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Featured Articles

Increased cerebrospinal fluid calpain activity and microparticle levels in Alzheimer's disease

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Abstract

Background: Calpain has been associated with the pathophysiology of Alzheimer's disease (AD) and with apoptotic neuronal cell death leading to microparticles (MPs) formation.

Methods: A total of 64 patients with AD and 52 age- and gender-matched cognitively healthy elderly controls were included in the study. We measured calpain activity and levels of MPs, amyloid beta (Aβ1–42), h-tau, and p-tau181.

Results: AD patients showed significantly increased calpain activity and higher levels of MPs in cerebrospinal fluid (CSF) and significantly decreased calpain activity and lower levels of MPs in serum and plasma compared with healthy controls. Combined assessment of calpain activity and Aβ1–42 levels in CSF improved diagnostic accuracy as compared with singular or combined traditional CSF biomarkers of AD.

Conclusions: This is the first study showing increased calpain activity and microparticle levels in CSF of AD patients. Calpain activity could represent a novel diagnostic and prognostic biomarker and promising treatment target for AD.

Keywords: Alzheimer's disease; Calpain activity; Microparticles; CSF; Serum; Plasma; Dementia

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Aging populations in developed countries ensure that AD will reach epidemic proportions unless therapies are developed to cure or prevent it. Unfortunately, to date all "disease-modifying" experimental therapies for AD have failed to demonstrate significant clinical benefit in individuals with symptomatic AD. Thus, besides earlier start of treatment there is also need to identify new treatment targets in AD.

The progressive formation of amyloid plaques and vascular deposits consisting of the 4 kD amyloid β-peptide (Aβ) is considered a pathological hallmark of AD [1]. Aβ is generated from the amyloid precursor protein (APP) by enzymatic digestion involving β- and γ-secretase activities [2]. Most β-secretase activity originates from a protease encoded by the β-site APP-cleaving enzyme 1 gene (BACE1) [3]. Growing evidence indicates that BACE1 activity is significantly increased in the brain [4] and cerebrospinal fluid (CSF) [5,6], of sporadic AD patients. According to experimental findings in an animal model of AD, BACE1 expression is promoted by activation of calpain [7].

Conflict of interest: The authors have no potential conflicts of interest to declare.

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Calpains represent a family of calcium-dependent proteases, which modify the function of their target proteins by partial truncation. Among more than 10 species of calpain isozymes, calpain 1 (μ-calpain) and calpain 2 (m-calpain) have been characterized most extensively [8]. These two isozymes are ubiquitously expressed in mammalian tissues, including brain and blood vessels. Although calpains are involved in different physiological processes, disrupted intracellular calcium homeostasis leads to hyperactivation of calpains. In the central nervous system, hyperactivated calpains have been shown to enhance the accumulation of β-amyloid peptide by increasing the expression levels of BACE1 [7] and induced tau hyperphosphorylation along with the formation of neurofibrillary tangles by mediating p35 cleavage into p25 and by activation of cyclin-dependent kinase 5 (CDK5) [9,10], both of which are the major mechanisms of neurodegeneration in AD. In line with these findings, calpain activity and levels were found to be increased in senile plaques and neurofibrillary tangles of AD brains [11,12]. A recent study has clearly demonstrated an enhancement of calpain activity in distinct plaque-surrounding compartments in the brains of patients with AD and APP-Tg mice [13]. Moreover, calpain plays an important role in neuronal apoptosis [14]. The involvement of calpain in the pathology of AD is further supported by the finding that Aβ amyloidosis, tau phosphorylation, and microgliosis were enhanced in mice lacking the endogenous calpain inhibitor calpastatin [13]. Finally, pharmacological inhibition of calpain has been shown to improve memory and synaptic transmission and to reduce AD-like pathology in a mouse model of AD [15] and to be neuroprotective in cell cultures [16,17].

Calpains are also involved in cell activation and/or apoptosis leading to microparticles (MPs) formation [18,19]. In addition, platelet-derived MPs (PL-MPs), which represent the majority of circulating MPs [20], are known to carry active calpain [21–23]. Thus, calpain activity in the plasma and the levels of PL-MPs seem to be directly associated. To the best of our knowledge, data concerning calpain activity and levels of MPs and PL-MPs in the blood and CSF from AD patients are still missing.

The aim of the present study was to measure calpain activity and MP levels in serum, plasma and CSF in AD patients and age and gender matched, cognitively healthy elderly controls and to examine the correlations with clinical parameters (age, Mini-Mental State Examination [MMSE] scores) and CSF biomarkers for diagnosis of AD (Aβ1–42, h-tau, and p-tau181).

2. Materials and methods

2.1. Reagents

Calpeptin was from Calbiochem (Darmstadt, Germany), the antibodies against μ- and m-calpain and all other drugs were from Sigma (Steinheim, Germany).

2.2. Subjects

A total of 64 patients with AD and 52 age and gender matched cognitively healthy elderly controls were included in the study. In cohort 1, consisting of 20 AD patients and 20 control subjects, we measured calpain activity in serum and CSF. In an independent cohort 2, consisting of 44 AD patients and 32 control subjects, we measured calpain activity in plasma. Baseline characteristics and demographic parameters of both cohorts are displayed in Table 1. AD patients were out-patients from our Memory Clinic at the University Hospital of Psychiatry and Psychotherapy Tübingen. Patients with AD fulfilled the criteria of *the International Classification of Diseases, Tenth Edition (ICD-10)* [24], *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)* [25], and the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association for probable AD [26]. The severity of dementia was assessed by MMSE [27]. Thirty-six AD patients in cohort 2 were followed-up and their cognitive status was reassessed 12 months after enrollment. The rate of cognitive decline was defined as fast when the Δ change of MMSE score was ≥4 and slow in case of Δ change of MMSE score <4. As control subjects we included in cohort 1 individuals from the Center of Neurology in Tübingen with other noninflammatory neurological diseases including headache or back pain, whose CSF did not show any evidence of inflammation. In cohort 2, controls were cognitively healthy elderly subjects without neurological diseases. All control subjects had a normal cognitive status according to clinical examination and MMSE score (Table 1).

Table 1
Baseline characteristics of Alzheimer's disease (AD) patients and healthy controls in both cohorts

Characteristics	AD patients (n = 20)	Healthy controls (n = 20)	*P* value
Cohort 1			
Female/male, n	12/8	11/9	.343*
Age (yr), mean ± SD	73.4 ± 8.3	70.3 ± 5.5	.174†
MMSE, mean ± SD	23.2 ± 3.1	28.6 ± 1.9	<.0001†
Characteristics	AD patients (n = 44)	Healthy controls (n = 32)	*P* value
Cohort 2			
Female/male, n	25/19	14/18	.819*
Age (yr), mean ± SD	72.0 ± 7.3	69.4 ± 7.3	.166†
MMSE, mean ± SD	19.9 ± 4.7	29.2 ± 0.7	<.0001†

Abbreviations: MMSE, Mini-Mental State Examination; n, number of subjects; SD = standard deviation.
*Fisher's exact test.
†Mann-Whitney U test.