease consist of intracellular and extra-cellular amyloid-β aggregates, intracellular neurofibrillary tangles, and dysfunction of neurons and synapses, resulting in brain atrophy and ultimately neuron loss that is restricted to specific brain regions (De, 2013). Although neuropathology of the brain is well defined, its etiology remains unclear (Liang, 2010). The prevalence of Alzheimer’s disease is predicted to increase significantly to affect over 100 million people worldwide by the year 2050 (Ferri, 2005). Mouse models are considered as one of the most important research tools for finding new treatments for Alzheimer’s disease (Hall, 2012).

MATERIALS AND METHODS

Animals and treatments: Ten each of nine month-old male APP<sub>swe</sub>/PS1<sub>E9</sub> and C57BL/6J strains were used in the experiment. The APP<sub>swe</sub>/PS1<sub>E9</sub> mice express a mouse human hybrid transgene containing Swedish mutations (K594N/M595L) and deleted exon-9. They were generated as previously described (Zong, 2011, and Wang, 2012) and genotyped by PCR. All the mice were housed in a 12 h light/dark cycle conditions, which were fed and given water regularly, Whereas, C57BL/6J mice were normal and served as control group.

Morris water maze: The behavioral test was carried out in the Morris water maze as previously described (Zhang, 2013, and Morris, 1984), starting on the first day as the mouse completed 9 month age. Briefly, a circular black pool (1.2 m in diameter, 55 cm deep) was filled with water (30 cm depth, 22°C). A clear circular platform (10 cm in diameter) was submerged 2 cm underwater in the northeast quadrant of the pool. Each mouse received four trials per day for 6 consecutive days. On the last day, a 2-
minute probe trial was performed with the platform removed. During place navigation trial, mice were placed randomly into the pool facing the wall individually from four preset starting points, and were allowed to swim for a maximum of 120 s or until they found the platform. During spatial probe trial, the platform was removed from the pool and the mice were allowed to swim for 120 s. The total swim time (escape latency), and the number of times the animal crossed the previous location of the platform were recorded by a video tracking system (SLY-WMS Morris Water Maze System; Sunny Instruments Co. Ltd., China).

Tissue preparation: After the Morris water maze tests, mice were deeply anesthetized with sodium pentobarbital (50 mg/kg intraperitoneal injection) and euthanized by decapitation. The brains were quickly removed and fixed in 4% paraformaldehyde in phosphate-buffered saline at 4 °C overnight and embedded in paraffin, cut into sections (5 µm), and stored at room temperature until stained for histopathological analysis.

Nissl’s staining: Sections mounted on poly-L-lysine-coated slides were dehydrated with ethanol and then treated with xylene for 5 min. After being washed with double-distilled water, the sections were incubated with 1% cresyl violet (Sigma-Aldrich) solution for 5 min at 50°C and then dehydrated with ethanol. Images were captured using a visible microscope objective (Li, 2011).

Modified Bielschowsky staining: Modified Bielschowsky gives a good compromise between sensitivity for plaques and tangles and thus can be used as a single stain for diagnosis of Alzheimer’s disease (Nobakht, 2011). Briefly, sections were deparaffinized through xylene and alcohols into tap water before being immersed into fresh 20% silver nitrate solution for 20 min. After washing thoroughly with distilled water, slides were immersed in 20% silver nitrate solution titrated with fresh sodium hydroxide and evaporated ammonium (200 ml of 28% ammonium hydroxide by leaving in an open beaker for 20 min in a fume cupboard). After 15 min, slides were washed with ammonia water before treatment with 100 ml of developer (20 ml of formaldehyde, 100 ml distilled water, 20 µl concentrated nitric acid, and 0.5 g citric acid) adding 50 ml of titrated silver nitrate solution. Slides were then rinsed in tap water, fixed in 5% sodium thiosulfate, and dehydrated through alcohols and xylene.

Immunohistochemistry (IHC): Standard avidin-biotinylated complex staining was performed to analyze the distribution of Aβ plaques in the APP/PS1 mouse brain. Briefly, paraffin sections were dewaxed, rehydrated, and treated in 0.1 M Tris-HCl buffer (Tris-buffered saline, pH 7.4) containing 3% hydrogen peroxide for 10 minutes to reduce endogenous peroxidase activity. After washing with Tris-buffered saline, sections were boiled in citric acid buffer for 3 minutes in a microwave oven. The sections were then rinsed, treated with 5% bovine serum albumin for 30 minutes, and subsequently incubated overnight with mouse anti-Aβ antibody (1:500; Sigma, A5213) at 4 °C. After rinsing, sections were incubated with biotinylated goat anti-mouse IgG (1:200) for 1 hour, followed by streptavidin peroxidase for 1 hour at room temperature. After rinsing, the sections were stained with 0.025% diaminobenzidine for 1 minute. The stained sections were dehydrated, cleared, covered with neutral balsam, and examined with a light microscope equipped with a digital camera (Olympus, Tokyo, Japan).

Statistical analysis: Results are expressed as mean ± S.E. Data of the water maze escape latency was analyzed using repeated measures ANOVA (RM ANOVA) with group as between-subjects factors, for single dependent variables in water maze probe trial, ANOVA were conducted with group as between-subjects factors. All data were analyzed using SPSS17.0 software, and differences were taken to be significant at P < 0.05.

RESULTS AND DISCUSSION

Morris water maze: The ability of the mice to learn and process spatial information was tested by the Morris water maze (Ferretti, 2011). The analysis of the place navigation trial showed that the escape latencies decreased from Day 1 to Day 5 in both the groups (p < 0.05). The APPsw/PS1E9 mice displayed longer escape latencies than C57mice (p < 0.05, Fig. 1A). The spatial probe trial showed that the platform-crossing times were significantly different between groups (p < 0.05, Fig. 1B). Taken together, the result indicated that the APPsw/PS1E9 mice were cognitive deficits on learning and memory performance.
**FIG 1:** Behavioral performance of animals in the Morris water maze. The average escape latency (A) during the place navigation trial, and the number of crossings to the previous location of the platform (B) during the spatial probe trial are presented as means ± SEM. n = 10 per group, *p < 0.05 vs C57 mice group.

**Nissl’s staining:** The Nissl’s staining showed that the neurons of the cerebral cortex of APPswe/PS1E9 mice were edematous and decreased in number.

**Modified Bielschowsky Staining:** The modified Bielschowsky staining showed that the neuro-fibers of the cerebral cortex of APPswe/PS1E9 double transgenic mice were enlarged, swollen, and dense. There were also senile plaques and nerve fiber tangles in the cerebral cortex of APPswe/PS1E9 double transgenic mice.

**Immunohistochemical (IHC):** The results of the IHC showed that there was large number of α-amyloid plaques in the APPswe/PS1E9 mouse brain.

Alzheimer’s disease is a progressive neurodegenerative disease characterized by neuropathological changes such as amyloid plaque deposition, neurofibrillary tangles (NFTs), neuronal loss, dystrophic neuritis, and gliosis, as well as behavioral phenotypes such as learning and memory impairment, anxiety, depression, and other psychological symptoms. It is the most common cause of dementia, affecting 35 million people today, and also, it is a typical age-dependent neurodegenerative disease that affects 5% of individuals > 65 years, 20% of those > 85 years, and more than one-third of those > 90 years.

Tremendous progress in understanding the pathophysiology of Alzheimer’s disease has been made in the last 20 years. Current emphasis is to find the new treatments of Alzheimer’s disease due to its increasing prevalence in aging population. Besides, in the absence of proper preventative and therapeutic efforts, its prevalence will continue to increase as life expectancy increases (Nojima, 2011). At the same time, animal model of Alzheimer’s disease are used to study the mechanisms underlying Alzheimer’s disease pathogenesis, genetic interactions with genes of interest, and environmental risk factors that cause sporadic Alzheimer’s disease as well as to test the therapeutic
FIG 3: Modified Bielschowsky staining. The cerebral cortex of C57 mice group (A, B, and C). The cerebral cortex of APP<sub>swe</sub>/PS1<sub>ΔE9</sub> mice group (D, E, and F). (A) The cerebral cortex of C57 mice (10×); (B) The cerebral cortex of C57 mice (20×); (C) The cerebral cortex of C57 mice (40×); (D) The cerebral cortex of C57 mice (10×), the neuron fibers of the cerebral cortex of APP<sub>swe</sub>/PS1<sub>ΔE9</sub> double transgenic mice reveal enlargement, swelling, and are more dense. (E) The cerebral cortex of C57 mice (20×); (F) The cerebral cortex of C57 mice (40×), the senile plaques are very clear.

FIG 4: Micrographs of immunohistochemistry (IHC)-prepared brain tissue reveal amyloid-β (Aβ)-positive plaques in the transgenic mouse brain. Large number of β-amyloid plaques in APP<sub>swe</sub>/PS1<sub>ΔE9</sub> mice (A, B), but no β-amyloid plaques in the mice of control group (C, D) are visible.

Effects of Alzheimer’s disease drug-candidates on neuropathology and cognitive function. Therefore, mouse models are one of the most important research tools for finding new treatments for Alzheimer’s disease.

Familial Alzheimer’s disease (FAD) is based on disease-causing mutations, there are strong evidence about the connection between FAD and specific genetic factors. Though FAD accounts for only a small percentage of Alzheimer's disease cases, the transgenic mouse models are considered the most common animal models of Alzheimer's disease. Moreover, the discovery of Alzheimer’s disease-associated genes has provided the foundation for development of mouse models. Small numbers of FAD cases have been linked to APP mutations (Goate, 1991) and a larger subgroup to Presenilin1/2 mutations (Armstrong, 2013). Presenilin 1 (PS1 or PSEN1) or presenilin 2 (PS2 or PSEN2) have already been identified in FAD (Bakulski, 2012). Thus, expressing these genes in mice is the basis of Alzheimer’s disease mouse models.

Alzheimer’s disease characterized by Aβ plaque accumulation, the most common of Aβ is Aβ42, is found largely in discrete Aβ deposits, whereas the more soluble Aβ40 found around blood vessels may develop later in the disease (Delacourte, 2002). Aβ is a cleavage product of APP. APP mutations are named according to the geographic location in which the affected family originated, and
it is the first gene mutation identified as a cause of autosomal dominant Alzheimer’s disease. It is one of the type I transmembrane proteins, which has a large amino-terminal extracellular domain. Alzheimer’s disease-causing mutations in APP occur predominantly at the two cleavage sites. There are four kinds of APP restructuring isomers, which are expressed in humans consisting of 695, 714, 751 or 770 amino acid residues. For example, The K670N/M671L double mutation was originally found in a Swedish family, and it increased both Aβ40 and Aβ42 production.

Another important mutation of autosomal dominant of Alzheimer’s disease occurs in the presenilin genes (Quiroz, 2013), and the most common type of FAD is linked to mutations of the PS genes. PS may have a variety of other functions. The PS1 gene may be involved in notch signalling (Steiner, 1999), and therefore is important in cell differentiation. PS1/2 genes may also be involved in cellular calcium homeostasis, thus, there the preseninil mouse models became important. Other genes encoding apolipoprotein E (apoE) (Reiman, 2009), Tau, UBQLN1 (ubiquilin-1), â2-M (Depboylu C, 2006), BIN1 (Hollingworth, 2011), CLUABCA7, PICALM (Koldamova, 2014), CR1 (Jun, 2010), shave also recently been associated with late-onset, sporadic Alzheimer’s disease through genome-wide association studies (Naj, 2011).

By and large, mice have a high degree of phylogenetic conservation with humans in the architecture and function of the hippocampal and entorhinal cortex circuits that mediate episodic memory and are vulnerable in Alzheimer’s disease. And also, they have a similar number of genes and considerable chromosomal synteny with humans. At the same time, mouse models provide a system that is reductionist enough to facilitate experimental manipulation. Therefore, the mouse models fill a unique niche in Alzheimer’s disease research. Especially with the wide variety of lines available and continuing technological advances, there is every reason to believe that mouse models will continue to be invaluable tools in the drug discovery pathway for AD treatment.

There are a wide variety of transgenic animal model of Alzheimer’s disease, including single genetically modified/double genetically modified and multiple genetically modified transgenic animal models. In our study, we used double transgenic APPswe/PS1ΔE9 mice to observe the learning memory and pathological changes in the cerebral cortex of Alzheimer’s disease. The mice expressed the human APP with the Swedish mutations (K670N/M671L) at the â-secretase cleavage site and PS1 (PS1ΔE9) (Yue Yang, 2013). These mice were on a pure C57BL/6J background. APPswe/PS1ΔE9 mice have previously been reported to demonstrate impaired learning and memory in the Morris water maze test (Cheng, 2014). And, we had the similar result. During the place navigation trial, the escape latency in the APPswe/PS1ΔE9 double transgenic mice was longer than that of the mice of C57BL/6J. During spatial probe trial, the platform-crossing times in the APPswe/PS1ΔE9 double transgenic mice were different from the mice of C57BL/6J. The modified Bielschowsky and Nissl’s staining showed significant pathological changes similar to those noticed in AD.

Behavioral features, histological, and immunohistochemical changes of the APPswe/PS1ΔE9 mice in this study, provided novel insights into phenotypic characteristic of APPswe/PS1ΔE9 mice. In conclusion, the double transgenic APPswe/PS1ΔE9 mice may serve as useful model of Alzheimer’s disease in man.

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