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Resolution of inflammation is altered in Alzheimer's disease

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Abstract

Background: Resolution is the final stage of the inflammatory response, when restoration of tissue occurs. Failure may lead to chronic inflammation, which is known as part of the pathology in the brain of individuals with Alzheimer's disease (AD).
Methods: Specialized pro-resolving mediators (SPMs), receptors, biosynthetic enzyme, and downstream effectors involved in resolution were analyzed in postmortem hippocampal tissue from AD patients and non-AD subjects. SPMs were analyzed in cerebrospinal fluid (CSF).
Results: SPMs and SPM receptors were detected in the human brain. Levels of the SPM lipoxin A_4 (LXA_4) were reduced in AD, both in the CSF and hippocampus. An enzyme involved in LXA_4 synthesis and two SPM receptors were elevated in AD brains. LXA_4 and RvD1 levels in CSF correlated with Mini-Mental State Examination (MMSE) scores.
Conclusions: A resolution pathway exists in the brain and the alterations described herein strongly suggest a dysfunction of this pathway in AD. MMSE correlations suggest a connection with cognitive function in AD.

Keywords: ChemR23; ELISA; FPRL1; Human; Immunohistochemistry; Lipoxin A_4; ALX/FPR2; 15-Lipoxygenase-2; Mild cognitive impairment; Resolvin D1; Specialized pro-resolving mediators; Tau

1. Introduction

Alzheimer's disease (AD) is the most common type of dementia and the number of AD patients is growing rapidly worldwide. Pathologic hallmarks in AD are the senile plaques, mainly composed of extracellular amyloid β (Aβ), and neurofibrillary tangles (NFTs), consisting of intraneuronal hyperphosphorylated tau [1]. Direct evidence of inflammation in the AD brain has been demonstrated by in vivo positron emission tomography (PET) studies, showing increased activation of microglia in AD patients [2]. Moreover, levels of pro-inflammatory cytokines are elevated in postmortem brain tissue [3] and serum from AD patients [4]. In vitro studies have demonstrated that Aβ can activate innate immune cells in the brain [5,6]. Epidemiologic studies suggest a neuroprotective role of nonsteroidal anti-inflammatory drugs (NSAIDs) [7], although prospective clinical trials have generally been ineffective, suggesting a more complex role of inflammation in AD [7].

Inflammation is normally terminated by resolution, with the purpose of promoting healing and a return to homeostasis. Resolution results in reduced numbers of immune cells at the site of insult by decreased infiltration and apoptosis, and clearance of apoptotic cells and debris by increased

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phagocytic activity [8,9]. The resolution is an active process mediated by fatty acid (FA) derivates called specialized pro-resolving mediators (SPMs). The lipoxin family of SPMs (LXs) was the first revealed [10], then characterized [11–13]. Novel SPMs that have since been discovered include the eicosapentaenoic acid (EPA)–derived resolvin E (RvE) series, docosahexaenoic acid (DHA)–derived resolvin D (RvD) series, the protectins that include (PD1)/neuroprotectin D (NPD1), and maresins (MaRs) [8]. The biosynthetic pathways of SPMs can involve oxygenation of their precursors by lipoxygenases (LOXs) or cyclooxygenases [14]. Multiligand receptors that recognize SPMs have been discovered [15–19] and, upon binding of the SPMs to their receptors, resolution is initiated [20]. However, few studies have hitherto addressed the subject of resolution in the brain. Neuroprotective effects have been shown for NPD1 [21] and LXA_4 [22], and reduced levels of NPD1 were described in AD brains [21]. LXA_4 can also reduce reactive oxygen species (ROS) in activated microglia [23] and inhibit interleukin (IL)-8 expression in astrocytoma cells [24], as well as reduce Aβ levels and improve cognition in a transgenic mouse model of AD [25]. RvD1 and RvE1 attenuate inflammation-associated pain in mice via central and peripheral action [26].

Chronic neuroinflammation in the AD brain indicates that the resolution of inflammation is dysfunctional. To investigate this, we have analyzed postmortem brain tissue and cerebrospinal fluid (CSF) samples from AD patients and controls with regard to production and transmission of pro-resolving signals.

2. Methods

2.1. Subjects

Human CSF samples were obtained from 15 AD patients (according to International Classification of Diseases version 10 [ICD-10] criteria [27]; age [mean ± SD] 67.87 ± 10.232 years), 20 mild cognitive impairment (MCI according to Winblad criteria [28]; age 65.65 ± 10.373 years), and 21 subjective cognitive impairment (SCI; no objective cognitive impairment; age 57.48 ± 5.409 years) subjects from the Memory Unit, Geriatric Clinic, Karolinska University Hospital, Huddinge, Sweden. As part of the diagnostic procedure these subjects were assessed by the Mini-Mental State Examination (MMSE) test, and levels of phosphorylated tau (phosphorylation site threonine 181) were measured by enzyme-linked immunosorbent assay (ELISA) kits (INNOTEST®; Innogenetics, Ghent, Belgium). Brain tissue samples were obtained from 10 AD patients (9 with Braak stage 5 or 6 definite AD [29] and 1 with Braak stage 3 or 4 probable AD), and 10 nondemented control subjects, all from the Brain Bank at Karolinska Institutet. There was no statistical difference in age or postmortem interval (PMI) between the AD and control groups (Table 1). Half of each brain was fixed in formalin and tissue samples embedded in paraffin. The other half was dissected according to region, and then frozen and stored at −80°C. The study was approved by the regional ethics committee of Stockholm.

Table 1
Subject information for hippocampal tissue samples

	Control (n = 10)	AD (n = 10)
Age (mean ± SD)	80.40 ± 6.275	80.20 ± 7.021
Gender (male)	6(4)	8 (2)
PMI (mean ± SD)	17.40 ± 12.131	23 ± 12.266

Abbreviation: PMI, postmortem interval (hours).

2.2. Tissue processing

Paraffin-embedded tissue blocks of the hippocampus were sectioned into 6-μm-thick sections and mounted onto polarized glass slides for morphologic analysis. Hippocampal tissues used for biochemical analysis were collected in the coronal plane at the level of the lateral geniculate nucleus, which contains the entire hippocampus region (cornu ammonis [CA] 1–4) and dentate gyrus (DG). Before analysis, each frozen tissue was pulverized (BioPulverizer; BioSpec Product, Inc.), mixed well, divided, and kept at −80°C until further processing.

2.3. Enzyme immunoassay

Free FAs (FFAs) were extracted from hippocampal tissue and CSF samples according to the supplier's instructions provided with the LXA_4 enzyme immunoassay (EIA; Oxford Biomedical Research). The brain tissues were collected over a period of 4 years, and stored at −80°C for a similar time period until further processing. The CSF samples were collected over a period of 3 years, and stored at −80°C until further processing. For tissue extraction, hippocampal samples were homogenized in ethanol by a pestle homogenizer, followed by centrifugation at 1500 × *g* for 15 minutes. The supernatant was collected and acidified to pH 3.5. These samples as well as the acidified CSF samples were extracted through Sep-Pak C18 columns (Waters). Briefly, the columns were equilibrated with methanol and ddH_2O, and acidified samples were loaded immediately. Bound FFAs were eluted with methyl formate, brought to dryness by nitrogen gas, and finally resuspended by extraction buffer supplied with the LXA_4 EIA kit. This kit was used for analysis of LXA_4, and RvD1 was measured with an RvD1 EIA kit (Cayman Chemical), following the manufacturer's instructions. Total FFAs were measured using an FFA assay kit (Cayman Chemical).

2.4. Liquid chromatography–tandem mass spectrometry

Three control and 3 AD tissue specimens were selected at random from the cohort of samples to carry out full lipid mediator (LM) profiling using liquid chromatography–tandem