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Chronic alpha-linolenic acid treatment alleviates age-associated neuropathology: Roles of PERK/eIF2α signaling pathway

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ABSTRACT

Aging is a principal risk factor for neurodegenerative diseases and especially shares similar pathologic mechanisms to Alzheimer's disease (AD). Amyloid-β (Aβ) plaques deposition and neurofibrillary tangles (NFTs) are the prominent age-dependent pathologies implicated in the cognitive deficits. Accumulation of mis-folded proteins in the endoplasmic reticulum triggers a cellular stress response called the unfolded protein response (UPR), the activation of which is increased in AD patients. However, the UPR relates to the pathological hallmarks of aging is still elusive. In this study, we report that long-term supplement of α-linolenic acid (ALA), starting before the onset of disease symptoms (6 month-old), prevents the age-related memory deficits during natural aging. The amelioration of the memory impairment is associated with a decrease in UPR related markers [glucose regulated protein 78 (GRP78), protein kinase RNA-like endoplasmic reticulum kinase (PERK), eukaryotic Initiation Factor 2α (eIF2α)]. ALA suppressed the PERK/eIF2α signaling, which may be responsible for multifaceted memory-deteriorating and neurodegenerative mechanisms, including inhibition of Aβ production by suppressing β-site APP-cleaving enzyme 1 (BACE1) expression, enhancement of cAMP response element binding protein (CREB) function via down-regulating activating transcription factor 4 (ATF4), and suppression of Tau phosphorylation by inhibiting glycogen synthase kinase 3β (GSK-3β) pathway. Taken together, our findings provide new insights into the link between ALA and PERK/eIF2α signaling, which could contribute to a better understanding of an ALA-mediated protective effect in aging-associated neuropathology.

1. Introduction

Cognitive deficits including learning impairment and delayed amnesia are considered to be one of the most prominent debilitating consequences of deterioration in brain function that occurs along with aging (Erickson and Barnes, 2003; Hatanpaa et al., 1999). Progression of neuropathology in aging shows that amyloid plaques can appear in the neocortex and hippocampus, whereas

Abbreviations: Aβ, amyloid-β; AD, Alzheimer's disease; ALA, α-linolenic acid; ANOVA, analyses of variance; APP, amyloid-β precursor protein; ATF4, activating transcription factor 4; BACE1, β-site APP-cleaving enzyme 1; BSA, bovine serum albumin; CAA, cerebral amyloid angiopathy; CREB, cAMP response element binding protein; DHA, docosahexaenoic acid; eIF2α, eukaryotic Initiation Factor 2α; ELISA, enzyme-linked immunosorbent assay analyses; EPA, eicosapentaenoic acid; ER, endoplasmic reticulum; FAME, fatty acid methyl esters; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GRP78, glucose regulated protein 78; GSK-3β, glycogen synthase kinase 3β; HFD, high fat diet; LSD, least significant difference; MUFA, monounsaturated fatty acid; MWM, Morris Water Maze; NFT, neurofibrillary tangle; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PUFAs, polyunsaturated fatty acids; RT-PCR, reverse transcription-polymerase chain reaction; SD, Sprague–Dawley; SFA, saturated fatty acid; ThT, Thioflavin-T; UPR, unfold protein response.

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neurofibrillary tangles (NFTs) are localized predominantly to the entorhinal cortex in cognitively intact aging individuals (Yankner et al., 2008), indicating that advancing age is associated with the accumulation of Alzheimer's disease (AD)-specific mis-folded proteins in the brain.

The presence of mis-folded proteins in the endoplasmic reticulum (ER) triggers ER stress and the subsequent unfolded protein response (UPR), a protective cellular mechanism that induces the transient shutdown of global protein synthesis through phosphorylation of eukaryotic Initiation Factor-2α (eIF2α) (Brown and Naidoo, 2012). However, sustained translational repression of global protein synthesis resulting from overactivation of the eIF2α phosphorylation pathway may lead to synaptic failure accompanied by abnormal expression of synaptic proteins, neurodegeneration, and memory deficits (Erguler et al., 2013). Remarkably, protein kinase RNA-like endoplasmic reticulum kinase (PERK), an eIF2α kinase, has been shown to play a key role in mediating persistently high levels of eIF2α phosphorylation following exposure to mis-folded proteins (Ohno, 2014). Whereas eIF2α phosphorylation inhibits general translation initiation, it is known to paradoxically cause translational activation of a subset of messenger RNAs including β-site APP-cleaving enzyme 1 (BACE1), and cAMP response element binding protein (CREB) repressor-transcriptional modulator activating transcription factor 4 (ATF4), which are closely associated with the development of Aβ production and deficient memory formation (Ohno, 2014). Moreover, the eIF2α kinases also control the nuclear localization and activation of glycogen synthase kinase 3β (GSK-3β), leading to the induction of Tau phosphorylation and even related NFT generation (Baltzis et al., 2007). Therefore, aberrant PERK/eIF2α signaling in UPR may underlie age-dependent pathologies and memory impairment, suggesting that targeting PERK/eIF2α signaling pathway may have great therapeutic potential in aging and age-associated disorders.

Converging epidemiological studies suggest that dietary n-3 polyunsaturated fatty acids (PUFA) are likely to be involved in the pathogenesis of mood symptoms and cognitive disorders linked to aging (Barberger-Gateau et al., 2002; Feart et al., 2008; Frasure-Smith et al., 2004). As a nutritionally essential PUFA, α-linolenic acid (ALA, 18:3 n-3) can act as the precursor of longer chain n-3 PUFA (docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3)) or compete with linoleic acid to reduce arachidonic acid content or direct interaction with ion channels and nuclear receptors, and thus may exert numerous beneficial effects in human body, such as anti-oxidative, anti-inflammatory and neuroprotective functions as well as accelerating brain growth in preterm and neonates (Kim et al., 2014). To date, the merit given to ALA is largely due to its conversion to DHA and/or EPA, and studies focus on the neuroprotective efficacies of ALA *per se* are limited. Recent *in vitro* studies have demonstrated that ALA protects against saturated fatty acid (SFA, including palmitic acid and stearic acid) induced-lipotoxicity through inhibition of ER stress and UPR (Katsoulieris et al., 2009; Zhang et al., 2011). However, whether ALA is capable to alleviate the cognitive impairment and AD-like pathology during natural aging via regulation of ER stress and UPR remains unclear.

In the present study, therefore, we investigated the effect of flaxseed oil and its major component ALA, which makes up almost 57.82% of the total oil, as dietary supplement during the rat life span on age-related changes in brain and the molecular mechanisms behind it.

2. Materials and methods

2.1. Rat model and treatment

Six-month old female Sprague–Dawley (SD) rats (Vital River Laboratory Animal Center, China) were fed with a powder diet for 1 week of acclimatization, and then randomly assigned to a standard diet or three high-fat diet (HFD) groups of 30 animals each (Table 1): Aged control (AIN-93M diet), HF (10% lard), L-ALA (5% lard + 5% flaxseed oil), and H-ALA (10% flaxseed oil). Commercial deodorized lard was purchased from a local supermarket. The flaxseed oil (food grade) was obtained from Caoyuankangshen Food Co., Ltd (Inner Mongolia, China). We used female rats because of their increased sensitivity to cognitive decline with normal aging and an earlier onset and faster progression of neurodegenerative disease (Halbreich et al., 1995; Hebda-Bauer et al., 2007). Moreover, they do not develop hypertension during the first year on a HFD (Roberts et al., 2000). With an addition of 10% (w/w) fat, the energy for fat in three HFD groups was equal and increased by 20% (Table 1). There is a common agreement that the best animal model of the normal and pathological aging in humans is the aging animal itself (Zhao et al., 2009). A diet rich in saturated fat can contribute to cognitive decline in aging (Wu et al., 2004) and accelerate the course of AD (Molteni et al., 2002). Importantly, the ability of dietary treatment to prevent cognitive decline may depend on when treatment is initiated (Zhang et al., 2010; Zhao et al., 2009), while limited intervention is possible for initiation of treatment in elderly individuals that present with memory complaints or those previously diagnosed with memory impairments (Visser and Verhey, 2008). Accordingly, the rat model of age-related phenotypes was established by natural aging rats with high fat diets, while ALA continuously supplement for 12 months. Thirty young female SD rats (3 months of age) were obtained from the same supplier and fed on the AIN-93M diet (Young group). Young and Aged rats were served as blank controls, while the HF rats were baseline controls.

The rats were provided food and water *ad libitum*, and were kept in standard conditions of temperature (22 ± 2 °C) and a 12 h light–dark cycle. All animals were weighed at the beginning and at weekly intervals during the study. The experiments were carried out according to the current regulations of the Institutional Animal Ethics Committee, and were approved by the Tongji Medical College Council on Animal Care Committee, Huazhong University of Science and Technology, China. During the experiment, food consumption was measured daily, body weights and health status were monitored weekly by a veterinarian, and any animals with overt signs of chronic respiratory distress, infection, or tumors were removed from the study.

Table 1
Composition of the experimental diets (g/kg diet).

Ingredients	Control	HF	L-ALA	H-ALA
Corn starch	495.7	367.2	367.2	367.2
Dextrinized cornstarch	125	125	125	125
Sucrose	100	100	100	100
Casein	140	157	157	157
L-Cystine	1.8	2	2	2
Soybean oil	40	40	40	40
Lard	0	100	50	0
Flaxseed oil	0	0	50	100
Cellulose	50	56	56	56
Mineral Mix	35	39	39	39
Vitamin Mix	10	11	11	11
Choline bitartrate	2.5	2.8	2.8	2.8
Total	1000	1000	1000	1000
Energy[1], kcal	3850	4308.5	4308.5	4308.5
ΣFat, % of energy	9.3	29.3	29.3	29.3

Control: regular diet; HF: 10% lard diet; L-ALA: 5% lard + 5% flaxseed oil; H-ALA: 10% flaxseed oil.
The energy value for the lard and/ or flaxseed oil diet was adjusted based on the formulation of AIN-93 M by reducing the amount of carbohydrate content (corn starch).
[1] Corn starch, dextrinised cornstarch, sucrose were calculated as carbohydrate; casein, L-cystine were calculated as protein; soybean oil, lard, flaxseed oil were calculated as fat; cellulose, minerals, choline bitartrate were ignore.