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Research article

Oxidative stress accelerates amyloid deposition and memory impairment in a double-transgenic mouse model of Alzheimer's disease

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HIGHLIGHTS

- We have established a novel mouse model of Alzheimer's disease (the APP/DAL mouse).
- Pathological changes and memory impairment are accelerated in the APP/DAL mouse.
- The APP/DAL mouse is useful for examining the role of oxidative stress in the progression and pathogenesis of Alzheimer's disease.

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ABSTRACT

Oxidative stress is known to play a prominent role in the onset and early stage progression of Alzheimer's disease (AD). For example, protein oxidation and lipid peroxidation levels are increased in patients with mild cognitive impairment. Here, we created a double-transgenic mouse model of AD to explore the pathological and behavioral effects of oxidative stress. Double transgenic (APP/DAL) mice were constructed by crossing Tg2576 (APP) mice, which express a mutant form of human amyloid precursor protein (APP), with DAL mice expressing a dominant-negative mutant of mitochondrial aldehyde dehydrogenase 2 (ALDH2), in which oxidative stress is enhanced. Y-maze and object recognition tests were performed at 3 and 6 months of age to evaluate learning and memory. The accumulation of amyloid plaques, deposition of phosphorylated-tau protein, and number of astrocytes in the brain were assessed histopathologically at 3, 6, 9, and 12–15 months of age. The life span of APP/DAL mice was significantly shorter than that of APP or DAL mice. In addition, they showed accelerated amyloid deposition, tau phosphorylation, and gliosis. Furthermore, these mice showed impaired performance on Y-maze and object recognition tests at 3 months of age. These data suggest that oxidative stress accelerates cognitive dysfunction and pathological insults in the brain. APP/DAL mice could be a useful model for exploring new approaches to AD treatment.

1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases involving cognitive impairment. The discovery of AD-responsible mutations in the human amyloid precursor protein (APP) has enabled construction of transgenic animal models. PDAPP [1], Tg2576 [2], and APP23 [3] are mouse models of AD harboring these mutations and showing several features of AD. However, such models rarely entirely replicate human pathological features, such as neurofibrillary tangles (NFTs) or neuronal degeneration [1–3].

Aging is the most important risk factor for AD. Growing evidence suggests that age-dependent oxidative stress is a characteristic feature of AD brains. Several studies have reported elevated oxi-

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dation of DNA, RNA, proteins, and lipids in AD brains [4,5]. The plasma levels of antioxidants, such as vitamin C and E, are lower in patients with AD than in the healthy aged population, and the activity of antioxidant enzymes, such as catalase and glutathione peroxidase, is also impaired in AD brains [6,7]. Furthermore, subjects with mild cognitive impairment exhibit elevated oxidative stress, lower antioxidant levels, and impaired antioxidant enzyme activity [8,9]. Thus, oxidative stress plays important roles in the onset and progression of AD pathogenesis.

We have constructed transgenic mice (DAL mice) expressing a dominant-negative mutant form of mitochondrial aldehyde dehydrogenase 2 (ALDH2), which detoxifies 4-hydroxy-2-nonenal (HNE), an end product of lipid peroxidation [10]. Lipid peroxidation is a major source of oxidative stress-mediated injury that directly damages neuronal membranes and contributes to oxidative damage in the pathogenesis of neurodegenerative disorders [4,5]. DAL mice exhibited a decreased ability to detoxify HNE in cortical neurons and an accelerated accumulation of HNE in the brain [10]. ALDH2 protein was localized in the mitochondria and we found increased levels of HNE adduct proteins in the mitochondrial fraction of DAL mice [11]. DAL mice show age-dependent neurodegeneration and cognitive decline and have a shortened lifespan. Additionally, ALDH2-deficient cells have a greater level of oxidative stress, as shown by oxidation–reduction sensitive green fluorescent protein (GFP) [11].

In order to investigate the role of oxidative stress in AD, we produced double-transgenic mice (APP/DAL mice) by crossbreeding DAL mice with APP mice. We investigated the pathological changes in this novel transgenic mouse model and assessed their performance on learning and memory tasks.

2. Materials and methods

2.1. Mice

Tg2576 (APP) mice [2], expressing human APP containing the Swedish mutation ($Lys^{670}Asn$ and $Met^{671}Leu$), were licensed from the Mayo Foundation for Medical Education and Research (Rochester, MN, USA). DAL101 (DAL) mice, expressing the dominant-negative form of mitochondrial aldehyde dehydrogenase 2 (ALDH2) [10], were purchased from Mitos Co., Ltd. (Kawasaki, Japan). We crossbred the APP with DAL mice to bear double transgenic (APP/DAL), single transgenic (APP and DAL), and wild-type mice. These mice were maintained by brother-sister mating to produce progeny with same genetic background. All experimental protocols were approved by both the Animal Care Committee of Nippon Medical School and Institutional Animal Care and Use Committee of Astellas Pharma Inc. Astellas Pharma Inc., Tsukuba Research Center was awarded Accreditation Status by the AAALAC International.

2.2. Immunohistochemistry

Mouse brains were collected after anesthesia and perfusion. Brains were fixed with 4% paraformaldehyde and embedded in paraffin. For Aβ staining, the sections were treated with 99% formic acid for 5 min at room temperature and incubated in Immunosaver (Nisshin EM Corp., Tokyo, Japan) according to the manufacturer's instructions. We used anti-Aβ40 (0.5 μg/mL, AnaSpec Inc., Fremont, CA, USA) and anti-Aβ42 (0.4 μg/mL, AnaSpec Inc., Fremont, CA, USA) primary antibodies and visualized the staining with an ABC kit (Vectastain; Vector Laboratories, Burlingame, CA, USA). The sections were counterstained with hematoxylin (Muto Pure Chemicals Co., Ltd., Tokyo, Japan). For paired helical filament (PHF)-tau staining, sections were incubated in anti-PHF-tau (1 μg/mL, Thermo Fisher Scientific Inc., Waltham, MA, USA) and counterstained with Congo red (Cosmo Bio Co., Ltd., Tokyo, Japan). Anti-glial fibrillary acidic protein (GFAP) antibody (0.1 μg/mL, Dako, Glostrup, Denmark) was used to stain astrocytes. Semi-quantification of Aβ deposits was carried out by counting the number of plaques per coronal section per mouse ($n = 5$). Semi-quantification of phosphorylated tau protein was measured as the mean area of PHF-tau protein on the periphery of each amyloid plaque per five visual fields (1.0 mm^2; × 20) per section per mouse ($n = 5$; ImageJ, National Institutes of Health, Bethesda, MD, USA), while that of astrocytes was measured by counting the number of GFAP-positive cells in the CA1 region of the hippocampus and in the cerebral cortex adjacent to the CA1 per visual field (×20).

2.3. Y-maze spontaneous alternation test

The Y-maze spontaneous alternation test, as previously described, examined recognition memory [12]. Briefly, mice were placed into the start arm and allowed to habituate to the maze environment for 10 min. The next day, the mice were placed at the end of the start arm and allowed to move freely through the maze for 8 min. The percentage of spontaneous alternations was calculated as the ratio of the number of alternations to the total number of arm entries.

2.4. Object recognition test

The object recognition test was used as a second measure of recognition memory [13]. Mice were habituated in a test chamber (25 cm wide × 25 cm long × 40 cm high) for 24 h before training. During the training session, two identical objects (round filter units, diameter 33 mm, height 27 mm) were placed in the chamber and mice were allowed to explore for 10 min. The following day, one of the familiar objects was replaced with a novel object (plastic cone, diameter 25 mm, height 30 mm). Object recognition index was defined as the percentage of time spent sniffing or touching the novel object with the nose during a 5 min session. All training and testing trials were video recorded and analyzed using EthoVision XT8.5 (Noldus Information Technology, Wageningen, Netherlands).

2.5. Statistical analysis

Data were analyzed using an unpaired two-tailed student's *t*-test or analysis of variance (ANOVA) followed by Tukey's test. The Kaplan–Meier method was used to calculate survival curves, and survival periods were compared with the log-rank test. A $P < 0.05$ was considered statistically significant. JMP9 software (SAS Institute Inc., Cary, NC, USA) was used for all statistical analysis.

3. Results

3.1. Life span and body weight of APP/DAL mice

Kaplan–Meier survival curves show that mice of all genotypes began dying by 120 days of age, but after 240 days, the rate of death among APP/DAL mice was more rapid than that of the other genotypes (Supplementary Fig. 1A). APP/DAL mice had a significantly shortened life span compared with other mice (log-rank test, $P < 0.0001$).

Body weights of all mice were recorded monthly (Supplementary Fig. 1B). APP/DAL mice weighed significantly lesser than WT or DAL mice (two-way repeated measures ANOVA, $P < 0.0001$). There were no significant differences in body weight between APP and APP/DAL mice ($P = 0.12$).

3.2. Onset of impairment in spatial learning and memory in APP/DAL mice

We used Y-maze and object recognition tests to examine whether oxidative stress leads to an acceleration of spatial learning