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Research report

Magnetic resonance spectroscopy analysis of neurochemical changes in the atrophic hippocampus of APP/PS1 transgenic mice

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ABSTRACT

Alzheimer's disease (AD) is characterized by neuropathological changes and progressive cognitive decline, which is associated with the volume loss and neurochemical alterations. However, the specific neurochemical alterations in cerebral regions that contribute to cognitive decline still remain unknown. In the present study, we measured cerebral morphological and neurochemical alterations using structural magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (^{1}H-MRS) in an AD model of APP/PS1 transgenic mice. Voxel-based morphometry (VBM) analysis indicated atrophy of the hippocampus, motor cortex, striatum, amygdaloid body, septal area, bed nucleus of the stria terminalis and accumbens nucleus in APP/PS1 transgenic mice. Furthermore, the hippocampus was selected as a region of interest (ROI) to explore neurochemical metabolism. The results showed that the ratios of *N*-acetylaspartate/creatine (NAA/Cr) and glutamate/creatine (Glu/Cr) were reduced, while *myo*-inositol/creatine (mIn/Cr) was increased in APP/PS1 transgenic mice compared to the wild type mice and accompanied by a decline in learning and memory. Taken together, the present study suggests that hippocampal atrophy and neurochemical changes in NAA, Glu and mIn may play a causative role in the cognitive decline associated with AD.

1. Introduction

Alzheimer's disease (AD) is an age-related progressive neurodegenerative disorder accompanied by cognitive decline [1]. *The World Alzheimer Report 2015* estimates that over 46 million people live with dementia worldwide and the cost of dementia is $818 billion [2]. Furthermore, the prevalence of AD increases with ageing and doubles every five years after the age of 65, which will cause great economic and social burdens [2,3].

Magnetic resonance imaging (MRI), including structural MRI, functional MRI and proton magnetic resonance spectroscopy (^{1}H-MRS), is becoming more useful in the understanding of the pathogenesis and diagnosis of AD [4–12]. Brain morphological abnormalities, such as hippocampus, entorhinal cortex, and amygdala have been gradually uncovered with the development of structural MRI in the study of AD patients [4,8,9]. Animal structural MRI also shows striatum and entorhinal cortex atrophy in AD models, including the TASTPM and APP/PS2/Tau mouse [13]. In addition, ^{1}H MRS studies have suggested that neurochemical alterations, such as those associated with glutamate (Glu) and *N*-acetylaspartate (NAA) in the hippocampus or cingulate, contribute to cognitive decline in AD patients [14–16]. In animal models of AD, neurochemical alterations were measured similarly with that of AD patients [17–19]. Glu is a primary excitatory neurotransmitter that is thought to represent the number of survival neurons [20]. NAA is a specific kind of amino acids that mainly exists in the neurons and axon [21]. As a neuronal marker, the concentration of

Abbreviations: AD, Alzheimer's disease; ANTs, Advanced Normalization Tools; CHESS, chemical shift-water suppression; Cr, creatine; CSF, cerebrospinal fluid; FOV, filed of view; Glu, glutamate; GM, grey matter; ^{1}H-MRS, proton magnetic resonance spectroscopy; MRI, magnetic resonance imaging; NAA, *N*-acetylaspartate; mIn, myo-inositol; PRESS, point-resolved spectroscopy; QUEST, quantum estimation; ppm, parts per million; RARE, rapid acquisition with relaxation enhancement; ROI, region of interest; SPM8, Statistical Parametric Mapping8; TE, echo time; TR, repetition time; VBM, voxel-based morphometry; WM, white matter

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NAA can be sensitive to neuron density. *myo*-inositol (mIn) is one of the most abundant metabolites visible on ^{1}H MRS in the brain and can act as a biomarker of glial activation[22]. A number of studies suggest that neurochemical alterations are associated with cognitive decline in AD [18,21,23–25].

However, few studies have contemporarily focused on the roles of structural MRI changes and neurochemical alterations in cognitive decline associated with AD. Therefore, the current study aimed to measure the learning and memory ability and regional volumes in APP/PS1 transgenic mice. Furthermore, some atrophic regions were selected as region of interest (ROI) to explore neurochemical metabolism using ^{1}H MRS in order to further understand cognitive decline in AD.

2. Materials and methods

2.1. Animals

Twelve-month-old APP/PS1 transgenic mice and age-matched littermate wild-type (C57Bl/6J) control mice were used for the water maze, MRI and ^{1}H MRS analyses, with 2 different groups per test (n = 7 mice/group, weights 25–35 g). APP/PS1 mice express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutanthuman presenilin 1 (PS1-dE9), which are associated with early-onset AD [26].

All animals were kept in standard size mouse cages (29*18*13 cm, up to 5 per same sex group) at 20–26 ˚C on a daily 12-h light-dark cycle with ad libitum access to food and water. All experiments were performed strictly in accordance with the International Ethical Guidelines and National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of Fujian University of TCM, P.R. China (protocol #FUTCM-2014012). All efforts were made to minimize animal suffering.

2.2. Morris water maze test

Cognitive function was tested using a water maze apparatus (Chinese Academy of Sciences, Beijing, China), which was a stainless steel circular tank with a diameter of 120 cm and a height of 50 cm. The tank was filled with water (21–23 ˚C) to a depth of 40 cm and divided into four equal quadrants. A platform (diameter, 6 cm) was placed in the third quadrant and submerged 1 cm below the surface of the water. For the place navigation trials, mice were trained for five days. Each trial was started by placing the mice in one of the four quadrants. Mice were allowed to swim for a period of 90 s to find the hidden platform. If a mouse did not find the platform within 90 s, it was removed from the water and placed on the platform for 15 s by a researcher. On the sixth day, the probe trial test was performed to assess memory consolidation and the platform was removed. Each mouse was allowed to swim freely for 90 s. The frequency of each mouse that crossed the position where the platform was once placed and the time spent in the target quadrant were recorded.

2.3. MRI scanning

Prior to MRI scanning, mice were initially anaesthetized with 1.5% isoflurane in a 1:4 oxygen and air mixture. During the MRI scan, the animals were placed prone in a MR-compatible stereotactic holder with the head cinched, the teeth hooked by a tooth bar, and the nose emplaced in a nose cone to exhaust isoflurane in a mixture of oxygen and air (ratio 1:4). The core body temperature was controlled to approximately 37 ˚C using a warm water circulated heating cradle, and respiration was monitored. Imaging was performed on a 7T small animal MRI scanner (70/20USR Biospec, Bruker Biospin GMbls, Germany). The body coil and surface coil were used as the exciting coil and receiving coil.

A 3D rapid acquisition with relaxation enhancement (RARE) T2-weigheted sequence was performed to assess anatomic changes with the following parameters: repetition time (TR) = 16000 ms, echo time (TE) = 65 ms, number of averages = 16; field of view (FOV) = 20*20 mm, slices = 75, slice thickness = 0.2 mm and matrix = 256*256.

2.4. Voxel-based morphometry (VBM) analysis of MRI data

All pre-processing was performed using Advanced Normalization Tools (ANTs) [27] and Statistical Parametric Mapping8 (SPM8, Wellcome Department of Clinical Neurology, London) with the SPMMouse plugin [28]. First, the images were corrected for intensity non-homogeneity with ANTs's N4biasFieldCorrection [29]. Then, the brain images were extracted from the corrected images by skull strip. The extracted brain images were normalized into the standard stereotactic space [30] and segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using SPMMouse toolbox [28]. Finally, the resulting GM images were smoothed with a 210 μm isotropic Gaussian kernel using SPM8. The two-sample *t*-test was performed by SPMMouse toolbox [28] voxel-by-voxel to determine the GM volume differences within the whole brain between control and APP/PS1 transgenic mice. $P < 0.01$ and clusters > 50 voxels were considered as the statistically significant threshold.

2.5. ^{1}H-MRS acquiring

Based on the results of the above VBM analysis, APP/PS1 transgenic mice revealed a significant reduction in volume of grey matter in the hippocampus, motor cortex, amygdaloid body, striatum, etc. The hippocampus is the area of most concern in AD studies. A number of studies have shown regional atrophy in the hippocampus of AD [8,9]. Therefore, we selected an area in the hippocampus as the region of interest (ROI) for ^{1}H-MRS measurement, where a significant reduction in volume of grey matter was detected by VBM. The ROI size was set as 1.5*1.5*1.5 mm.

A T2-weighted image using RARE sequence was first acquired to ensure the mouse head position. The parameters were as follows: TR = 4200 ms, TE = 35 ms, number of averages = 4, FOV = 20*20 mm, slice thickness = 0.5 mm, slices = 30, and matrix = 256*256. The selected ROI was shimmed, and the water suppression pulse was adjusted for chemical-shift-water suppression (CHESS) prior to the point-resolved spectroscopy (PRESS) acquisition. MR spectroscopic data were collected with TR = 2500 ms, TE = 16.168 ms and scan duration = 5 min 20 s.

2.6. ^{1}H-MRS spectral processing

The spectral data were processed using the software package TOPSPIN (v3.1, Bruker Biospin, Germany). The areas under the peak for various metabolites, including NAA, mIn, Glu and creatine (Cr), were calculated automatically using a quantum estimation (QUEST) method with a subtraction approach for background modelling. Using Cr as the criterion [18,31], NAA/Cr, Glu/Cr and mIn/Cr were statistically evaluated. The metabolites chemical shift position in the brain, including NAA 2.02 parts per million (ppm), Glu 2.2 ppm, mIn 3.5 ppm and Cr 3.05 ppm.

2.7. Statistical analysis

Both the water maze and ^{1}H MRS data were analysed using SPSS 21.0 software (SPSS, Armonk, NY, USA) with a two-tailed Student's *t*-test, and the results were used to compare APP/PS1 transgenic mice versus wild type mice. The data were presented as the mean ± standard error of the mean (S.E.M). $P < 0.05$ was considered statistically significant.