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Increased apoptosis in the platelets of patients with Alzheimer's disease and amnestic mild cognitive impairment

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ABSTRACT

Objectives: Alzheimer's disease (AD), the most common cause of dementia, is a progressive, incurable neurodegenerative disorder. Platelet is a suitable source of human peripheral tissue to study pathological mechanisms occurring in the brain. The present study aims to investigate (1) whether abnormal apoptotic events besides involved in AD within the central neurologic system, could also occur at peripheral platelet level; (2) whether apoptosis at peripheral platelet level starts at the early stage of AD.
Patients and methods: Amnestic mild cognitive impairment (MCI), AD, and age-matched healthy individuals were recruited, and each group had 50 person. In the present study, we investigate whether alterations of caspase family and Bcl2 family could be found in the platelets in Alzheimer's disease (AD) and amnestic mild cognitive impairment (MCI) patients. The platelet levels of caspase protein and Bcl2 family were analyzed by western blot.
Results: The results show that the platelet levels of caspase-3, caspase-9, Bad, and Bax significantly increased in AD and amnestic MCI. The increased apoptosis proteins levels in amnestic MCI were found between AD and normal controls. The anti-apoptosis protein Bcl2 increased in amnestic MCI, while decreased in AD.
Conclusion: We suggest that increased apoptosis exist in the platelet and might mirror apoptosis within the brain. Abnormal apoptosis may appear in the early of AD, and the ratio between pro- and anti-apoptotic protein levels partially determines the susceptibility of platelet to a death signal. In conclusion, platelet may be a good model to study apoptotic pathways of AD.

1. Introduction

Alzheimer's disease (AD), the most common cause of dementia, is a progressive, incurable neurodegenerative disorder. Characteristic neuropathological hallmarks of AD are extracellular senile plaques and intracellular neurofibrillary tangles [1]. Cultured neurons exposed to amyloid peptide (Aβ), a major protein component of the plaques, undergo apoptotic cell death, which suggests that apoptosis may be one of the mechanisms leading to neuronal cell death in AD [2–4].

Apoptosis is a form of cell death triggered by the activation of an intrinsic cellular program deliberately invoked by the cell in response to environmental or developmentally associated signals [5]. Over the last decade, apoptosis of platelet has been recognized. Although platelet is anucleate, they do contain mitochondria and mitochondrial DNA, metabolically stable mRNA, and is capable of protein synthesis [6]. A number of apoptosis of nucleate cells have also been recognized in the platelet, such as activation and mitochondrial translocation of Bax, activation of caspases-3, -8 and -9, collapse of the mitochondrial inner membrane potential, B cell lymphoma/lewkmia-2 (Bcl-2) family proteins expression, and phosphatidylserine (PS) exposure [7–9]. In search for peripheral apoptotic changes of AD, platelet recently gains increasing attention. Growing evidence show that slow accumulation of AD pathology starts decades before the disease is clinically recognizable [10]. Hence, researchers continue to pursue pathophysiological changes by studying amnestic mild cognitive impairment (MCI), a high risk of developing AD [11].

The present study aims to investigate (1) whether abnormal apoptotic events besides involved in AD within the central neurologic system, could also occur at peripheral platelet level; (2)

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whether apoptosis at peripheral platelet level starts at the early stage of AD.

2. Materials and methods

2.1. Participants

Patients with aMCI ($n = 50$; age range: 50–80) and AD ($n = 50$; age range: 50–80) were recruited from Shanghai Jiaotong University Affiliated First People's Hospital and Shanghai No. 10 People's Hospital between April 2008 and April 2009 for enrollment in this community-based prospective cohort study. At the same time, 50 age-matched healthy individuals were recruited for the control group through the nursing home and included volunteers in the city of Shanghai and its proximal regions. All subjects performed a general physical examination and neurologic examination. Participants underwent a computed tomography (CT) or magnetic resonance imaging (MRI) brain scan within six months before the screening. The behavioral and global cognitive evaluation was conducted according to a standardized battery, which included the following tools: Mini-Mental State Examination (MMSE), Clinical Dementia Rating (CDR), Chinese version of the Montreal Cognitive Assessment (MoCA-C), Instrumental Activities of Daily Living scale (IADL), and Auditory Verbal Learning Test (AVLT). All amnestic MCI patients met the criteria for MCI as proposed by Petersen et al. [12], including: (1) a subjective memory complaint by the patient or his/her caregiver; (2) no or minimal impairment of daily life activities; (3) objective memory impairment adjusted for age and education; (4) preservation of general cognitive functioning; and (5) no dementia. An additional criterion, that cognitive impairment must be restricted to memory loss, was added. A diagnosis of probable AD was based on National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [13]. The final diagnosis of amnestic MCI and AD was made by the consensus of two highly qualified neurologists. The criteria [14] for healthy volunteers were: (1) an absence of memory complaints or any other cognitive symptoms; (2) preservation of general cognitive functioning; and (3) no active neurological or psychiatric disease.

Participants were excluded if they had any of the following: serious physical illness, including, but not limited to, cancer and myocardial infarction; neurological disease, including epilepsy, significant head trauma, central nervous system infection, brain tumor, and subdural hematoma; psychiatric illness, such as, depression or substance abuse; less than 4 years of education; visual or hearing disabilities, and any other relevant health conditions that could either affect cognition or limit administration of the neuropsychological tests. To avoid potential pharmacologic confounding effects on platelet physiologic findings, patients taking psychotropic agents, nootropic drugs, cholinergic or anticholinergic agents, antiplatelet agents, anticoagulants, corticosteroids, or serotoninergic drugs entered the study only after being drug free for at least 14 days before blood collection and platelet preparation. Patients and controls gave their informed consent to the research center, and the study was conducted in accordance with the provisions of the Shanghai Hospital Development Center, China.

2.2. Platelet preparation

Patients' blood samplings were carried out after an overnight fast. Blood was collected from the cubital vein and placed in three 3 mL vacutainers containing sodium citrate, for subsequent use to collect platelet. Hemolyzed samples were discarded. Anticoagulant blood was centrifuged at 800 × g at 4 °C for 10 min, and platelet-rich plasma (PRP) were collected by centrifugation at 3000 × g at 4 °C for 10 min. Platelet pellets were washed with tris-buffered saline and resuspended in lysis buffer containing tris–HCl, ethylene glycol tetraacetic acid, phenylmethanesul-phonyl fluoride, and protease inhibitors. Platelet aliquots were stored at −20 °C until analysis. The supernatant was collected and stored at −20 °C until use in other research.

Table 1
Demographic characteristics and neuropsychological assessments in the AD group, aMCI group and in healthy controls.

	Normal control ($n = 50$)	aMCI ($n = 50$)	AD ($n = 50$)	P value
Age	69.80 ± 5.391	70.94 ± 4.834	71.54 ± 5.136	0.229
Edu	22 (44%)	22 (44%)	25 (50%)	0.909
9–12 years	26 (52%)	27 (54%)	23 (46%)	
>12 years	2 (4%)	1 (2%)	2 (4%)	
Male,%(n)	64% (32)	56% (28)	56% (28)	0.644
MMSE	27.46 ± 1.843	26.44 ± 1.864	22.62 ± 1.894	0.000
MoCA	26.28 ± 2.232	23.42 ± 2.348	16.28 ± 2.907	0.000

Three groups did not differ in sex, education, or age. The levels of MMSE, MoCA-C of three scoring methods in patients with amnestic MCI and AD were found to be significantly decreased in comparison to healthy control subjects. In addition, the patients with amnestic MCI had higher scores than the patients with AD.

2.3. Western blotting

Protein concentrations were determined in each platelet sample by a modified Lowry method (Bio-Rad DC Protein Assay) before the Western blot Assay. Measurements of apoptosis-related protein in platelets, such as caspase-3 and caspase-9, Bcl2, Bad, and Bax, were carried out by western blot kits (Beyotime Institute of Biotechnology, Shanghai, China), according to the manufacturer's instructions. Platelets were processed for western blot with antibody of caspase-3 and caspase-9, Bcl2, Bad, and Bax (Fushen biotechnology company, Shanghai,China), separately. Quantitative analysis of western blot was performed by means of computer-assisted imaging, Quantity one 4.62, specifically. GADPH was used as the internal standard. Images of blots were captured with a scanner (Scanprisa 640UT, Acer, Shanghai, China) and proteins of interest were quantified by measuring optical densities of the protein bands using Image Pro-Plus image analysis software (Media Cybernetics, Inc., Bethesda, MD, USA).

2.4. Statistical analyses

The data were analyzed with the Statistical Package for the Social Sciences (SPSS, v.16.0 for Windows; SPSS Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) was used to compare measurement data among normal controls, amnestic MCI, AD patients, and the least significant differences method was selected. Post hoc tests were used to compare the MMSE and MoCA scores between the two groups. Pearson's chi-squared (χ^2)-test was used to compare gender, education level, neuropsychological assessments, optical density of apoptosis-related proteins between two groups.

The tests of significance were two-tailed and P values <0.05 were considered statistically significant throughout the analysis. The results are expressed as mean ± standard error (SE).

3. Results

3.1. Demographic characteristics and neuropsychological assessments

As shown in Table 1, the three groups did not differ in sex, education, or age. Amnestic MCI group had significant lower MMSE scores than the control group ($P = 0.019$), while higher scores than