Neuroscience 332 (2016) 160–169

GENDER DIFFERENCES OF PERIPHERAL PLASMA AND LIVER METABOLIC PROFILING IN APP/PS1 TRANSGENIC AD MICE

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Abstract—Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive impairment. Currently, there is less knowledge of the involvement of the peripheral biofluid/organ in AD, compared with the central nervous system. In addition, with reported high morbidity in women in particular, it has become very important to explore whether gender difference in the peripheral metabolome is associated with AD. Here, we investigated metabolic responses of both plasma and liver tissues using an APP/PS1 double mutant transgenic mouse model with NMR spectroscopy, as well as analysis from serum biochemistry and histological staining. Fatty acid composition from plasma and liver extracts was analyzed using GC-FID/MS. We found clear gender differences in AD transgenic mice when compared with their wild-type counterparts. Female AD mice displayed more intensive responses, which were highlighted by higher levels of lipids, 3-hydroxybutyrate and nucleotide-related metabolites, together with lower levels of glucose. These observations indicate that AD induces oxidative stress and impairs cellular energy metabolism in peripheral organs. Disturbances in AD male mice were milder with depletion of monounsaturated fatty acids. We also observed a higher activity of delta-6-desaturate and suppressed activity of delta-5-desaturate in female mice, whereas inhibited stearoyl-CoA-desaturase in male mice suggested that AD induced by the double mutant genes results in different fatty acids catabolism depending on gender. Our results provide metabolic clues into the peripheral biofluid/organs involved in AD, and we propose that a gender-specific scheme for AD treatment in men and women may be required.

Key words: Alzheimer's disease, gender difference, metabonomics, peripheral, liver extracts, fatty acids.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia among older people and often displays progressive, irreversible problems with memory, thinking and behavior (Ferri et al., 2006). AD affects around 35 million people globally, with forecasted numbers of 115 million by 2050 (Ferri et al., 2006; WHO, 2012). AD is a complex neurodegenerative disorder with multifactorial etiology and there is currently no reliable method to diagnose the disease, nor effective interventions to delay, to prevent or to cure the disease (Hardy and Selkoe, 2002). In the absence of a recognized non-invasive method, the only definitive gold standards for a positive diagnosis of AD is a post-mortem autopsy, by detecting beta amyloid plaques from amyloid precursor protein (APP) and neurofibrillary tangle (NTF) derived from hyper phosphorylated tau protein (Querfurth and LaFerla, 2010). While some brain regions such as the cortex and hippocampus are involved in this neurological disease (Hardy and Selkoe, 2002), more information on the molecular characteristics of the body fluids/organs related to this complex neurological disease is important to underpinning the mechanisms of the disease, which could offer non-invasive methods of diagnosing AD. Recent studies conducted in multiple animal models and human population studies strongly suggest that abnormalities in peripheral biofluids and tissues are associated with brain networks connected with brain functions (Sassone et al., 2009; Lan et al., 2009), implying that the manifestation of brain disease is not only limited to regions in the brain. Urinary formaldehyde level is found to be inversely correlated to mini mental state examination scores in senile dementia patients (Tong et al., 2011). Levels of F2A-isoprostane in plasma and urine are reported as an

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Abbreviations: AD, Alzheimer's disease; AMP, adenosine monophosphate; APP, amyloid precursor protein; D5D, delta-5-desaturase; D6D, delta-6-desaturase; FADS, fatty acid desaturase genes; FT, female transgenic group; GC-MS, gas chromatography-mass spectrometry; MT, male transgenic group; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; PUFA, polyunsaturated fatty acids; SCD, stearoyl-CoA-desaturase; UMP, uridine monophosphate; UDP, uridine diphosphate.

http://dx.doi.org/10.1016/j.neuroscience.2016.06.049
0306-4522/

in vivo quantitative biomarker for oxidative damage in AD and a biomarker for differentiating mild cognitive impairment during AD (Montine et al., 2005; Mufson and Leurgans, 2010). Plasma lyso PCs, sphingosine and tryptophan are found to be potential early markers for AD patients (Li et al., 2010). In addition, the function of peripheral liver (Astarita et al., 2010), intestines (Van Ginneken et al., 2011), spleen and thymus (Gonzalez-Dominguez et al., 2015) are also reported to be affected during AD progression. However, systematic information of possible involved biochemical pathways during AD development is still lacking.

As a valuable approach for investigating systematic metabolic changes during disease development, nuclear magnetic resonance (NMR) based metabonomics combined with multivariate data analysis have been applied in diverse fields (Lindon et al., 2011). With advantages including non-invasiveness, simple sample preparation, high throughput and excellent reproducibility, this method has been applied to investigate the metabolic profiles of biofluids and brain regions of interest, in neurological diseases such as Huntington's disease (Mochel et al., 2007) and psychiatric diseases such as bipolar disorder (Lan et al., 2009). Previous studies on the plasma and brain regions of senescence-accelerated mice (Jiang et al., 2008) and CRND8 transgenic mice (Salek et al., 2010) have shown that amino acids and lipid composition are disturbed in mildly cognitive impaired patients (Tukiainen et al., 2008; Barba et al., 2008). In addition, gender differences in neurological and psychiatric disorders are increasingly recognized and have become an important therapeutic issue. For example, Gillies et al. describes that the Y-chromosome gene may directly influence the nigrastriatal dopamine system, which contributes to the gender differences in Parkinson's disease (Gillies et al., 2014). Li et al. explores the gender differences in cognitive performance (Li and Singh, 2014), which also highlights the importance of investigating gender-related systematic metabolic effects of AD.

In our study, NMR and gas chromatography-mass spectrometry (GC-MS) based metabolic profiling techniques were used to identify metabolic perturbations in both plasma and liver tissues obtained from an AD animal model (APP/PS1 transgenic mice). In addition, we also investigated the metabolic characteristics of sexual dimorphisms in our AD animal model. Our results could provide important clues for deepening the understanding of peripheral metabolic mechanisms in AD and highlight the need for gender-specific therapeutic strategies for AD surveillance in the future.

EXPERIMENTAL PROCEDURES

Animal experiments and sample collection

A total of 60 specific pathogen free transgenic mice ($APP_{swe}PSEN1_{dE9}$)85Dbo, approximately 4 weeks old, were purchased from a national resource center for mutant mice, Nanjing University. This strain of double mutant transgenic mouse has been widely used as an AD animal model (Francis et al., 2009). Mice were divided into four groups according to gender and transgenic background: female transgenic group (FT, $n = 11$) and their female wild-type counterparts (FW, $n = 15$), male transgenic group (MT, $n = 16$) and their male wild-type counterparts (MW, $n = 18$). All mice were housed in an environmentally-controlled facility (temperature: 18–22 °C; humidity: 40–70%; light–dark cycle: 12–12 h) at the animal laboratory center of Wuhan University. All experimental procedures were performed according to national guidelines for experimental animal welfare (No 398-2006) and approved by the committee for animal care of the animal laboratory center of Wuhan University.

Mice were sacrificed at the age of 9 months, and plasma and serum samples were collected from orbital venous plexus. Plasma samples were prepared by transferring blood to Eppendorf containing 5 μL sodium heparin. The tubes were centrifuged at 3000*g* for 10 min. The supernatants were immersed into liquid nitrogen immediately, and kept at −80 °C until NMR analyses. Blood serum samples for clinical biochemistry analysis were prepared by centrifugation of whole blood directly without adding anticoagulant.

The middle lobe of the liver of each mouse was excised following cervical dislocation and immediately snap-frozen in liquid nitrogen and stored at −80 °C. Brain slices of three mice from each group were stored in formalin solution for thioflavin-S dyeing assessment, which is a typical histological method to stain the amyloid plaques (Braak and Braak, 1991).

^{1}H NMR spectroscopy

The plasma samples for NMR were prepared according to a previously protocol (Wu et al., 2010). The mixed liquid was transferred into 1.7 mm diameter micro NMR-tubes (CortecNet, Paris, France) using a micro syringe. Liver samples of 50 mg were extracted with methanol/water three times. After removal of the organic solvent followed by freeze-drying, the obtained powder were dissolved in 0.1 M K_2HPO_4/NaH_2PO_4 buffer made with 50% D_2O and 0.002% sodium-3-trimethylsilylpropionate-2,2,3,3-D_4 (TSP, Cambridge Isotope Laboratories, USA), which was used as the lock signal and reference, respectively.

A standard one dimensional (1D) NMR experiment with the first increment of NOESY pulse sequence (recycle delay (RD)-90°-t_1-90°-t_m-90°-acquisition (ACQ)) was employed for plasma and liver extracts. The water peak was suppressed during the RD of 2 s and mixing time (t_m) of 100 ms, with t1 fixed to 4 μs. A spin relaxation edited ^{1}H NMR experiment with Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (RD-90°-(τ-180°-τ)$_n$-ACQ) with total spin–spin relaxation delay of 70 ms was used for plasma in order to detect the signals from small molecular weight metabolites. The 90° pulse length was adjusted to 10 μs. For each sample, 256 scans for plasma and 64 scans for liver extracts were respectively recorded into 32k data points with a spectral width of 20 ppm. Additionally, two-dimensional (2D) NMR experiments, such as ^{1}H–^{1}H correlation spectroscopy and total correlation spectroscopy were carried out to assist metabolite identification on selected plasma or liver extracts utilizing standard acquisition parameters.