

Original Article

Investigation of metabolism in posterior cingulate cortex in patients with Alzheimer's disease using magnetic resonance spectroscopy

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Abstract: Background: The neurodegenerative changes of Alzheimer's disease (AD) may cause a series of molecular and cellular cascades, leading to dysfunctions in awareness, memory and other fields. Purpose: To investigate the metabolism in posterior cingulate cortex (PCC) in patients with AD by applying point resolved spectroscopy (PRESS) and spin-echo single-voxel spectroscopy (SE SVS). Methods: 20 AD patients and 20 healthy people were enrolled into the study with minimum mental state examination (MMSE) and clinical dementia rating score. The T1WI-3D image was used to perform the spectral positioning of PCC. PRESS and SE SVS were used to collect the PCC spectrum. The levels of N-acetylaspartate (NAA), myo-inositol (ml), choline (Cho) and creatine (Cr) and the relative ratio of NAA/Cr, ml/Cr, NAA/ml and Cho/Cr were analyzed. Results: Compared with the control group, the NAA peaks in the AD group were reduced to various degrees, while the ml peaks were increased. NAA/Cr and NAA/ml in PCC in the AD group were lower than the control group, while ml/Cr in the AD group was higher than the control group. ml/Cr was negatively correlated with the related MMSE score. Conclusion: Studying PCC with PRESS and SE SVS is helpful towards the screening and early diagnosis of AD.

Keywords: Alzheimer's disease, magnetic resonance spectroscopy, posterior cingulate cortex

Introduction

The neurodegenerative changes of Alzheimer's disease (AD) might cause a series of molecular and cellular cascades, thus secondarily leading to the biochemical metabolic abnormalities in local brain regions and the loss of a large number of neurons, followed by such dysfunctions in awareness, memory and other fields [1]. AD accounted for 60% of all senile dementia [2], with the increasing of aged population in China, the proportion of AD patients would continue to rise, and it had become the high-incidence disease second to cardiovascular disease, cancer and stroke, and it would threaten the life and health of the elderly [3-5]. The clinical diagnosis of AD was the preclusive diagnosis [6], while AD could only be confirmed through the pathological examination of brain tissues [7, 8], but up to 20% clinical diagnosis of AD had no pathological basis [9].

Currently, a variety of imaging technologies, including magnetic resonance spectroscopy (MRS), had been used for the diagnosis of AD [10, 11]. MRS was a noninvasive technology that could determine the metabolite concentrations *in vivo*, so it could be used for the semi-quantitative analysis of such very important chemical substances in the pathophysiological metabolism of *in vivo* brain tissues as N-acetylaspartate (NAA), myo-inositol (ml), choline (Cho) and creatine (Cr), these substances could reflect the amounts of neurons and axons, as well as gliosis and the metabolic changes of energy, so it could help in evaluating the progression and treatment of AD patients [12, 13]. Studies had shown that the increased ml, accompanied with the reduced NAA, was positively correlated with the damage degrees of memory [14, 15].

Posterior cingulate cortex (PCC) was an important part of limbic system, closely related to the

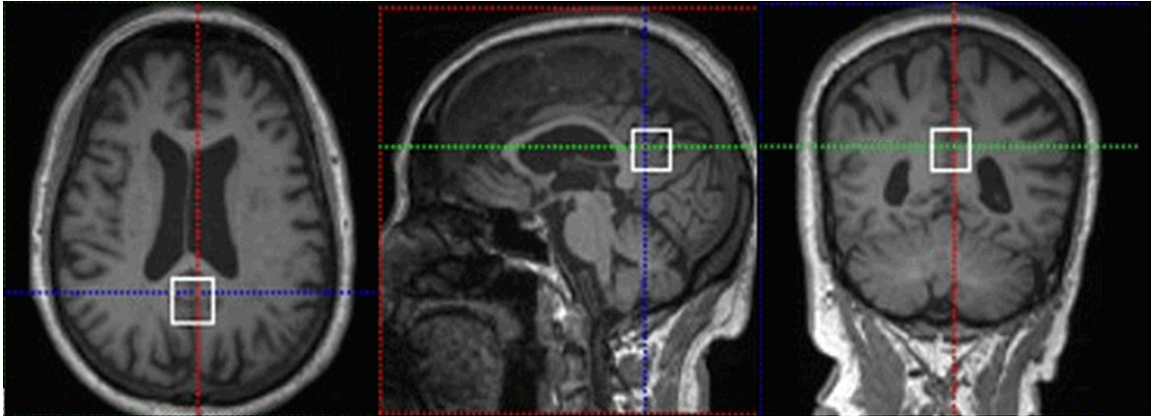


Figure 1. Transverse, sagittal and coronal positioning images acquired by PCC SVS.

neural circuits of learning and memory, and one of the first involved brain regions in AD patients. AD patients exhibited atrophy in anterior part of PCC, the cortex became thinner, the connection with other brain regions' functions became gradually waning [16, 17], and the corresponding markers such as NAA, Cho, ml and Cr, as well as the ratios of NAA/Cr, ml/Cr and Cho/Cr, in partial brain tissues exhibited abnormal changes [18, 19]. The phenomena that NAA/Cr was reduced, while Cho/Cr was increased, were the classic changes of AD patients in MRS, with the disease worsened, the NAA/Cr values in hippocampus and PCC were gradually decreased, while the ml/Cr value was gradually increased, and NAA/ml in PCC was significantly reduced than in hippocampus [20]. In addition, the peri-PCC brain structures were relatively simple, and no skull and gas would interfere, so it would be easy to obtain stable and reliable spectral data. Therefore, this study set PCC as the region of interest for the acquisition of spectral data.

Materials and methods

Clinical data

20 AD patients treated in the elderly outpatient of our hospital were enrolled, including 9 males and 11 females, with an average age of 80.4 ± 6.37 years old, the minimum mental state examination (MMSE) score was 18.68 ± 4.04 points, the clinical dementia rating (CDR) score was 1.6 ± 0.76 points, and the educational level was: 15 cases of high school or less, 5 cases of high school and more. All patients were right-handed. Another 20 normal healthy volunteers,

who matched with the AD group in age, sex, educational level and handedness, were selected, including 10 males and 10 females, with the average age of 72.0 ± 6.92 years, the MMSE score was 28.4 ± 0.82 points, the CDR score was 0, and the educational level was: 14 cases of high school or less, 6 cases of high school and more. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Fuzhou General Hospital of Nanjing Military Region. Written informed consent was obtained from all participants.

Inclusion criteria of AD group

The inclusion criteria of AD group were as follows: i) Met the diagnostic criteria of AD, published by National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA, USA) (11); ii) MMSE score ≤ 17 points (illiteracy), ≤ 20 points (primary school), ≤ 24 points (secondary school and above); iii) CDR score ≥ 0.5 points; iv) HIS ≤ 4 points to exclude the vascular dementia; v) excluded vasogenic dementia and other neurological diseases that could cause dementia by conventional MRI; vi) no serious physical illness, no abuse of alcohol, tobacco and other substances; vii) did not meet the diagnostic criteria of depression.

Inclusion criteria of normal control group

The inclusion criteria of normal control group were as follows: i) MMSE score ≥ 28 points; ii)

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Table 1. Comparison of gender, age, educational level, MMSE score and CDR score between 2 groups

Group	Gender (male/female)	Age (year)	Educational level*	MMSE score (point)	CDR score (point)
AD	9/11	80.4±6.37	15/5	18.68±4.04	1.6±0.76
Control	10/10	72.0±6.92	14/6	28.40±0.82	0
t	$\chi^2 = 0.1$	3.99	$\chi^2 = 0.125$	-10.768	9.49
P	0.752	0	0.723	0	0

* < high school/≥ high school; AD, Alzheimer's disease; MMSE, minimum mental state examination; CDR, clinical dementia rating.

CDR score was 0 point; iii) with normal results of routine physical examination and routine laboratory tests; iv) without neurological and psychiatric disorders, and other organic diseases by routine MRI examination; v) without serious abuse of alcohol, tobacco and other substances.

Inspection methods

Siemens 3.0 T Trio Tim MR scanner, with 12-channel phased-array head coil (Germany, Siemens). The conventional MRI scanning included the cross-sectional T1WI, T1WI-3D, T2WI, DWI and FLAIR sequence scanning. Before the conventional MRI scanning, T1WI-3D was selected to performed the PCC spectral positioning from transverse, sagittal and coronal planes (**Figure 1**) and the PRESS and SE SVS-30 sequences were used to collect the PCC spectra, with region of interest (ROI) set at the top of parietooccipital sulcus and behind splenium corporis callosi (**Figure 1**). The voxel size was 20 mm × 20 mm × 20 mm, TR 2000 ms, TE 30 ms, the acquisition times were 96, with the flip angle as 90°, and the matrix as 1024 × 1024. Firstly, all the directions of ROI were added the pre-saturated zone, then performed the manual shimming, water suppression and fat suppression, the spectra were then acquired at FWHM (FHMW) < 15 HZ and water suppression > 95%, with the acquisition time as 3'8".

T1WI scanning parameters were as follows: TR = 2000 ms, TE = 9.2 ms, FOV = 230 mm × 230 mm, Flip angle = 130°, Slice thickness = 5.0 mm, Slice = 20; T2WI scanning parameters: TR = 3000 ms, TE = 98 ms, FOV = 230 mm × 230 mm, Flip angle = 130°, Slice thickness = 5.0 mm, Slice = 20; DWI scanning parameters: TR = 5100 ms, TE = 90 ms, FOV = 230 mm × 230

mm, Flip angle = 130°, Slice = 20; FLAIR scanning parameters: TR = 7000 ms, TE = 93 ms, FOV = 230 mm × 230 mm, Flip angle = 130°, Slice thickness = 5.0 mm, Slice = 20. Sagittal T1WI-3D structural image, covered the whole brain, scanning parameters: TR = 1900 ms, TE = 2.5 ms, FOV = 240 mm × 240 mm, Flip angle = 9°,

Slice thickness = 1.0 mm, Gap = 0, Matrix = 256 × 256, NEX = 1, Slice = 160, voxel size 1 mm × 1 mm × 1 mm, for the subsequent spectral positioning.

Data collection

Siemens Trio Tim 3.0T MRI workstation Syngo B15 spectral analysis software (Siemens, Germany) was used for the automatic curve fitting, zero padding, Fourier transforming, and phase-baseline adjustment, then the spectrum of each metabolite in PCC was generated, furthermore, the integral value of the area under each metabolite's curve was calculated. Such metabolite peaks as NAA, ml, Cho and Cr were selected as the research targets, and calculated the relative ratios of NAA/Cr, Cho/Cr, ml/Cr and NAA/ml. Due to the different chemical shifts of nuclei in different compounds, the chemical shift was expressed as parts per million (ppm) in MRE, and the chemical shifts of the above metabolites were 2.02, 3.56, 3.22 and 3.02 ppm, respectively.

Statistical analysis

PEMS3.1 package (Huaxi School of Public Health, Sichuan University) was used to statistically process the data of the 2 groups. The data were expressed as mean ± SD. Comparison between two groups was performed using two-sample t test. $P < 0.05$ considered as statistically significant.

Results

General information

There was no significant difference in sex, age and educational level between the 2 groups ($P > 0.05$); while the MMSE scores and the CDR scores had significant difference ($P < 0.05$, **Table 1**).

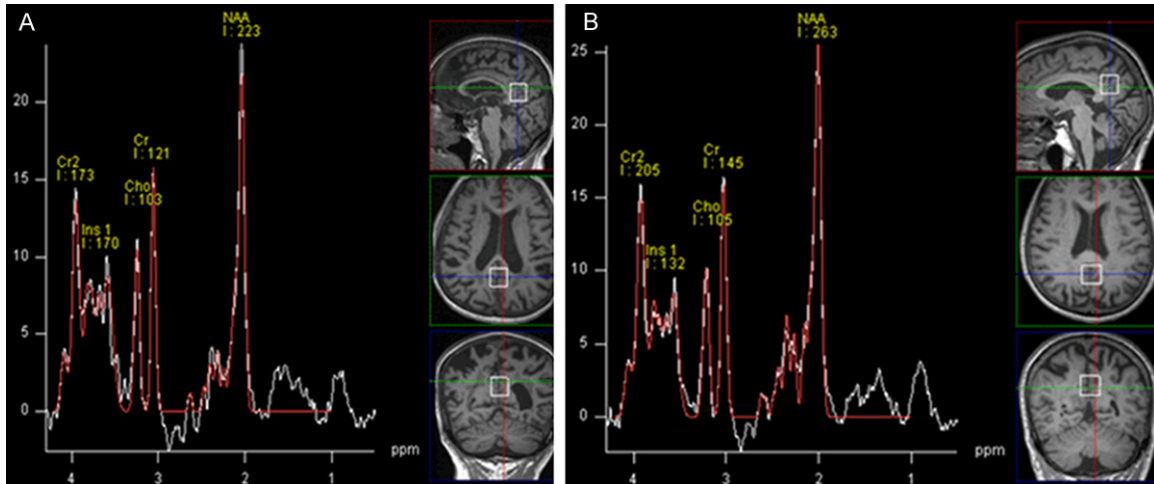


Figure 2. A. PCC spectra of the AD patients; B. PCC spectra of the normal control, the NAA peaks of the AD group were reduced than the control group, while the ml peaks were increased than the control group.

Table 2. Two-sample t test results of metabolite ratios in PCC

Group	NAA/Cr	ml/Cr	Cho/Cr	NAA/ml
AD	1.84±0.22	1.27±0.16	0.76±0.09	1.5±0.17
Control	2.03±0.27	0.74±0.18	0.713±0.095	2.49±0.44
t	-2.502	9.78	1.76	-9.16
P	0.017	0.00	0.086	0.00

PCC, posterior cingulate cortex; AD, Alzheimer's disease; NAA, N-acetylaspartate; Cr, creatine; ml, myo-inositol; Cho, choline.

Spectral results of PPC

Compared with the control group, the NAA peaks in the AD group were reduced to various degrees, while the ml peaks were increased (**Figure 2**). The metabolite ratios of the 2 groups were shown in **Table 2**. NAA/Cr of the AD group was lower than the control group ($t = -2.502$, $P = 0.017$), NAA/ml was lower than the control group ($t = -9.16$, $P = 0$), ml/Cr was higher than the control group ($t = 9.78$, $P = 0$), and the difference was statistically significant. Cho/Cr of the AD group was increased, compared with the control group, but the difference was not statistically significant ($t = 1.76$, $P = 0.086$). The ml/Cr ratios were negatively correlated to the related MMSE scores ($r = -0.648$, $P = 0.002$).

Discussion

In 1907, Alzheimer firstly reported the pathological changes of AD, and it was found later [21, 22] that the main pathological features of

AD were neurofibrillary tangles, senile plaque formation, nerve cell loss, amyloidosis, neuron loss, Hirano small body, neuronal vacuolar granular degeneration and others, which were the major causes to the changes of compounds at the cellular level inside AD patients.

In the clinical studies towards the central nervous system, ^1H -MRS and ^{31}P -MRS were the most widely used spectroscopies currently. Through analyzing the contents and changes of metabolites and neurotransmitters in brain by MRS, the pathophysiological process of AD could be understood and thus helpful to the early diagnosis and differential diagnosis of AD. In the researches about AD and other neurological degenerative brain diseases, the metabolites mainly included NAA, Cho, ml and Cr, and the MRS software could calculate the relative ratios of NAA/Cr, ml/Cr, NAA/ml and Cho/Cr for the further analysis [12, 13].

NAA was a neuronal marker, its resonance peak was at 2.02 ppm, almost all NAA only existed inside the neuronal cells, while the mature glial cells did not contain NAA [23]. The AD pathological study revealed that $\text{A}\beta$ protein and Tau protein were highly cytotoxic, they could cause the degeneration and necrosis of nerve cells, and destroy the neuronal functions, therefore, this pathological change would be bound to cause NAA abnormality inside brain. Kantarci *et al.* [24] dissected the corpses of AD

patients and found a significant decrease of NAA in AD patients. Schott *et al.* [25] performed long-term MRS follow-up study towards the AD patients, and also found the NAA content was reduced in AD patients. Another study confirmed that, the reduction of NAA/Cr in AD patients was related with the early pathology of PCC Tau protein [13]. The results of this study exhibited that the NAA/Cr ratio in AD patients was lower than the control group, the difference was statistically significant ($P < 0.05$), consistent with most literatures.

Cho was the precursor of neurotransmitter acetylcholine, and could reflect the changes of cell numbers and catabolism of membrane phospholipid. Certain domestic researcher found that, the Cho/Cr ratio in AD patients was higher than the control group [19]. However, most studies considered that the Cho/Cr ratio had no significant difference between the two groups. The results of this study showed that, the Cho/Cr ratio in the AD group was higher than the normal control group, but the difference was not statistically significant.

Cr was the reserve of high-energy phosphate compounds and the buffer of adenosine triphosphate (ATP) and adenosine diphosphate (ADP), uniformly distributed inside brain, including phosphocreatine and creatine. Its MRS peak was at 3.02 ppm [22]. Cr could reflect the high-energy phosphate metabolism and its content could remain relatively stable in various physiological and pathological conditions, so it was often used as a reference to compare the concentration changes of other metabolites in MRS.

ml existed only inside glial cells, with the resonance peak at 3.56 ppm, ml played an important role in maintaining the volume stability of glial cells, as a metabolite to reflect gliosis, the increasing of ml might prompt gliosis. Currently, most studies showed that the ml/Cr ratio was increased in the brains of patients with mild cognitive impairment and AD [26, 27], the increasing of ml/Cr was earlier than NAA/Cr in the AD process [28]. The results of this study exhibited that ml/Cr was significantly increased in the AD group, while NAA/ml was decreased, the differences between the two groups were statistically significant ($P < 0.05$). Rose *et al.* [27] pointed out that NAA/ml was positively correlated with the severity of demen-

tia. Murray *et al.* [13] found that the increasing of ml/Cr ratio was related with the occurrence of A β protein plaques in AD patients. In this study, NAA/Cr, NAA/ml and ml/Cr in the AD group were performed the correlation analysis with the related MMSE scores, and it was found that NAA/ml was positively correlated with the MMSE score, while ml/Cr was negative related with the MMSE score.

This study found that NAA/Cr and NAA/ml in PCC was decreased, and ml/Cr was increased, which exhibited certain values from the molecular imaging level towards the early diagnosis of AD. 1H-MRS of PCC was simple to operate, and easy to obtain stable images and spectral data, thus it could be used as a new method for the screening and early diagnosis of high-risk groups, and provide relevant evidence for the clinical implementation of early intervention to AD patients.

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Disclosure of conflict of interest

None.

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