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Review Article

The role of the 5-lipoxygenase pathway in Alzheimer's disease

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Abstract

Many neurological diseases are now recognized to involve neuroinflammation as a major pathological feature. Neuroinflammation can be both a cause and a consequence of the disease process in Alzheimer's disease (AD). Chronic use of nonsteroidal antiinflammatory drugs (NSAIDs) has been found to delay the onset of AD and slow disease progression, a finding that has stimulated substantial interest in the role of these drugs in the prevention and treatment of AD. Unfortunately, recent clinical trials involving NSAIDs either have produced negative results or have been suspended due to toxicity issues concerning cyclooxygenase inhibition (*e.g.*, ADAPT). Consequently, there is an urgent need to develop and test new antiinflammatory drugs devoid of the toxic effects associated with cyclooxygenase inhibition but which still suppress neuroinflammation and β-amyloid-induced neurotoxicity. In addition to the cyclooxygenase pathway, arachidonic acid is also metabolized by the lipoxygenase pathway to form leukotrienes and lipoxins. There is some evidence indicating that 5-lipoxygenase inhibitors may offer protection against various aspects of the pathogenesis of AD. Based on these findings, we suggest that 5-lipoxygenase inhibitors may have therapeutic potential in the prevention and treatment of AD.

Introduction

The brain of patients with Alzheimer's disease (AD) shows chronic inflammatory responses characterized by activated microglia and astrocytes and enhanced expression of cytokines and complement factors surrounding β-amyloid (Aβ) deposits. Several epidemiological studies have demonstrated a lower risk for AD in chronic users of nonsteroidal antiinflammatory drugs (NSAIDs). This finding has stimulated substantial interest in the role of these drugs in the prevention and treatment of AD. Unfortunately, recent clinical trials on the use of these drugs have produced negative results or have been suspended due to toxicity associated with cyclooxygenase inhibition. Therefore, there is an urgent need to develop and test new antiinflammatory drugs without the toxic effects associated with cyclooxygenase inhibition but retaining the ability to suppress neuroinflammation. This review article concerns the potential use of 5-lipoxygenase (5-LOX) inhibitors for the prevention and treatment of AD.

Neuroinflammation and age-related neurodegenerative diseases

Neuroinflammation is widely considered as a possible pathophysiological mechanism of aging-associated neurodegeneration. It is thought to be an integral component of normal aging, as well as in the pathogenesis of age-related neurological diseases such as AD, Parkinson's disease and amyotrophic lateral sclerosis (ALS). These neurodegenerative diseases share neuroinflammatory characteristics such as elevation of proinflammatory cytokines (*i.e.*, IL-1 and TNF-α), microglial activation and the presence of reactive astrocytes. Numerous research reports have provided evidence of neuroinflammation in the brain of AD patients (*e.g.*, 1-4). Extracellular deposition of Aβ, a pathological characteristic of AD, has been reported to trigger many signal transduction pathways that contribute to neuroinflammation. Amyloid deposits are found to be co-localized with various inflammation-related proteins, such as complement proteins, acute-

phase proteins and clusters of activated microglia, indicating a strong glial response to amyloid deposits. Elevated levels of cytokines, cytokine receptors and reactive astrocytes suggesting inflammation have also been reported in post mortem AD brains (5-7).

Neuroinflammation as a cause or a consequence of amyloid pathology

Although the extent of involvement of neuroinflammation in AD pathogenesis is still unclear, it appears to be both a consequence and a cause of amyloid pathology. The theory that chronic inflammation may accelerate AD pathogenesis is supported by recent genetic findings showing that polymorphisms in proinflammatory genes (*e.g.*, IL-1α, IL-1β and TNF-α) enhance the risk for AD (8, 9). The extensive neuronal damage observed in AD patients is also thought to be a consequence of glial activation and inflammation, since glial activation and cytokine overexpression are observed decades before the pathological and behavioral changes selective for AD occur in individuals with Down's syndrome and traumatic brain injury (10).

Activated microglia and reactive astrocytes in AD brains were initially considered to be a response to amyloid deposition. However, there is a consensus that chronic microglial activation may also accelerate the amyloid cascade by stimulating the secretion of proinflammatory cytokines and reactive molecules that further enhance inflammation (10). This is supported by observations whereby microglia, when stimulated *in vitro*, generate free radicals, proinflammatory cytokines and neurotoxic lipid mediators. Proinflammatory cytokines may further be involved in activating β-secretase and stimulating more amyloid production. Enhancement of amyloid deposition has been observed in APPV717F transgenic mice upon administration of bacterial lipopolysaccharide (LPS). Chronic glial activation was shown to enhance the hyperphosphorylation of tau, resulting in the subsequent development of neurofibrillary tangles, an important pathological feature of AD (11). These observations confirm that neuroinflammation may accelerate amyloid deposition and the formation of neurofibrillary tangles in a high-risk group for AD (12, 13).

Based on this link between neuroinflammation and AD pathology, it appears logical that suppression of microglial activation and free radical generation should delay the progression of the disease. Therefore, numerous research laboratories have focused their efforts on antiinflammatory drugs and their effects on various aspects involved in the pathogenesis of AD.

Chronic NSAID treatment reduces the risk of AD

The first data associating chronic neuroinflammation with the pathogenesis of AD came from epidemiological studies. These studies showed a reduction of up to 50% in the risk of AD and lower rates of cognitive decline in chronic NSAID users (14-16), and subsequent studies by various groups confirmed these findings. The results of these epidemiological observations were further supported by observations from experimental studies using animal models of AD. Chronic treatment with a subset of NSAIDs (*e.g.*, ibuprofen, flurbiprofen, indomethacin) reduced neuroinflammation, Aβ levels and the deposition of Aβ in rat brain (17-23). In a study by Lim *et al.* (22), 6 months of oral treatment with ibuprofen decreased amyloid plaque number, as well as SDS-soluble and -insoluble Aβ levels in transgenic Tg2576 mice. Ibuprofen treatment suppressed the number of activated microglia and brain levels of IL-1β, a key cytokine implicated in AD pathogenesis (17, 21, 22). These observations were confirmed by Yan and coworkers (23).

However, the antiinflammatory mechanism underlying the beneficial effects of NSAIDs on AD pathogenesis was challenged by recent findings demonstrating selective suppression of Aβ42 generation by certain NSAIDs, such as ibuprofen, indomethacin and sulindac sulfide (24). These compounds were suggested to change the conformation of the γ-secretase complex by binding to a novel site distinct from the catalytic center. However, further support for the antiinflammatory mechanism of NSAIDs in protecting against AD comes from the finding of their ability to suppress various markers of inflammation, which are considered to be important in animal models of AD (25, 26). These studies have shown that NSAIDs may exert their beneficial effects via multiple mechanisms of action (reviewed in 27).

Based on the epidemiological studies suggesting a protective role for NSAIDs, these drugs were expected to be excellent drug candidates for the treatment and/or prevention of AD. However, the results of clinical trials in AD patients have been quite discouraging. Also, the low tolerability of these drugs due to gastric and renal sensitivity and liver damage in elderly patients has clouded the results from these trials. The dropout rates from some of these clinical trials were as high as 50%.

At present, there is substantial evidence that both cyclooxygenase COX-1 and COX-2 isoforms are not only involved in homeostasis but are also modulators of inflammatory reactions. Recent observations indicate that treatment with COX-2-selective NSAIDs, such as rofecoxib, is associated with increased cardiovascular risk. This compound was withdrawn from the market by Merck & Co. after a colon cancer prevention trial revealed that it was associated with double the rate of strokes and heart attacks compared to placebo. It was thought that selective targeting of COX-2 could lead to cardiovascular effects by altering the fine balance between the fatty acids prostacyclin and thromboxane, which control blood clotting. In a study conducted by Pfizer, patients with cardiac surgery who were taking both aspirin and COX-2 inhibitors reported nearly 3 times the rate of cardiovascular events compared with those on placebo, indicating that inhibition of COX-2 increases the risk for cardiovascular events. This implies that the use of NSAIDs, which inhibit both COX-1 and COX-2, might also increase the risk for cardiovascular events.

The concern for toxicity of NSAIDs was reflected in the announcement by the National Institutes of Health that the use of naproxen (nonselective COX inhibitor) and celecoxib (selective COX-2 inhibitor) was being suspended in a large AD prevention trial. The trial, called the Alzheimer's Disease Anti-Inflammatory Prevention Trial (or ADAPT), was designed to assess the potential benefit of long-term use of NSAIDs in decreasing the risk of developing AD in people 70 years of age or older, who were considered to be at an increased risk for the disease. Even though the mechanism implicated in lowering toxic Aβ production by NSAIDs is considered not to involve COX inhibition, all NSAIDs reported to reduce Aβ42 also potently inhibit COX activity at much lower doses and are therefore likely to have toxicity problems. The (*R*)-enantiomers in the profen NSAIDs supposedly lack COX-inhibitory activity but retain the ability to reduce Aβ42 production. These (*R*)-enantiomers are currently the focus of research by many investigators (*e.g.*, 28, 29).

In conclusion, the efficacy of NSAIDs in AD has not yet been proven and safety issues have significantly limited their use. Consequently, there is an urgent need to develop and test new antiinflammatory drugs without the toxic effects associated with COX inhibition but retaining the ability to suppress neuroinflammation and toxicity induced by Aβ42.

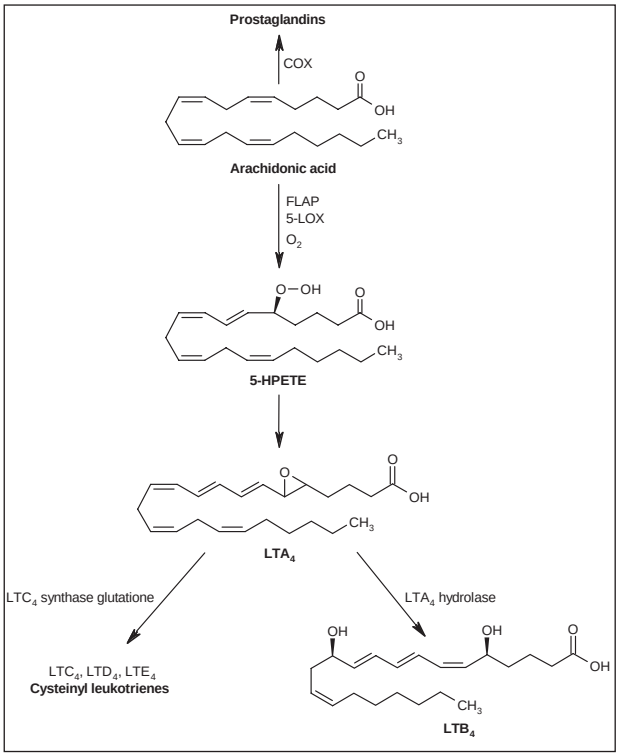

Fig. 1. The 5-lipoxygenase pathway of arachidonic acid metabolism.

Arachidonic acid metabolism

Arachidonic acid and lysophospholipids are released by the action of phospholipase A_2 (PLA_2) on phospholipids. In mammalian brain, there are different forms of phospholipases and they are implicated in inflammation, neurodegeneration and the intracellular as well as intercellular signal transduction network. Elevated immunoreactivity for PLA_2 has been observed in association with amyloid deposits in AD brain. Aβ peptides have also been observed to activate this enzyme in *in vitro* studies. In mammalian cells, the release of arachidonic acid constitutes the rate-limiting step in the biosynthesis of eicosanoids, such as prostaglandins, leukotrienes, thromboxanes and platelet-activating factor, all of which act as potent inflammatory mediators. High levels of these metabolites are neurotoxic and are associated with neurodegeneration. NSAIDs block the production of prostaglandins by inhibiting COX-mediated metabolism of arachidonic acid.

In addition to the cyclooxygenase pathway, arachidonic acid is also metabolized by the lipoxygenase pathway (Fig. 1) to form leukotrienes and lipoxins. There are 6 mammalian lipoxygenases that site-specifically oxidize arachidonic acid to lipid hydroperoxides and are classified as 5-, 8-, 12- and 15-lipoxygenases according to the carbon atom of arachidonic acid at which oxygen is inserted. The action of 12-lipoxygenase leads to the formation of oxidized lipids such as 12(*S*)-hydroxyeicosatetraenoic acid [12(*S*)-HETE] (30, 31).

The major lipoxygenase of the central nervous system is 5-lipoxygenase (5-LOX), which is present in neurons and glial cells at high levels, and its expression increases in the CNS as a function of age (32, 33). 5-LOX catalyzes the first step in a pathway leading to leukotriene end products (33-36). When unstimulated, 5-LOX resides as a cytosolic enzyme which is activated by p38 mitogen-activated protein kinase and gets translocated to the nucleus (34, 38). In the nucleus, the interaction between 5-LOX and the substrate arachidonic acid is mediated by the 5-LOX-associated protein, FLAP (34, 38). Thus, 5-LOX activity appears to be regulated at multiple levels, both pre- and posttranscriptional.

The first product of 5-LOX catalysis is leukotriene A_4 (LTA_4), which can be hydrolyzed to the dihydroxy lipid product leukotriene B_4 (LTB_4) or, alternatively, LTA_4 can be conjugated with glutathione and further processed to yield a series of cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4; Fig. 1). Leukotrienes can be released from the cell as paracrine agents to promote bronchoconstriction, platelet aggregation or other tissue-specific functions (34-37). Accordingly, leukotriene antagonists are being investigated for the treatment for asthma but have not been investigated for their role in the pathogenesis of neurodegenerative disorders.

Natural polyphenols that act as 5-LOX inhibitors

The two classical natural products that inhibit 5-LOX are nordihydroguaiaretic acid (NDGA) and the curry spice component curcumin (Fig. 2). NDGA inhibits 5-LOX, 12-LOX and 15-LOX with reported K_i values of 200 nM, 30

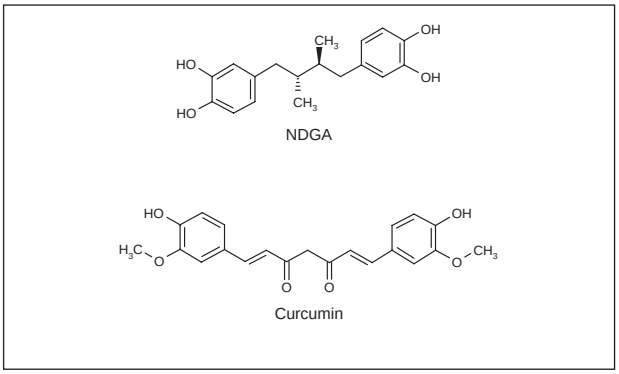

Fig. 2. Structures of the 5-lipoxygenase antagonists NDGA and curcumin.

μM and 30 μM, respectively (39). The mechanism underlying NDGA-induced 5-LOX inhibition is thought to involve reduction of the iron center in the enzyme by the catechol group of the compound (35, 40). Similar to NDGA, curcumin (turmeric) also has a polyphenolic structure (Fig. 2). Curcumin also inhibits 5-LOX, but less efficiently than NDGA (41). Interestingly, recent work from Cole's laboratory shows that curcumin reduces amyloid deposition and IL-1β expression in a transgenic model of AD amyloidopathy (42). Oral curcumin also reduces loss in synaptic markers following intracerebroventricular treatment with Aβ.

While considerable importance has been given to cyclooxygenase as an inflammatory contributor to neurological diseases (reviewed in 43), partially due to the protective benefits against AD that have been associated with chronic use of NSAIDs (1), much less attention has been given to the lipoxygenase branch of arachidonate metabolism. However, recent data from the literature and some data from our laboratory suggest that lipoxygenase inhibitors are excellent drug candidates for clinical development for the treatment of neurodegenerative diseases (reviewed in 44). In support of this argument, we have recently found that dietary NDGA delays neurodegeneration in a mouse model of amyotrophic lateral sclerosis, indicating a broader role of the lipoxygenase pathway in causing neuronal degeneration (45).

There is evidence implying that the 5-LOX pathway may be involved in AD pathogenesis. Frautschy *et al.* (46) have shown that dietary curcumin (a 5-LOX inhibitor and antioxidant) treatment prevents Aβ-induced spatial memory deficits and reduces Aβ deposits and microgliosis. Dietary curcumin but not ibuprofen also reduced oxidative damage and the loss of synaptophysin in this mouse model of AD.

Since Aβ accumulation is a pathological characteristic of AD, inhibition of the accumulation of Aβ peptide and the formation of Aβ fibrils from Aβ, as well as the destabilization of preformed Aβ fibrils in the CNS, would be attractive therapeutic targets for the treatment of AD. Natural polyphenols like curcumin are much more potent in inhibiting Aβ aggregation than ibuprofen and naproxen. Ono *et al.* (47, 48) reported that both curcumin and NDGA concentration-dependently (5-30 μM) inhibited Aβ fibril formation from Aβ(1-40) and Aβ(1-42). These compounds also dose-dependently destabilized preformed Aβ fibrils. Soluble Aβ oligomers are more diffusible and more toxic and increasingly viewed as playing an important role in AD pathogenesis. Low micromolar and even submicromolar concentrations of curcumin effectively block soluble Aβ oligomer formation and toxicity. In a report by Ono *et al.*, NDGA was also shown to disaggregate Aβ protofibrils (47), whereas observations by Moss *et al.* indicated inhibition of direct protofibril-protofibril association of Aβ by NDGA (49). It is possible that the antiamyloidogenic activity of NDGA and curcumin is 5-LOX-independent and may be due to their polyphenolic nature, as well the propensity of these compounds to bind to specific sites of Aβ or their metal-binding property (47, 48). The effects of NDGA and curcumin on Aβ aggregation could result in reducing the toxicity of Aβ. Consistent with this, Goodman *et al.* (50) previously reported that NDGA concentration-dependently reduces the cytotoxicity of Aβ to cultured rat hippocampal neurons by suppressing Aβ-induced accumulation of reactive oxygen species and intracellular free Ca^{2+}. These findings suggest that NDGA interferes with a neurodegenerative pathway important to the pathophysiology of AD.

5-LOX and AD

Naturally occurring polymorphisms in the 5-LOX promoter may influence expression levels of this protein in humans (51). Approximately 25% of the human population has a mutation in the 5-LOX promoter, which diminishes the expression of the 5-LOX gene. Manev and coworkers (52) hypothesized that 5-LOX promoter polymorphism could affect the onset of AD and/or influence the response of AD patients to antiinflammatory treatment with 5-LOX inhibitors. The epidemiological implications of such promoter variation have not yet been rigorously investigated.

We have some evidence that 5-LOX levels are dysregulated in AD patients and in transgenic animal models of ALS and AD. Preliminary Western blot analysis of AD and age-matched brains of individuals who did not suffer from AD indicate highly variable expression of 5-LOX in AD brain cortex. However, the average level of 5-LOX was 2.8-fold greater in AD cortex than in normal cortex. Similarly, 5-LOX levels are increased in the cortex of the APP/PS1 mouse model of AD. 5-LOX mRNA was increased by at least 2-fold at 120 days in the spinal cord of transgenic G93A mice (a model of ALS) relative to the levels in nontransgenic mice, suggesting that changes in 5-LOX expression may be a factor associated with neurodegeneration.

Other studies have reported that in addition to 5-LOX, 12/15-LOX may also be important in AD pathogenesis. Studies have shown the presence of 12/15-LOX in the brain (53), and recently, levels of the 12/15-LOX metabolite (12/15-HETE) were found to be markedly elevated in AD brains compared to controls. The increase in the lev-

els of this metabolite was also directly correlated with brain lipid peroxidation (54).

Neuroprotective actions of 5-LOX inhibitors

Woo and coworkers have shown previously that another 5-LOX metabolite, LTB_4, mediates the release of free radicals from cultured fibroblasts exposed to TNF-α (55). EOC-20 cells stimulated with TNF-α produce robust amounts of reactive oxygen species as evidenced by nitrite accumulation in the medium. Inhibitors of arachidonic acid metabolism, especially 5-LOX inhibitors, are potent antagonists of TNF-α-induced nitrite production (56). TNF-α has been reported to play an important role in AD neurodegeneration (reviewed in 57). There are also reports on the involvement of nitric oxide in AD pathology (58, 59) and elevated levels of nitrotyrosine-modified proteins have also been found in AD brains (59-61). Thus, a potent inhibitory role of NDGA in suppressing TNF-α-induced nitrite production in glial cells (56) could be an important mechanism of NDGA's protective effect against AD pathogenesis.

Modulation of signal transduction by 5-LOX inhibitors

Recent data from several laboratories suggest that leukotrienes, particularly LTB_4, may modulate signal transduction. For instance, Funk and colleagues suggest that nuclear LTB_4 binds specific transcription factors including PPARγ and/or AP1 (62), increasing the potency of gene induction. 5-LOX inhibitors have been reported to suppress the nuclear factor NF-κB activation pathway (63). The 5-LOX inhibitor curcumin is known to directly inhibit IκB kinase activity and IκBα phosphorylation and subsequently block NF-κB activation (64). There is evidence that NF-κB is activated by Aβ as well as free radicals and inflammatory stimuli such as TNF-α and IL-1, etc. (Mhatre, unpublished observations). In human neuron-like cells, binding of Aβ to p75NTR (a member of the cell death receptor family) was found to activate NF-κB and induce DNA fragmentation (65). Inhibition of NF-κB activation by curcumin prevents Aβ-induced cell death, thus implicating NF-κB in Aβ-induced cell death (66).

Conclusions

The use of anticholinesterase drugs and NSAIDs in the treatment of AD is limited by serious toxicity issues and contraindications such as gastric sensitivity, which is common in elderly patients. There is considerable hope that inhibition of neuroinflammatory processes might lead to a treatment approach to delay the onset of AD in individuals at high risk. Research from our laboratory and others suggests that some natural product inhibitors of 5-LOX might provide lead compounds for such pharmacotherapeutic development.

The 5-LOX inhibitors curcumin and NDGA possess both antioxidant and antiinflammatory activity. 5-LOX inhibitors have been reported to block the increase in APP secretion by IL-1β (66) and reduce Aβ aggregation (46, 47), which is considered critical for Aβ-induced neurotoxicity. 5-LOX levels are increased in neurons during aging and this could significantly increase the brain's vulnerability to neurodegeneration. As mentioned above, we have found that NDGA delays neurodegeneration in a mouse model of amyotrophic lateral sclerosis, implying a broader role of the LOX pathway in neuronal degeneration (45). Thus, based on these observations, we propose that the inhibition of arachidonic acid-5-LOX has therapeutic potential in the treatment and prevention of AD.

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