REVIEW

New Anti-inflammatory Treatment Strategy in Alzheimer's Disease

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Received August 17, 1999

ABSTRACT—Numerous reports have indicated that patients suffering from inflammatory diseases (e.g., arthritis) who take anti-inflammatory medication have a reduced risk of developing Alzheimer's disease (AD). Thus, the first generation of anti-inflammatory cyclooxygenase (COX) inhibitors, such as aspirin and indomethacin, have been tested as potential therapeutics in AD. Because the inhibition of COX-1 is also known to cause tissue damage in the gastrointestinal system from the resultant reduced cytoprotection, selective COX-2 inhibitors are being investigated and tested clinically as potentially better therapeutics for AD patients. However, such drugs may also trigger unwanted effects; for example, the COX-2 inhibitors, which reduce the production of one type of eicosanoids, the prostaglandins, may increase the production of other eicosanoids; i.e., the leukotriene B₄ (LTB₄), which is one of the most potent endogenous chemotactic/inflammatory factors. LTB₄ production is initiated by the enzyme 5-lipoxygenase (5-LOX). The expression of the 5-LOX gene is upregulated during neurodegeneration and with aging. In spite of the fact that 5-LOX and leukotrienes are major players in the inflammation cascade, their role in AD pathobiology/therapy has not been extensively investigated. We propose that the 5-LOX inflammatory cascade may take part in the process of aging-associated neurodegenerative diseases, and we point to the role of 5-LOX in neurodegeneration and discuss its relevance for anti-inflammatory therapy of AD.

Keywords: Inflammation, Cyclooxygenase, 5-Lipoxygenase, Nonsteroidal anti-inflammatory drug, Dementia

1. Introduction

In general, interventions aimed at reducing the risk factors are a useful strategy for the prevention of illnesses such as Alzheimer's disease (AD) that are otherwise poorly understood. An understanding of the specific risk factors or the protective mechanisms in AD may also provide clues to its pathogenesis. The first strong indication of the involvement of inflammatory processes in AD was derived from epidemiological evidence for a link between anti-inflammatory treatments and an altered course of this disease. Thus, it was reported that patients who take anti-inflammatory drugs or suffer from inflammatory diseases (e.g., arthritis) have a reduced risk of developing AD. McGeer et al. reviewed 7,490 hospital discharges of elderly patients and found a 0.39% occurrence of concomitant diagnoses of rheumatoid arthritis and AD (1). This is 6–12 times lower than would have been predicted assuming independence of the two diagnoses. A Canadian study revealed that a history of arthritis resulted in a low risk of AD, as did a history of use of nonsteroidal anti-inflammatory drugs (2). A

co-twin control study of 50 elderly twin pairs that examined the onsets of AD suggested that anti-inflammatory drugs may prevent or delay the initial onset of AD symptoms (3). Breitner et al. (4) reported that among siblings at high risk for AD, sustained use of nonsteroidal anti-inflammatory drugs (NSAIDs) was associated with delayed onset and reduced risk of AD. More recently, population-based studies that focussed on rheumatoid arthritis and NSAID use as risk factors, strongly suggested that anti-inflammatory drugs may have a protective effect against AD (5, 6). Based on these studies, it can be proposed that sustained use of NSAIDs, which block the synthesis of eicosanoids [mostly by inhibiting the binding of arachidonic acid to the active site of cyclooxygenase (COX), but probably also by inhibiting 5-lipoxygenase (5-LOX)] might also interfere with the mechanism involved in the pathobiology of AD.

2. Evidence of inflammation in Alzheimer's disease

The cells expressing nitric oxide synthase, which may also have a protective mechanism against oxidative stress,

survive in aging and AD (7–9). It is becoming increasingly more evident that inflammation coupled with oxidative stress occurs in the brain of AD patients (10–15) and that the classical mediators of inflammation, eicosanoids and cytokines, may contribute to the neurodegeneration. The source of inflammatory processes in the brain is usually sought among the peripheral inflammatory cells, which may invade the brain, and in activated glia (in particular microglia). More recent data indicate that neurons also can express enzymes such as COX and LOX, which typically are considered important in inflammatory cells. Research on the role of neuronal eicosanoid-synthesizing enzymes is just beginning to emerge.

Eicosanoids (prostaglandins and leukotrienes): Eicosanoids are synthesized from arachidonic acid by two families of enzymes: COX and LOX. These enzymes are capable of inserting oxygen into the molecule of arachidonic acid in a specific manner, thereby producing prostaglandins (PGs), by an action of COX, or leukotrienes (LTs), by an action of 5-LOX. Neuropathological role of 5-LOX and COX pathways are summarized in Fig. 1.

PGs in the brain were first described by Samuelsson over 30 years ago (16). Since then a large number of studies have shown that PGs are formed in certain regions of the brain and in the spinal cord in response to a variety of stimuli. The key enzyme in the synthesis of PGs from arachidonic acid, COX, was purified in 1976. After cloning of the COX-1 gene in 1988, several laboratories identified a second gene with COX activity (COX-2) in 1991. The constitutive isoform of COX, COX-1, appears to possess a physiological function that involves the synthesis of cytoprotective PGs. These may protect the stomach lining and maintain normal renal function in the kidney. The inducible isoform, COX-2, is induced by pro-inflammatory stimuli in migratory cells and in inflamed tissues. Because COX-2 is capable of regulating both pro-inflammatory cytokines and growth factors, this implies that it may play a role in inflammation and in the control of cell growth. Not only are COX-1 and COX-2 expressed in the brain, but an increase in their neuronal expression, particularly that of COX-2, has been implied in the progression of ischemic brain damage (17). Although there is no direct evidence of elevated prostaglandin production in AD, mRNA and protein of COX-2, and to a lesser extent also of COX-1, are upregulated in affected areas of AD brain (18–20). One possible mechanism of increased COX-2 gene expression in the brain may involve the inflammation-related transcription factor, NF- κ B; namely, there is a strong correlation between NF- κ B DNA binding and COX-2 mRNA expression in AD brain (21). Moreover, the immediate COX-2 promoter possesses multiple recognition sites specific for NF- κ B DNA binding (22).

PGs have been shown to cause rapid direct degenerative

Fig. 1. Putative role of 5-LOX and COX pathways in neuropathology. Phospholipase A₂ (PLA₂) participates in liberating arachidonic acid (AA) from membranes. AA is subjected to the action of either 5-lipoxygenase (5-LOX) or cyclooxygenases (COX); as a result, leukotrienes and prostaglandins are formed that may trigger the inflammatory cascade and/or alter gene expression, which, in turn, may lead to neuropathologic changes. When over-expressed, 5-LOX and COX may also cause lipid peroxidation. The crosses indicate that 5-LOX or COX pathway can be blocked by selective inhibitors.

changes in differentiated murine neuroblastoma cells in culture (23). In vivo, the primary role of PGs may be the regulation of cytokines and maintenance of the inflammatory cascade. For example, when released from activated microglia, PGE₁ and PGE₂ increase the expression of the cytokine interleukin (IL)-6 in astrocytes (24, 25). This, in turn, triggers the formation of additional PGs. PGE₂ also induces polymerization of alpha-1-antichymotrypsin (ACT), an acute-phase inflammatory protein (26). Indomethacin and nordihydroguaiaretic acid (NDGA), known inhibitors of COX and LOX pathways, respectively, block increased secretion of amyloid precursor protein (APP) by IL-1 β , which suggests the involvement of prostaglandins and leukotrienes in IL-1 β -mediated APP processing (27). Thus, eicosanoids may promote amyloid deposition and neurodegeneration in AD brains by inactivating protease inhibitors and by altering of APP processing.

5-LOX, the enzyme responsible for the key step in leu-

kotriene synthesis, is also expressed in the brain, including the neurons (28, 29). The regulation of 5-LOX gene expression involves hormonal influences; it is suppressed by melatonin (30) and stimulated by glucocorticoids (31). The likely mechanism of how these hormones affect the expression of 5-LOX includes the hormone-responsive elements in the promoter region of the 5-LOX gene, as well as the action of these hormones on specific nuclear receptors. Aging-associated hormonal changes, i.e., relative melatonin deficiency and hyper-glucocorticoidemia, may be responsible for the increase in neuronal expression of 5-LOX during aging (32, 33), which, in turn, might lead to increased synthesis of leukotrienes in elderly subjects (34).

Several lines of evidence suggest that the process of leukotriene formation might be deleterious to neurons, either because of the associated lipid peroxidation or due to a direct but as yet poorly understood action of leukotrienes on neuronal cells (35, 36). The major inflammatory mediator produced by 5-LOX is the leukotriene LTB₄. In the CNS, its production has been observed in oligodendrocytes (37) and astrocytes (38), and it has been proposed that 5-LOX may be induced in microglia. These cells are considered to be a resident-type of monocyte in the brain, and they possibly participate in AD pathology. No direct evidence for a role of 5-LOX in AD has been demonstrated as yet. Nevertheless, it has been hypothesized that mutations of the 5-LOX gene might influence the progression of AD (39).

Cytokines: AD patients show a generalized increase in levels of cytokines, which include IL-1 β (40, 41), tumor necrosis factor alpha (TNF- α) (40, 42) and IL-6 (43). Production of these cytokines in the CNS appears to be extensive in those brain regions in which AD neuropathology is most prominent. For example, IL-6 has been found mostly in plaques in which neuritic pathology has not yet developed, which suggests that activation of the immune cascade may be an early event leading to neurodegeneration rather than just a consequence of neuritic degeneration. Inflammatory stimuli (associated with AD) may up-regulate the expression of cytokines and chemokines by activation of the nuclear factor NF- κ B (44–46). Its activation may also increase the production of APP. The latter mechanism involves recognition by NF- κ B of an identical sequence to that of NF- κ B binding sites (APP- κ B sites) in the 5'-regulatory region of the APP gene (44). Since the NF- κ B binding site is also present in the regulatory region of ACT, the nuclear factor's activation may increase brain levels of ACT as well (45, 47, 48).

IL-1 is capable of increasing eicosanoid formation (i.e., the accumulation of 5-LOX and COX products) (49, 50), as well as of modulating the extent of COX expression. The latter occurs via activation of the mitogen-activated protein kinase (MAPK) pathway (51–53). On the other

hand, inhibition of IL-1 receptors down-regulates the production of both PGs and the leukotriene LTB₄ (50, 54). Hence, it can be proposed that inflammation, when it occurs in AD, may initiate a self-propagating cycle whereby the cytokine cycle becomes closely related to the deposition of amyloid beta (A β) and ACT in the amyloid plaques of AD patients.

A β and glial activation: Although the brain has been traditionally viewed as immunologically privileged, the neuroglia can be involved in immune system-like inflammatory responses in the CNS. Our study revealed the involvement of glial activation and increased APP expression in age-related cognitive impairment (55, 56). It is becoming increasingly more evident that prominent glial activation and local acute phase responses occur around the amyloid plaques in AD. This has led to the hypothesis that A β may play a causative role in the formation of the inflammatory responses associated with AD. A β activates astrocytes (57–59) and microglia (60–62) through NF- κ B modulation (57, 60). In mixed primary cultures of neurons and astrocytes, A β caused a concentration- and time-dependent activation of NF- κ B, with subsequent upregulation of IL-1 β and IL-6 (63). Moreover, A β was shown to be capable of binding to neuronal receptors for the advanced glycation endproduct (RAGE) and of inducing the macrophage-colony stimulating factor via the NF- κ B-dependent pathway (64). Such actions by A β may influence both neurons and glia to set in motion inflammatory events, which could become a positive feedback to A β cytotoxicity (65) and may favor amyloidogenic A β processing (27). A β cytotoxicity and amyloidogenic A β processing are both increased by IL-1 and decreased by NSAIDs, which indicates the importance of the cytokine system and of the eicosanoid cascade in modulating these aspects of AD pathology. The recent report by Schenck and his colleagues in *Nature* (66) raised the possibility of using vaccination for AD therapy. In the study, PDAPP transgenic mice, which over-express familial AD type APP, were immunized with synthetic A β -42. These mice develop amyloid plaques, astrogliosis and dystrophic neuritis as they age. Immunization of six-week-old mice with A β -42 essentially prevented these AD-like pathologies and immunization of 11-month-old mice, which already have the pathology, significantly reduced progression of the pathology. We have to be careful to evaluate this result. While these transgenic mice accumulate amyloid in their brains, they do not display other AD pathologies. Since we did not find a tight relation between apoptotic cell death and amyloid plaques in AD brain (67), extracellular A β deposition may not be a cause of the neurodegeneration, and is merely a consequence of the disease.

The classical complement pathways are also closely connected with A β deposition. Various molecules associated with senile plaques, including A β itself, bind to C1q of the

complement pathway, and activate it (68–71), thereby initiating the complement cascade in AD (72). Such complement activation can further propagate the inflammatory processes by releasing the leukotriene LTB₄. Although several types of cells could produce complement in the brain (73), microglia are probably mainly responsible for A β -related activation of the complement pathway (74).

It is now clear that resident brain microglia express immune-related antigens (75) and can function as antigen-presenting cells (76). Increased MHC class II antigens and increased complement factors (77) indicate that glia cells become activated in AD. Activated microglia were found within or near A β plaque formations in postmortem AD brain (78) and were also found in the brain of transgenic mice expressing human APP with a familial AD gene mutation (61, 79). Although microglia are primarily involved in phagocytic activity and may be responsible for inducing further neuronal damage by generating reactive oxygen species (8, 9, 80, 81) or activating complement and proteolytic enzymes, they are also an important source of inflammatory molecules such as PGE₂ (82). Thus, chronic activation of glia may be yet another factor capable of setting in motion molecular events that involve neurodegenerative inflammatory processes (83).

3. Anti-inflammatory treatments in AD

Retrospective studies that compared the frequency of use of NSAIDs and the progression of AD suggested a possible therapeutic benefit with conventional anti-inflammatory medications in delaying the onset of AD (1, 3, 84). Furthermore, an analysis of longitudinal changes over a 1-year period revealed a lesser decline of verbal fluency, spatial recognition and orientation in AD patients treated with NSAIDs than in AD patients without NSAID usage (85). The effect of NSAIDs on the risk for incidence of AD was investigated in the recent Rotterdam Study (86). In this study, NSAIDs appeared to reduce the risk for AD when taken for 6 months and longer and in subjects younger than 85 years; however, overall there was no significant association of NSAID use with the risk for the incidence of AD.

First generation of anti-inflammatory drugs for AD:

Because most of the commonly used anti-inflammatory NSAIDs are basically nonselective COX inhibitors (i.e., effective in inhibiting both COX-1 and COX-2), the COX inflammatory pathway is currently being considered an important mechanism in the pathophysiology of neurodegeneration, including AD. In a recent study, Yasojima et al. (18) found that both COX-1 and COX-2 are constitutively expressed in human brain; however, only COX-2 is significantly up-regulated in the affected areas of the AD brain. Ho et al. also reported that COX-2 is up-regulated in hippocampal neurons of AD patients compared with

age-matched controls (87). A limited randomized trial of the nonselective COX inhibitor indomethacin was conducted in patients with mild AD by Rogers et al. (88). In this 6-month study, 100–150 mg/day indomethacin appeared to reduce the extent of cognitive decline in mild- to moderately impaired AD patients. Based on the assumption that inflammation should also be suppressed by glucocorticoids, a preliminary clinical study using a glucocorticoid, prednisone, was conducted by Aisen (89). However, the results of this study were not conclusive. A hint as to why glucocorticoids might not be effective in reducing CNS inflammatory mechanisms is provided by our recent finding that glucocorticoids increase the expression of the pro-inflammatory 5-LOX gene in the brain (31). Although further work is needed to clarify whether steroids, NSAIDs, or both prevent or attenuate the symptoms of AD, the available data suggest that NSAIDs exert a stronger protective influence than steroids.

One possible mechanism by which the inhibition of COX (COX-1 and COX-2) may provide neuroprotection can be deduced from the fact that PGs, which are produced by the action of these enzymes, could promote neurotoxicity mediated via the excitatory neurotransmitter glutamate – namely, it has been demonstrated that an excessive stimulation of glutamatergic neurotransmission can lead to neurotoxicity. Since PGs are capable of stimulating the signal transduction cascade in cells that express NMDA-type glutamate receptors (90) and since they may also potentiate glutamatergic transmission by inhibiting the removal of excessive glutamate content that normally occurs via astrocytic re-uptake, they can favor the conditions of glutamate-mediated excitotoxicity. Another mechanism of action of NSAIDs may involve the suppression of the immune/microglial response in the brain. For example, Mackenzie et al. reported that AD patients with plaques who were not treated with NSAIDs had almost three times the number of activated microglia as the NSAID-treated patients, whereas the number of plaques and the extent of neurofibrillary tangle pathology were not significantly different between the two patient groups (91). These results point to a mechanism of action of the NSAIDs that involves the suppression of microglial activity.

The drawback of nonselective COX inhibitors, i.e., NSAIDs, is that they reduce the overall synthesis of PGs, including the PGs involved in protection of the gastric mucosa. Therefore, they are known to trigger gastric and duodenal ulcers. Although many AD patients obtained benefit with nonspecific COX inhibitor treatment, they also suffered from severe side effects such as gastrointestinal lesions and bleeding. To prevent these side effects, an exogenous PG, the PGE₁ analog (misoprostol), has been studied (92) as possible cytoprotective therapy. With the discovery that cytoprotective PGs are mainly synthesized by

COX-1 and that the brain pathology may be due to COX-2, the focus of developing NSAIDs as therapeutic agents for AD has shifted towards the search for specific COX-2 inhibitors.

Second generation of anti-inflammatory drugs for AD:

It has been established that there are at least two isoforms of COX, COX-1 and COX-2. The two COX isoenzymes share structural and enzymatic similarities but are differently regulated at the molecular level and may have distinct functions, although some physiological overlap between them does occur. In most tissues, COX-1 is the isoform that is constitutively expressed and is responsible for physiological prostanoid synthesis. Selective COX-1 inhibitors reduce the production of the PGs that have a cytoprotective effect in these tissues. Although questioned by some (93), most of the results published so far support the hypothesis that the unwanted side effects of NSAIDs, such as damage to the gastric mucosa and the kidneys, are due to their ability to inhibit COX-1, whereas their anti-inflammatory (therapeutic effects) are due to the inhibition of COX-2.

In contrast to COX-1, COX-2 expression can be induced. This usually occurs as a consequence of tissue injury by cytokines, growth factors or mitogens. Thus, selective COX-2 inhibitors are currently being developed with the expectation that these compounds will selectively suppress the prostanoid biosynthesis triggered by pathological events and that they will be devoid of the side effects associated with the inhibition of the constitutive prostanoid biosynthesis. New compounds with a high selectivity for COX-2, especially those that are non-acidic, are expected to be better tolerated by the gastrointestinal tract. Although these compounds might have potential use in diseases such as colorectal cancer and neurodegenerative diseases of the Alzheimer-type, it is still premature to exclude the possible appearance of adverse effects, perhaps those related to the functioning of the kidney. Additional roles for COX-2 inhibitors will probably be found in the near future. For example, COX-2 is potently expressed in human colon cancer cells, and NSAIDs delay the progress of colon tumors, possibly by causing apoptosis of the tumor cells. The new highly selective inhibitors of COX-2 might, therefore, lead to advances in cancer therapy in addition to their likely action in protecting against Alzheimer's disease (94). As with the classical NSAIDs, well-designed large clinical trials with COX-2 inhibitors are required to adequately estimate the promising therapeutic advantages and the potential drawbacks that may be inherent with prolonged COX-2 inhibition (95).

4. Prospective anti-inflammatory treatments in AD

Potential pitfalls in using only the COX-2 inhibitors:

In spite of the fact that gastrointestinal side effects are

reduced with selective COX-2 inhibitors, these drugs may not be the ultimate compounds for reducing inflammatory processes, in particular as they relate to the therapy of AD. Gilroy et al. (96) compared the selective COX-2 inhibitor NS-398, which selectively prevented the synthesis of the PGs family of eicosanoids, with other anti-inflammatory drugs. They found that selective COX-2 inhibition resulted in increased formation of eicosanoids (i.e., LTB₄) synthesized via the 5-LOX pathway. Such induction of leukotriene production indicates that the selective inhibition of COX-2 may lead to a compensatory activation of the 5-LOX inflammatory pathway, which, in turn, could reduce the anti-inflammation efficacy of the COX-2-selective treatment. Moreover, in a recent review article, Wallace pointed out to the fact that in some studies, the anti-inflammatory efficacy was not observed unless doses of the COX-2 inhibitors were used that were greater than those required for COX-2 inhibition (93). Moreover, mice devoid of the gene for COX-2 exhibited inflammatory responses similar in magnitude to those observed in wild-type controls, whereas mice without the COX-1 gene exhibited diminished inflammatory responses. These new findings stress the point that further refinement in the development of successful anti-inflammatory therapeutics is necessary.

Putative role of 5-LOX in neurodegeneration: A significant body of evidence points to the involvement of 5-LOX induction and activation in peripheral inflammation. However, only a limited number of studies has addressed the role of this inflammatory pathway in pathologies of the central nervous system. For example, 5-LOX was found to be increasingly expressed in human brain tumors (97) and in the brains of experimental animals exposed to reperfusion after ischemia (98), or in response to excitotoxic brain injury (33). Stimulation of the 5-LOX pathway in brain cells also occurs during blood-brain cell contact (99). 5-LOX is expressed in neuronal cells in resting condition (28), and its expression is increased during aging (33), in melatonin deficiency (32) and in conditions of hyperglucocorticoidemia (31). Under conditions of such uncontrolled and exaggerated activity, 5-LOX may significantly contribute to membrane lipid peroxidation, cytotoxicity and neurodegeneration (100, 101). An activation of 5-LOX might even participate in the neurodegenerative processes associated with HIV infection (101). 5-LOX inhibitors have decreased brain edema (102), and more directly, have reduced the extent of traumatic (103), hypoxic (104) and excitotoxic (33, 104) brain injury. Thus, several lines of evidence suggest that increased 5-LOX expression may enhance the neurodegenerative response of the brain to insults (33, 35).

The major inflammatory mediator produced by 5-LOX is the leukotriene LTB₄. It is known to be a major product of activated neutrophils and macrophages (105). It

produces its biological effects by interacting with specific G protein-coupled cell surface receptors (106). It is not clear whether these receptors are present on brain cells and whether they are involved in the effects of the 5-LOX pathway on neuronal functioning; namely, alternative mechanisms, including the action of LTB₄ on other intracellular sites (e.g., nucleus), as well as the recently proposed non-enzymatic actions of the 5-LOX protein (107), could also play a role. Since LTB₄ has been found in oligodendrocytes (37) and astrocytes (38), it has been proposed that the 5-LOX pathway may play an important role in the activation of these cells. Nordihydroguaiaretic acid (NDGA), a relatively nonspecific inhibitor of lipoxygenases, was found capable of inhibiting APP secretion in experimental animals. These results suggest that eicosanoids of the 5-LOX pathway may promote APP, whereas eicosanoids of the COX pathway inhibit APP secretion (108). The relevance of these findings to AD pathology is not yet clear.

Attempts are being made to synthesize selective 5-LOX inhibitors. The first generation of 5-LOX inhibitors were antioxidants that lacked specificity and also were toxic. Further attempts were made using nonsteroidal NSAID cyclooxygenase inhibitors such as ibuprofen, naproxen and indomethacin as orally bioavailable scaffolds to design selective 5-LOX inhibitors. Replacement of the NSAID's carboxylic acid group with a *N*-hydroxyurea group provided congeners with selective 5-LOX inhibitory activity (109). The best examples of the new generation of 5-LOX inhibitors are zileuton, which was designed as a chelator for the non-heme iron of 5-LOX, and MK-866, which is an indirect inhibitor that appears to block the stimulatory action of FLAP (5-LOX-activating protein) on 5-LOX (110). Currently, zileuton is the only clinically available selective 5-LOX inhibitor, and it is used for the treatment of asthma. To our knowledge, no studies have been conducted to explore its efficacy in AD patients.

Proposed combined use of COX-2 and 5-LOX inhibitors: On the basis of presently available information, it is plausible to propose that selective inhibition of COX will reduce the production of only one type of eicosanoids, PGs, but that at the same time it may increase the formation of eicosanoids of the leukotriene-type. Such a compensatory activation of the LOX pathway could lead to an activation/attraction of inflammatory cells, causing local sites of inflammation and degeneration. This hypothesis was tested in studies in rats. Kirchner et al. evaluated the effects of tepoxalin, a dual LOX/COX inhibitor, on COX-inhibitor-induced changes of leukotriene (LTB₄) levels in gastric mucosa and on neutrophil adhesion in mesenteric venules. Chronic oral administration of the unselective COX inhibitor indomethacin (2 mg/kg daily over 4 days) resulted in 40% mortality, accompanied by intestinal adhe-

sions and perforations (evaluated 24 h after the fourth dose of indomethacin) (111). Additionally, neutrophil adhesion was increased in the mesenteric venules, and cell infiltration was evident in the mesenteric interstitium. These gastrointestinal side effects were inhibited by the LOX/COX inhibitor tepoxalin (20 mg/kg, p.o.; 30 min prior to each daily indomethacin treatment). Further studies were performed to determine tepoxalin's effects on early events associated with COX inhibitor-induced gastrointestinal inflammation, including neutrophil adhesion, and generation of lipid peroxide and LTB₄. In these studies, indomethacin (100 mg/kg, p.o.) increased the levels of LTB₄ in rat gastric mucosa 90 min after administration. Pretreatment with tepoxalin (ED₅₀ = 7.5 mg/kg, p.o.) or the selective 5-LOX inhibitor zileuton (100 mg/kg, p.o.) prevented the increases in gastric mucosal LTB₄ levels and in neutrophil adhesion induced by the COX inhibitor indomethacin (100 mg/kg, p.o.) (111). These data suggest that LOX inhibition could possibly play a vital role in the prevention of NSAID-induced gastric inflammation and provide insight into possible new approaches to anti-inflammatory therapy that may also prevent gastric side effects.

As stressed by Muller-Peddinghaus (110), the use of appropriate tools determines the outcome of an experiment. Before the selective 5-LOX inhibitors (e.g., zileuton) were discovered, unspecific and broad-spectrum inhibitors (e.g., phenidone, BW755C, NDGA, querecetin) were used to investigate the role of 5-LOX in biological processes; data from such experiments are difficult to interpret. The emerging new and selective 5-LOX inhibitors will provide better tools to test the hypothesis that a combined COX-2/5-LOX inhibition could provide an efficacious neuroprotective therapy devoid of serious side effects.

5. Conclusion

It is becoming more and more evident that inflammatory pathways play a role in the pathophysiology of AD. Their contribution to this pathology may be via the action of classical inflammatory cells and their counterparts in the CNS (e.g., microglia), but also through the neuronal expression and action of COX-1/COX-2 and 5-LOX, the enzymes typically associated with inflammation. Currently available results from epidemiological studies and clinical trials support the view that anti-inflammatory drugs (typically COX inhibitors) may benefit AD patients. Substantial efforts are being directed towards developing selective COX-2 inhibitors as putative therapeutics for AD. Evidence is also emerging that points to a significant role of 5-LOX in neurodegeneration and, presumably, in AD. No studies about the efficacy of selective 5-LOX inhibitors in AD have been reported as yet. We propose that such trials should be conducted and that it is possible that combined COX-2/5-LOX inhibition could provide additional benefits.

Acknowledgments

Supported, in part, by NIH-NIA grant R03 AG16474-01 (K.S.), by NIH-NIA grant RO1 AG15347 (H.M.) and by a University of Illinois at Chicago Campus Research Board Award, CRB F98 (H.M.).

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