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Review - Part of the Special Issue: Alzheimer's Disease – Amyloid, Tau and Beyond

Novel lipid signaling pathways in Alzheimer's disease pathogenesis

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. With an increasing longevity and the absence of a cure, AD has become not only a major health problem but also a heavy social and economic burden worldwide. In addition to the presence of abundant intra- and extra-cellular neurotoxic amyloid β (Aβ) peptides, which form the amyloid plaques, and intracellular hyperphosphorylated tau protein, the main component of neurofibrillary tangles, consistent evidence indicates that the AD brain is characterized by extensive neuroinflammatory processes. The 5-lipoxygenase (5LO) is a pro-inflammatory enzymatic pathway widely distributed within the central nervous system and is up-regulated in AD. In the last five years our group has been involved in unraveling the neurobiology of this protein and investigating its relationship with cellular and molecular events of functional importance in AD pathogenesis. By using a combination of in vitro and in vivo experimental tools and implementing genetic as well as pharmacological approaches today we know that 5LO is likely an endogenous regulator of Aβ formation via the modulation of the γ-secretase complex, and tau metabolism by modulating its phosphorylation state at specific epitopes via the cyclin-dependent kinase-5 (cdk-5). In addition, 5LO influences synaptic function and integrity and by doing so significantly affects learning and memory in the Tg2576 and 3xTg AD transgenic mouse models. Taken together our data establish this protein as a pleiotropic contributor to the development of the full spectrum of the AD-like phenotype in these mouse models of the disease, making it a viable therapeutic target for the treatment of AD in humans.

Contents

1. Introduction

Alzheimer's disease (AD) is the most common cause of aging-associated neurodegenerative dementia, characterized by profound, irreversible memory impairment and global cognitive decline. The hallmark brain pathologies in AD are the deposition of extracellular amyloid plaques composed of amyloid-β (Aβ) protein as well as aggregation of intraneuronal neurofibrillary tangles consisting of hyperphosphorylated tau protein [1,2]. Although the prevalence of AD is currently thought to be in excess of 35 million people worldwide, due to the aging of the "baby-boomer" generation as well as increasing human longevity, this burden is thought to quadruple by 2050 [3]. Given this public health challenge, and that the current pharmacological armamentarium approved for AD is limited to symptomatic treatment (i.e., cholinesterase inhibitors and NMDA receptor antagonists), exploration of new molecular pathways as novel therapeutic targets in AD remains an attractive option for disease modifying drug development.

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2. 5-Lipoxygenase (5LO)

The lipoxygenase (LOs) proteins are a group of lipid-peroxidizing enzymes that insert molecular oxygen into esterified and free polyunsaturated fatty acids such as arachidonic acid, generating bioactive lipid moieties. The 5-lipoxygenase (5LO) in particular catalyzes the conversion of arachidonic acid to 5-hydroxy-peroxy-eicosatetrenoic acid (5-HPETE), which can subsequently be metabolized to 5-hydroxy-eicosatetrenoic acid (5-HETE) as well as different leukotrienes [4] (Fig. 1). Since leukotrienes are potent pro-inflammatory molecules, much work has investigated the role that 5LO plays in vascular inflammation, atherosclerosis, allergy, and cancer biology [5]. Currently, clinical application of 5LO modulation is focused on control of asthma by the 5LO inhibitor, zileuton (Zyflo).

Despite this investigation in the periphery, 5LO's role in the central nervous system has only recently received attention. The 5LO enzyme is widely expressed in the central nervous system, where it localizes mainly in neuronal cells [5–7]. In human and murine brains, 5LO is abundantly expressed in the hippocampus and cerebral cortex, with its levels and activity rising as a function of aging [8]. Since aging is an unavoidable risk factor in the development of sporadic AD, and 5LO is expressed in regions of the brain that are particularly affected by AD, we originally hypothesized that 5LO could play a function role in its progression. Although one limited study with 34 individuals had previously hinted that polymorphisms in the 5LO gene (*ALOX5*) may be associated with AD risk [9], ours was the first group to show that 5LO steady-state protein levels were significantly increased in the brains of patients with sporadic AD compared to age-matched controls [10]. The work was later confirmed by another group by using an immunohistochemistry approach [11]. Additionally, a more recent report showed a significant up-regulation in 5LO gene expression and enzyme activation, as measured by leukotriene B4 levels, in peripheral blood mononuclear cells of AD subjects compared to healthy controls [12].

Interestingly, we also found that compared to wild-types animals, 5LO levels were also elevated in a transgenic mouse model of AD-like brain amyloidosis (the Tg2576 mouse, see below), affording us the opportunity to experimentally study if and how 5LO could participate in the AD phenotype development. Below, we summarize the data so far available supporting a functional role for 5LO in AD-related Aβ metabolism, tau phosphorylation, synaptic integrity and function as well as behavior. Based on this knowledge, we suggest that the development of selective and specific 5LO inhibitors hold significant promise as novel disease-modifying agents. Considering that the 5LO inhibitor Zyflo has already been approved by the FDA for use in humans and is readily commercially available, our work underscores the tremendous translational importance of developing 5LO-centered therapeutics for AD.

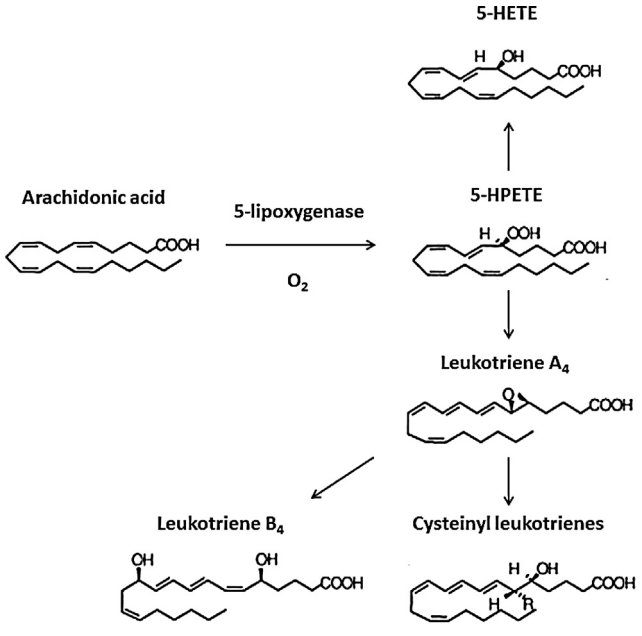

Fig. 1. The 5-lipoxygenase enzymatic pathway. The 5-lipoxygenase (5LO) by inserting molecular oxygen into carbon 5 catalyzes the conversion of arachidonic acid to 5-hydroxy-peroxy-eicosatetrenoic acid (5-HPETE), which can subsequently be metabolized to 5-hydroxy-eicosatetrenoic acid (5-HETE) as well as different leukotrienes such as leukotrienes A4 and leurkotriene B4.

3. 5LO and Aβ

The Aβ peptides are formed from sequential cleavages of the Aβ precursor protein (APP) by β-site secretase-1 (BACE-1), followed by the γ-secretase complex, which is composed at least of four different intramembrane proteins: presenilin 1 (PS1), presenilin-enhancer 2 (PEN2), anterior pharynx-defective 1 (APH1) and nicastrin (NCT), in a 1:1:1:1 ratio [13]. Once produced, Aβ peptides are prone to oligomerize and it is thought that aggregation of low-*n* oligomers eventually forms fibrils of amyloid, leading to the formation of the amyloid plaques [14]. Genetic analyses in families with early-onset AD have revealed multiple mutations in the synthetic pathway of Aβ, particularly in APP and presenilin which then predispose these individuals to greater production of Aβ [15]. Therefore, of the two pathologies in AD, Aβ has received significantly more attention in clinical trials attempting to alter AD progression.

Since in our initial studies we found that 5LO expression increases with age and is expressed in regions of the brain in which Aβ plaques are also found, we asked whether 5LO modulation would change amyloid plaque burden in vivo. To address this question we utilized Tg2576 mice, a transgenic mouse model that expresses the K670N/M671L APP mutation found in a Swedish family with early-onset AD, which develop age-dependent brain amyloidosis similar to that seen in humans. We crossed the Tg2576 with mice genetically deficient for 5LO (i.e., homozygous knockout of 5LO) and compared them with regular Tg2576 animals to see how brain amyloidosis was affected when 5LO was not genetically available. We found that both soluble and insoluble Aβ peptides were significantly reduced in the brains of Tg2576 animals lacking 5LO, and that this reduction was even more apparent as the animals aged from middle- to late-life. On immunohistochemical analyses, this reduction in Aβ peptides translated to fewer amyloid β plaques and reduced total amyloid burden [10]. What was singular about this initial work was that Aβ reduction caused by 5LO knockout did not seem to change steady state levels of APP or increase several proteins thought to participate in Aβ clearance in the brain. To verify our knockout results, we also conducted studies of pharmacological inhibition of 5LO. Thus, we fed Tg2576 animals rodent chow supplemented with the 5LO inhibitor zileuton, from early adulthood, and found as with our knockout studies, that this pharmacologic approach also reduced brain Aβ peptide levels and amyloid plaques burden [16]. Interestingly, a recent paper showed that pharmacologic blockade of 5LO activating protein reduces amyloid angiopathy in TgCRND8 mice and this reduction was associated with a significant decrease in the steady state levels of nicastrin, one of the components of the γ-secretase complex [17].

Following these results, we searched for an explanation of how 5LO modulates Aβ production in vivo. We first considered the BACE-1 metabolic pathway: if steady-state levels of this secretase were to be decreased or its net activity reduced, this could lead to lower levels of Aβ without any change in APP. However, we found