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ENRICHED ENDOGENOUS n-3 POLYUNSATURATED FATTY ACIDS ALLEVIATE COGNITIVE AND BEHAVIORAL DEFICITS IN A MICE MODEL OF ALZHEIMER'S DISEASE

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Abstract—Alzheimer's disease (AD) is a progressive neurodegenerative disorder that accompanied by memory deficits and neuropsychiatric dysfunction. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) have seemly therapeutic potential in AD, but the benefit of n-3 PUFAs is still in debates. Here, we employed a transgenic mice carry *fat-1* gene to encode n-3 desaturase from *Caenorhabditis elegans*, which increase endogenous n-3 PUFAs by converting n-6 PUFAs to n-3 PUFAs crossed with amyloid precursor protein (APP) Tg mice to evaluate the protective effects of endogenous n-3 PUFAs on cognitive and behavioral deficits of APP Tg mice. We fed APP, APP/*fat-1* and *fat-1* mice with n-6 PUFAs rich diet. Brain tissues were collected at 3, 9 and 12 months for fatty acid and gene expression analysis, histology and protein assays. Morris Water Maze Test, open field test and elevated plus maze test were performed to measure the behavior capability. From the results, the expression of *fat-1* transgene increased cortical n-3: n-6 PUFAs ratio and n-3 PUFAs concentrations, and sensorimotor dysfunction and cognitive deficits in AD were significantly less severe in APP/*fat-1* mice with endogenous n-3 PUFAs than in APP mice controls. The protection against disturbance of spontaneous motor activity and cognitive deficits in AD was strongly correlated with increased n-3: n-6 PUFAs ratio and endogenous n-3 PUFAs, reduced APP generation, inhibited amyloid β peptide aggregation, suppressed nuclear factor-kappa B and astroglia activation, and reduced death of neurons in the cortex of APP/*fat-1* mice compared with APP mice controls. In conclusion, our study demonstrates that an available medication with the maintenance of enriched n-3 PUFAs in the brain could slow down cognitive decline and prevent neuropsychological disorder in AD.

Key words: Alzheimer's disease, polyunsaturated fatty acids, amyloid precursor protein.

INTRODUCTION

Alzheimer's diseases (AD) are a progressive, degenerative brain disease that accompanied by progressive memory disorders, cognitive dysfunction, and psychiatric symptoms (Small et al., 2007; Amihaesei et al., 2013; Reitz and Mayeux, 2014). Current reports indicate that aging, which is one of the biggest challenges worldwide, is the primary risk factor for AD (Alzheimer's, 2013). The characterized neuropathological changes in AD patients are presence of amyloid β (Aβ) deposits, formation of neurofibrillary tangles, and loss of neurons (De-Paula et al., 2012; Godoy et al., 2014). Aggregated Aβ has been proved to play a key role in the initiation of AD (Pimplikar et al., 2010). As the disease progresses, aggregated Aβ causes loss of neurons by releasing neurotoxic inflammatory mediators and introducing oxidative stress, which eventually leads to progressive cognitive dysfunction and mental behavior change (Pimplikar et al., 2010; Zhu et al., 2014). Therefore, a potential beneficial method for AD control is to attenuate Aβ depositions and protect neurons.

Over the past 20 years, omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been widely recognized for reducing AD risk as a potential nutritional product (Palacios-Pelaez et al., 2010; Cederholm et al., 2013; Mohajeri et al., 2015). n-3 PUFAs compose approximate 20% of the brain's dry weight, and one third of total fats in the central nervous system (Bourre et al., 1991; Prior and Galduroz, 2012). In addition, n-3 PUFAs are involved in the regulation of oxidative stress, neurogenesis and

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Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; APP, amyloid precursor protein; EDTA, ethylenediaminetetraacetic acid; GFAP, glial fibrillary acidic protein; MAP2, microtubule-associated protein 2; n-3 PUFAs, Omega-3 polyunsaturated fatty acids.

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neurons apoptosis (Zhao et al., 2011). Evidences suggest that high dose n-3 PUFA supplementation is associated with increased volume of gray matter and right hippocampus, which are found pathologically atrophic in AD patients (Conklin et al., 2007). Hence, most animal or *in vitro* studies in AD with supplemental n-3 PUFAs showed positive outcomes (Hossain et al., 2009; Oster and Pillot, 2010; Cunnane et al., 2013). Nevertheless, the negative results from clinical trials indicate that n-3 PUFAs dietary intake does not improve cognitive decline in advanced AD (Fotuhi et al., 2009; Quinn et al., 2010). Thus, in our view there is still insufficient evidence to link supplemental n-3 PUFAs with protection of AD, and some studies suggested that the conventional animal models with supplement of dietary n-3 PUFAs are inappropriate for AD research (Cunnane et al., 2013).

n-3 PUFAs cannot be synthesized endogenously in mammals, therefore depend on dietary intake. Traditional animal models using n-3 PUFAs diet are difficult to handle because of poor control of PUFAs composition and ratio. They are also easily interfered by feeds (Balk et al., 2007). One of reasons is that n-3 PUFAs are very susceptible to reactive oxygen species and lipid peroxidation, approximately 1% oxidized DHA was sufficient to significantly increase Aβ production (Grimm et al., 2016). Another reason associated with a reduction of DHA crossing the blood–brain barrier in 3xTg-AD mice was a deficit in n-3 PUFAs in the brain (Bourasset et al., 2009).To avoid potential confounding dietary factors, Kang et al. introduced transgenic *fat-1* mouse that can endogenously synthesize n-3 PUFAs and therefore increase n-3/n-6 PUFAs ratio (Kang et al., 2004). The *fat-1* gene from *Caenorhabditis elegans* encodes n-3 desaturase that converts n-6 PUFAs to n-3 PUFAs by feeding high n-6 PUFAs diet, which have been proved to decrease the n-3/n-6 PUFAs ratio and may negate the positive benefits of n-3 supplementation irrespective of n-3 intake in AD (Jicha and Markesbery, 2010). The *fat-1* mice have high n-3 PUFAs and low n-6 PUFAs concentrations in numerous tissues including the brain (Kang et al., 2004; Das and Puskas, 2009). Although a previous report demonstrated that expression of endogenous n-3 PUFAs in 3xTg-AD/*fat-1* mice decreases soluble $A\beta_{42}$ levels and both phosphorylated tau levels and therefore improved AD-like neuropathology (Lebbadi et al., 2011), there's still a lack of longitudinal behavioral data. Besides, the 3xTg-AD mice showed independent Aβ and tau pathologies that differed from human AD. Comparing with 3xTg-AD mice developed extracellular Aβ deposits around 4–6 months of age (Oddo et al., 2003), the same symptom in APP mice reported to start at 9 months (Dong-mei et al., 2011), which likes human age of onset. Therefore, to obtain accurate preclinical data of the impact of n-3 PUFAs on Aβ in the progression of AD, we crossed the Swedish mutation human amyloid protein precursor $695^{K595N/M596L}$ (APP) transgenic mice with the *fat-1* mice. The aim of this study is to further evaluate the protective effects of endogenous n-3 PUFAs on cognitive and behavioral deficits of APP mice.

EXPERIMENTAL PROCEDURES

Animals and diets

Transgenic animals were of C57BL/6 background. *Fat-1* mice were obtained from Dr. Jing X. Kang (Kang et al., 2004). APP transgenic mice, containing the Swedish mutation human amyloid protein precursor $695^{K595N/M596L}$ (APP), were obtained from Institute of Laboratory Animal Science (License No. SYXK -BJ-2009-0003) (Zhang et al., 2003). The heterozygous APP, APP/*fat-1*, *fat-1* mice and WT littermates were acquired by crossing male heterozygous *fat-1* mice with female heterozygous APP mice. The *fat-1* and APP genotypes of each mouse were identified by PCR assay. Ear punches were incubated with 10 mM NaOH and 0.1 mM EDTA for 2 h at 95 °C and submitted to 2-step PCR with Titanium Taq (Takara Bio, Inc., China). The following sets of primers were used: *fat-1*-F: 5′-CTGCACCACGCCTTCACCAACC-3′; *fat-1*-R: 5′-CACAGCAGCAGATTCCAGAGATT-3′; APP-F: 5′-GACTGACCACTCGACCAGGTTCTG-3′; APP-R: 5′-CTTGTAAGTTGGATTCTCATATCCG-3′. Amplification of a 250-bp band confirmed the *fat-1* genotype, another 344-bp band conformed the APP genotype. In this study, weight-matched male mice aged 4 weeks were divided into four groups: APP, APP/*fat-1*, *fat-1* and WT. All of the mice were fed in the same conditions, using a modified diet containing 10% corn oil (TROPHIC Animal Feed High-tech Co., Ltd, China), with a fatty acid profile rich in n-6 PUFAs (mainly linoleic acid) and low in n-3 PUFAs (< 0.05% of the total fat content). All animal care and procedures were performed in Guangdong Laboratory Animal Monitoring Institute (GDLAMI) animal facility. The study was approved by the Institution Animal Care and Use Committee at the GDLAMI and the methods were carried out in accordance with the approved guidelines.

Tissue preparation

The mice were euthanized at 3, 9 and 12 months of age under deep anesthesia with 10% chloral hydrate (3.5 ml/kg each) and transcardially perfused with 1× PBS (pH7.4). Brains were collected, the left hemisections were immersion fixed with 4% paraformaldehyde overnight, embedded in paraffin with regular procedures. Sections with thickness of 8 μm were prepared for histology. The cortex of right hemisection was dissected on ice and stored at −70 °C for fatty acid analyses and Western blot assay. The remaining sections were also dissected respectively and stored as frozen stock at −70 °C.

Brain fatty acid analysis

The composition and concentration of PUFAs in the cortex were determined using GS-FID as described previously (Masood et al., 2005). Briefly, the cortex tissues were ground to powder in liquid nitrogen and homogenized in 1× PBS (pH7.4). The bottom phase was collected, after centrifugation at 4000 rpm for 10 min. The extracts were dried in nitrogen and subjected to fatty acid methylation 14% boron trifluoride-methanol reagent