Current and future directions for targeting lipoxin A_4 in Alzheimer's Disease

Irundika HK Dias[1*] and Helen R. Griffiths[2]

1. Aston Medical School, Aston University, Birmingham, B4 7ET, UK

2. Swansea University Medical School, Swansea University, SA2 8PP, UK

*Corresponding Author

Irundika HK Dias
Aston Medical School
Aston University
B4 7ET
UK
Tel: 0044 121 204 4678

Email: diashki1@aston.ac.uk

Abstract

Neuroinflammation has been implicated in Alzheimer's disease onset and progression. Chronic neuroinflammation is initiated by amyloid beta-activated microglial cells that secrete immuno-modulatory molecules within the brain and into the vasculature. Inflammation is normally self-limiting and actively resolves by "switching off" the generation of pro-inflammatory mediators and by non-phlogistic clearance of spent cells and their debris to restore tissue homeostasis. Deficits in these anti-inflammatory/pro-resolution pathways may predispose to the development of chronic inflammation. The synthesis of endogenous lipid mediators from arachidonic acid (AA), lipoxins via cyclooxygenase 2 and lipoxygenases, and conversion of exogenous polyunsaturated fatty acids, namely docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) to resolvins contributes to effective, timely resolution of acute inflammation.

Work by Xiuzhe et al., 2020 in the Journal of Alzheimer's Disease (AD) reported that plasma level of LXA_4 is related to cognitive status in ischemic stroke patients suggesting that decreased LXA_4 may be a potential risk factor for post post-stroke cognitive impairment. As evident by recent clinical trials and development of drug analogues, there is recent drive to search for lipoxin analogues as therapeutics for inflammatory diseases. Understanding how bioactive lipid signalling is involved in resolution will increase our understanding of controlling inflammation and may facilitate the discovery of new classes of therapeutic pro-resolution agents for evaluation in AD prevention studies.

Commentary

The initiation of an inflammatory response and its timely resolution is critical to maintain a healthy physiological state. When the resolution of inflammation is compromised, homeostasis may be skewed towards chronic inflammatory status resulting in bystander tissue damage. Emerging evidence over the last decade suggested that the presence of a sustained immune response in the brain is a common pathology in Alzheimer's disease (AD) [1, 2]. The interaction of microglia and other brain resident immune cells with fibrillar amyloid beta (Aβ) may lead to their activation and the conversion of these cells into a classically 'activated' phenotype producing of chemokines, neurotoxic cytokines, eicosanoids and reactive oxygen (ROS) and nitrogen species (RNS) that are deleterious to the central nervous system [3]. Moreover, a role for inflammation in AD has gained strong support from genome-wide association studies that have identified genes involved in inflammation that are associated with increased risk of developing AD [4, 5]. While neuroinflammation is elicited to clear "damage" and initiate repair processes, uncontrolled inflammation can result in neurotoxicity that exacerbates a neurodegenerative pathology. However, merely inhibiting the inflammatory response may weaken its protective effect and potentially be more detrimental. Therefore, a deeper understanding of the resolution of inflammation generally and AD is needed to enable its protective while minimising its detrimental effects.

It is now recognised that resolution of inflammation is a dynamically regulated, active process that is orchestrated by specialised pro-resolving lipid mediators (SPMs) such as lipoxins, resolvins, protectins and maresins. These SPMs are enzymatically synthesised from essential polyunsaturated fatty acids namely docosahexaenoic acid (DHA; 22:6 ω3), eicosapentaenoic acid (EPA; 22:5 ω3) and arachidonic acid (AA; 20:4 ω6) via sequential steps involving cyclooxygenase (COX) and lipoxygenase (LOX) enzymes [6]. AA and DHA can be metabolised by lipoxygenases to form hydroxy derivatives and leukotrienes and AA is metabolised by cyclooxygenases to prostaglandins and thromboxanes or lipoxins via sequential lipoxygenase activity. Lipoxins (LXA_4 and LXB_4) are generated by two main routes in circulating immune cells. The first described route inserts molecular oxygen to AA by 15-LOX followed by 5-LOX based transformation. The second route involves the conversion of 5-LOX-derived LTA4 and subsequent conversion to lipoxins via 12-LOX activity [7]. The generation of lipoxins is known to increase during cell-cell interactions in blood and mucosal tissues. When both 15-LOX and 5-LOX are co-expressed, the biosynthesis of lipoxins can occur in the same cell such as in eosinophils, macrophages and resolution phase neutrophils [8, 9]. They have been reported to act at both temporal and spatially distinct sites from other eicosanoids produced during the inflammatory-resolution process. However, it is still unclear how lipoxins are formed within the intact human brain and in pathological conditions.

Epidemiological studies of non-steroidal anti-inflammatory drug (NSAID) interventions have suggested their use may delay disease onset [10, 11] providing support for inflammation in the aetiology of AD. A meta-analysis study has revealed as much as 50% reduction in the risk of disease onset among chronic none steroidal anti-inflammatory drugs (NSAID) users [12]. NSAIDs exert their anti-inflammatory effect by either inhibiting the COX-2 pathway or modifying COX-2 to promote biosynthesis of SPMs. For example, aspirin triggers acetylation of cyclooxygenase-2 to increase lipoxin production [7]. This inducible enzyme increases the synthesis of the pro-inflammatory mediators, PG from dietary or membrane-originated AA via phospholipase A2 activity. NSAIDs inhibit the COX-2 enzyme without affecting 5-LOX activity and do not prevent the formation of leukotrienes (LTs). Elevated 5-LOX mediated AA metabolism and accumulation of cysteinyl LTs, such as LTC4, LTD4, LTE4, and leuko-attractive LTB4 are observed in several organs including the brain of mammals in response to NSAID consumption [13].

The positive outcome of a number of small (n~50) pilot intervention studies over 3-6 months to address the hypothesis that chronic NSAID use can prevent or delay the rate of cognitive decline in AD patients has led to the establishment of clinical trials [14]. However, large-scale double-blind placebo-controlled clinical trials have not supported the use of NSAIDS in treating established AD [11]. The disappointing outcome of these clinical trials has raised questions as to our understanding of mechanisms and pathways that link systemic inflammation and neurodegeneration, and prompted questions about whether NSAIDs can act only as preventative agents following long term use, and whether their clinical failure to treat established AD has resulted from inappropriate choices of NSAIDs and the doses employed.

The article by Xiuzhe et al., 2020 in Journal of Alzheimer's disease is timely in reporting that levels of the plasma LXA_4 were significantly reduced in post-stroke cognitive impairment (PSCI) patients compared with post-stroke non-cognitive impairment (PSNCI) patients [15]. This work has implications not only for the management of post stroke events, but also for conditions such as metabolic diseases [16], and diabetic chronic kidney disease [17], lupus nephritis [18] that increase risk of stroke in which similar low serum level of LXA_4 is reported. There is growing interest to validate low LXA_4 as a biomarker for inflammatory diseases as indicated by clinical trials such as COPD (id: NCT03609541) and asthma (id: NCT01898767 and NCT03423693).

Xiuzhe et al., found that the formation of plasma LXA_4 and the ratio of LXA_4/LTB_4 were positively correlated with Mini-Mental State Examination scores within the first 7 days of stroke onset. The study design was careful not to include any persons with previous known cognitive decline. Interestingly, lower levels were not observed for other SPMs such as resolvin D1, resolvin D2 or maresin1, highlighting the suitability of LXA_4 as a biomarker for cognitive decline. As the authors suggest, the mechanisms behind the decreased formation of plasma LXA_4 and any contribution to PSCI pathogenesis has yet to be explored. It is possible that LXA_4 regulates polarization of microglia to M2 phenotype and may enhance neuronal survival against Aβ toxicity, reduce tau phosphorylation and reduce Aβ secretion but lower rates of formation in PSCI patients compromise its protective effects driving towards neuronal damage and cognitive decline. Longitudinal data for such a study will be able to shed more light into circulatory level of LXA_4 and cognitive health in post-stroke patients. Other in vivo studies supported that intravenous treatment with lipoxin analogues that resist metabolic degradation can provide neuroprotection and reduce neurobehavioral deficits at 4 weeks after ischemic stroke in rats [19]. In addition to LXA4, the ability of other SPMs such as resolvins and maresins to reduce inflammation in AD has also been described previously [13, 20]. Based on these common observations, the possibilities of developing novel "resolution-targeted" therapy is being investigated.

Initial efficacy studies for early analogues of LXA_4 studies have now been developed into four generations of LXA_4 drug analogues: 1st generation- stable analogues of LXA_4 [21], 2nd generation- 3-oxa-LXA_4 analogues [22], [23], [24], 3rd generation- pyridine/benzo-LXA_4 analogues [25], [26] and 4th generation- Imidazole- and Oxazole-Containing Synthetic Lipoxin A4Mimetics (SLXms) [27]. These are being studied in various biological systems and a phase I trial is ongoing to evaluate the safety and efficacy of the LX analogue BLXA4-ME as an oral rinse for gingivitis (id: NCT02342691). Another study, ASTHMIRINE trial (id: NCT02906761, phase 3, n = 180) investigates the ability of Aspirin to enhance SPM formation with special attention to aspirin triggered LXA_4, which is an epimer of LXA_4. Even though these investigations are at early stage, they showcase the importance of future research for targeting LXA_4 in common inflammatory diseases.

The next challenges will be to understand the timing of the treatment for patients with cognitive impairment, and optimal drug doses, pharmacodynamics and pharmacokinetics. Research

developments to improve resolution of inflammation could lead to combat not only neurodegenerative diseases but also improve health outcomes in a range of inflammatory diseases.

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