CHAPTER 11

NSAIDs FOR THE CHEMOPREVENTION OF ALZHEIMER'S DISEASE

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Abstract: Epidemiologic and laboratory studies suggest that non-steroidal anti-inflammatory drug (NSAID) use reduces the risk of Alzheimer's disease (AD). Initial reports in the early 1990's indicated that a history of arthritis, a presumed surrogate of NSAID use, was associated with a lower risk of AD. [1] These reports were followed by epidemiologic studies in which NSAID use was assessed directly and the majority of these reports confirmed the inverse association with risk for AD. [2, 3] Postmortem studies in humans [4], studies in animal models of AD [5, 6], and in vitro studies [7, 8] generally support the notion that NSAIDs can reduce the deleterious inflammation which surrounds amyloid beta (Aβ) plaques in the AD brain. In addition, some studies conducted in vitro and in rodents point to a subgroup of NSAIDs that may work by inhibiting amyloidogenic APP metabolism rather than through traditional anti-inflammatory mechanisms. [9–11] This novel property of NSAIDs is currently being explored in epidemiologic studies. Results from randomized clinical trials of NSAIDs and established AD and one trial on secondary prevention have not been promising and there have been no prevention trials completed. The feasibility of using NSAIDs as a chemopreventive agent in AD is discussed

R. E. Harris (ed.), Inflammation in the Pathogenesis of Chronic Diseases, 229–248.

1. INTRODUCTION

AD is a neurodegenerative disease that causes progressive decline in cognitive function and behavior, culminating in dementia. Clinical disease onset is insidious and symptom progression is typically slow. It is estimated that between 2.5 and 4.5 million people in the United States are currently afflicted with AD. [12, 13] Age is the strongest risk factor and a meta-analysis of the annual incidence of AD indicates that rates approximately double for every five year age group over 60 starting at 0.06% in 60–65 year olds and increasing to 6.69% in those 95 years of age and older. [14] This, coupled with the fact that the age distribution of the population is shifting upward, may lead to a major public health problem in the future. Estimates in the United States alone indicate that the proportion of the population older than 65 years of age was 12% (35 million) in 2000 but is expected to increase to 20% (82 million) by 2050. [15, 16]

As noted by Alzheimer nearly a century ago, the brains of AD patients show three hallmark neuropathological and diagnostic features – neurofibrillary tangles, Aβ or "senile" plaques, and gliosis. These pathological features are present in more abundance in people with AD but are also present in brains of non-demented individuals. The neurofibrillary tangles are the main intracellular pathology of the disease and are primarily comprised of hyper-phosphorylated tau protein. [17] Neurofibrillary tangles gradually develop over decades and are used post-mortem to stage AD pathology. [18] In the AD brain, neurofibrillary tangles are thought to be the first stage preceding neuronal degeneration and loss, although their etiology still remains elusive. [19]

The extracellular "senile" plaques are primarily comprised of insoluble Aβ deposits and are often surrounded by activated astrocytes and microglia, and, in the case of mature plaques, contain dystrophic neurites. Aβ peptides are proteolytically derived from a much larger parent molecule, amyloid precursor protein (APP), a type I transmembrane protein. APP metabolism is a highly regulated process that is coordinated by a family of enzymes known as the secretases. There are two pathways for APP metabolism: non-amyloidogenic and amyloidogenic. In the former, APP is first acted upon by α-secretase and then γ-secretase cleavage, precluding the formation of Aβ. Amyloidogenic processing of APP occurs through sequential cleavage by extracellular β-secretase and intramembrane γ-secretase, and results in peptides from 38 to 43 amino acids in length. [20] $A\beta_{40}$ is a more abundant and more soluble variant of the peptide whereas $A\beta_{42}$ is not as readily cleaved (and therefore not as abundant), but is thought to be more toxic because it characteristically forms aggregates that may start the process of amyloid deposition in the AD brain. [21] Diffuse plaques, which are present early in the disease, are comprised mainly of $A\beta_{42}$ [22] and are also found in non-AD brains as early as the fourth or fifth decade. [18] It seems that, as the disease progresses, the diffuse plaques mature to form senile neuritic plaques that contain both $A\beta_{42}$ and $A\beta_{40}$. [21–23]

In addition to these two neuropathological features, Alzheimer originally identified a third type of pathology known as glial inflammation or simply "gliosis". [24] Although acute inflammatory responses are often beneficial in

clearing foreign material from the brain, it is the chronic inflammation present in AD that may be pathogenic as it causes neurotoxicity, damage to neighboring cells, and is even thought to promote plaque pathology. [19, 25] The cellular and biochemical processes involved in brain inflammation in AD have been extensively studied (for detailed reviews see Akiyama et al. 2000 [26], McGeer et al. 2003 [27], and Eikelenboom et al. 2002 [28]). It is still not known whether this brain inflammation is an epiphenomenon (i.e., resulting from the disease process) or is pathoetiologically involved in disease initiation and/or progression. [19, 24] However, it is clear that the inflammatory response observed in the AD brain is typically localized around the Aβ plaques. For example, the numbers of proinflammatory cells such as microglia and astrocytes are elevated in AD brains compared to controls and these cells are co-localized with Aβ plaques. [29] Activated microglia, identified in vivo using positron emission tomography, have been found in patients with mild AD suggesting that the inflammatory process occurs at early stages of the disease. [30] Also, biochemical markers of inflammation, such as cyclooxygenase (COX), complement factors, chemokines, and cytokines, are in higher concentration in the AD brain. [31] Aβ-associated increases in activated microglia and related inflammatory markers have also been shown in studies using transgenic mouse models of AD. [32–35] Whether or not Aβ deposition precedes inflammation, once the deposition begins it is thought to activate proinflammatory glial cells which in turn produce a myriad of inflammatory molecules 26 which may lead to a feed-forward continuous cycle of deposition and inflammation.

A growing body of evidence suggests that non-steroidal anti-inflammatory drugs (NSAIDs), which are commonly used for pain relief and treatment of symptoms in inflammatory conditions such as arthritis, may protect against the neuropathogenesis of AD. [1–3] Epidemiologic data have accumulated over the last 15 years showing that the use of NSAIDs is associated with a reduced risk of AD. And, data from laboratory studies in cell culture and animal models are helping to clarify the mechanisms through which NSAIDs might exert their protective effects. These data are summarized below, and the potential for using NSAIDs as part of an intervention strategy in AD is discussed.

2. MECHANISMS OF ACTION

There are at least two possible mechanisms by which NSAIDs might protect against AD. One is by reducing inflammation via a COX-mediated pathway and the other is by altering APP metabolism to Aβ via a COX-independent pathway. Regarding the more traditional anti-inflammatory mechanism, NSAIDs competitively inhibit the active site of COX preventing it from binding arachidonic acid and converting it to prostaglandins, which are locally-acting hormones involved in inflammatory responses. [36, 37] At least two isoforms of the COX enzyme, referred to as COX-1 and COX-2, have been identified. [38] COX-1 is constitutively expressed in most tissues including the brain and gastric mucosa. By contrast, COX-2 is inducible and typically only detected in response to inflammation, except in the

brain where it is constitutively present. [38] Commonly-used NSAIDs such as aspirin, ibuprofen, naproxen, and indomethacin, are non-selective in that they inhibit both COX-1 and COX-2 to varying degrees. The degree to which an NSAID blocks COX-1 relative to COX-2 is typically related to its toxicity. For example, NSAIDs that preferentially inhibit COX-1 generally have more gastrointestinal side effects. [38] Recently, COX-2 selective NSAIDs, such as celecoxib, rofecoxib, and valdecoxib, were approved for use in humans. These NSAIDs were widely touted at first because they have desirable anti-inflammatory properties presumably without the side effects of other traditional NSAIDs. However, they have since attracted considerable scrutiny due to emerging concerns over their potential cardiotoxicity. [39–41] Aspirin, unlike the other NSAIDs, inhibits COX by irreversibly acetylating the enzyme's active site. As a result, any new COX activity must be mediated by newly synthesized enzyme. [36] In platelets, the COX enzyme also plays an important role in converting arachidonic acid to thromboxanes, which are locally-acting hormones involved in the clotting activities of platelets. Because platelets cannot synthesize new COX enzyme, aspirin has potent anti-clotting effects even at low doses. It is this property which provides the rationale for using low-dose aspirin to prevent cardiovascular disease. Higher doses of aspirin may be required to see an anti-inflammatory effect.

Evidence from neuropathologic studies supports the notion that NSAIDs may protect against inflammation in the brain. MacKenzie and colleagues examined the brains of cognitively normal people who had osteoarthritis (OA) or rheumatoid arthritis (RA) and had been taking NSAIDs for one year or longer and compared them to age-matched controls. [42] They found, as expected, that some OA/RA brains and some control brains contained amyloid plaques. However, the number of activated microglia was lower in chronic NSAID users compared to non-users and this decrease was not dependent on the number of amyloid plaques. This decrease in inflammatory cells has been found in some studies [4, 42], but not all. [43]

The effect of NSAIDs on brain inflammation has also been investigated in cell culture and animal model studies. Aside from one contradictory finding [44], these studies have provided consistent evidence that NSAIDs have significant anti-inflammatory effects in the brain. [5–8, 45, 46] For example, in a mouse model of AD, Lim and colleagues found that chronic three or six-month treatment with the NSAID, ibuprofen, or the naturally-occurring NSAID, curcumin, reduced plaque-associated microglial activation, astrocytosis, and inflammatory products. [5, 6, 47] Similar anti-inflammatory properties have been found by others using ibuprofen [46] and indomethacin. [7, 8, 45]

Aside from their anti-inflammatory properties, recent evidence suggests that some NSAIDs may protect against AD independently of COX by directly reducing the metabolism of APP to Aβ. [9, 11] As described above, the metabolism of APP to Aβ is thought to play a central role in the pathogenesis of AD. APP is a type I transmembrane protein found in many cell types including neurons. Its normal physiological role is unknown, but it has been shown to inhibit certain enzymes, promote cell adhesion, and is associated with neuroprotection and neurodevelopment. [20, 48]

In AD, it undergoes sequential enzymatic cleavage first in the extracellular region by the enzyme β-secretase, which results in the release of a soluble protein denoted $sAPP_{\beta}$. The remaining piece of APP, embedded in the cell membrane, is then cleaved by the intramembrane enzyme, γ-secretase, after which the resulting Aβ fragment is secreted from the cell. [20] Gamma-secretase cleavage can occur at four sites resulting in Aβ fragments varying in length with 38, 40, 42, or 43 amino acids. Both the $A\beta_{40}$ and $A\beta_{42}$ peptides accumulate in the "senile" plaques that are pathognomic of AD, but it is the $A\beta_{42}$ species that is thought to be more damaging. Recent studies suggest that NSAIDs may modulate the activity of γ-secretase and shift the cleavage of APP towards the more benign $A\beta_{40}$ species. [9, 49]

Both in vivo [5, 6, 9, 11, 44–46, 50] and in vitro [9–11, 46, 50–55] studies have now documented that NSAID administration can modulate Aβ levels or Aβ plaques. Interestingly, some of these studies suggest that the effect on Aβ production may depend upon the type of NSAID. *In vivo* studies with ibuprofen [5, 6], indomethacin [45], and a derivative of flurbiprofen [44] all showed reduced Aβ levels in brain regions classically associated with Alzheimer-like pathology. However, one study could not replicate the ibuprofen finding and failed to show any evidence that celecoxib treatment mitigated Aβ pathology. [44] In a more detailed series of studies, Weggen and colleagues fed ibuprofen or naproxen to Alzheimer's mice and measured both soluble $A\beta_{42}$ and $A\beta_{40}$ levels. [11] They found that although ibuprofen selectively lowered $A\beta_{42}$ compared to $A\beta_{40}$, naproxen did not. In a follow-up study, they fed numerous NSAIDs to mice for three days and, as before, found that only some NSAIDs (e.g., diclofenac, diflunisal, fenoprofen, flurbiprofen, ibuprofen, indomethacin, meclofen, piroxicam, R-flurbiprofen, S-flurbiprofen, and sulindac) selectively lowered $A\beta_{42}$ compared to $A\beta_{40}$ whereas other NSAIDs (e.g., aspirin, ketoprofen, nabumetone, and naproxen) did not. [9] Yan and colleagues [46] found a similar reduction in $A\beta_{42}$ after mice were fed ibuprofen for four months, but a recent study by Lanz et al. [50] failed to show consistent reductions in brain $A\beta_{42}$ after three-day administration of flurbiprofen, ibuprofen, or sulindac. Cell culture studies appear to support the notion that some, but not all, NSAIDs selectively decrease $A\beta_{42}$ levels. However, the results are more difficult to interpret because many different cell types (i.e., derived from humans vs. other animals, and from peripheral vs. glial cells or neuronal cells) and varying doses of NSAIDs have been used. For example, in hamster ovary, human neuroglioma, human kidney, or mouse fibroblasts, a selective reduction in $A\beta_{42}$ was found with diclofenac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, meclofen, R-ibuprofen, R-flurbiprofen, S-flurbiprofen, and sulindac, but not with aspirin, celecoxib, diflunisal, etodolac, fenbufen, ketorolac, ketoprofen, mefanamic acid, meloxicam, nabumetone, naproxen, phenylbutazone, piroxicam, sulindac sulphone, and suprofen. [9, 11, 46, 52] In contrast, Gasparini and colleagues tested a neuroblastoma cell line and found that flurbiprofen, sulindac sulfide, and aspirin produced a reduction in both $A\beta_{42}$ and $A\beta_{40}$, while in primary neurons, sulindac sulfide reduced both $A\beta_{42}$ and $A\beta_{40}$ and celecoxib reduced $A\beta_{40}$ and increased levels of $A\beta_{42}$. [54] Although the results from the cell culture studies are not entirely

consistent with the animal model studies, there appears to be some consensus that NSAIDs such as ibuprofen, flurbiprofen, indomethacin and sulindac tend to lower $A\beta_{42}$ whereas others such as naproxen and celecoxib do not.

The mechanisms underlying the apparent differences between NSAIDs need clarification, but in any case $A\beta_{42}$ reduction is probably not mediated through COX as compounds that lack COX activity (e.g., R-flurbiprofen, R-ibuprofen) still showed the capacity to decrease $A\beta_{42}$. [9–11, 51, 52, 55] To demonstrate that the effect on $A\beta_{42}$ reduction is independent of COX, Weggen and colleagues treated COX-deficient cells with sulindac sulphide and then monitored $A\beta_{42}$ levels. [11] Interestingly, the COX-deficient cells did not demonstrate any alteration in basal $A\beta_{42}$ vs. wild-type cells, but treatment with sulindac did reduce $A\beta_{42}$. In another study, Sagi et al. showed that other known targets of NSAIDs besides COX, including lipoxygenases, peroxisome proliferator-activated receptor, or nuclear factor kappa B, are also not required for $A\beta_{42}$-reduction. [10] Studies with a broken cell γ-secretase assay have suggested that the $A\beta_{42}$ lowering NSAIDs may in fact directly target the γ-secretase complex. [9, 49] In order to elucidate how such NSAIDs might interact with γ-secretase, Lleo and colleagues utilized a fluorescence resonance energy transfer technique. [56] They found that these NSAIDs influence the proximity between APP and presenilin-1 (PS1), which is thought to activate or be part of the γ-secretase complex, and as a result alter PS1 conformation. They proposed a model in which the allosteric change in PS1 conformation shifts the cleavage of APP toward shorter Aβ species. [56]

However these compounds work to reduce amyloidogenic APP processing, a key question is whether such drugs will be effective in the prevention or treatment of clinical AD. One study with a transgenic mouse model of AD showed that treatment with ibuprofen was associated not only with a reduction in amyloid burden but also with mitigation of behavioral deficits as assayed by an open field task. These results are encouraging because they suggest that AD-like clinical features can be ameliorated by NSAID treatment. [6] However, another study with a transgenic mouse model suggested that the neuroprotective effects of such NSAIDs may depend upon when they are used. Jankowsky and colleagues made an inducible APP transgenic mouse (where the mutant APP transgene is controlled by the antibiotic tetracycline and its analogues, designed to mimic γ-secretase inhibition) and turned the transgene "off" once AD-like pathology was established. Strikingly, these authors were unable to reverse Aβ plaque, astroglial, or neuritic pathology in these mice, even after six months of transgene inactivation. [57] These results suggest that inhibition of γ-secretase would need to be started early in the course of the disease in order to have any efficacy.

3. EVIDENCE FROM EPIDEMIOLOGIC STUDIES

3.1. Observational Studies

At least 25 epidemiologic studies have reported on the relationship between NSAIDs and the risk of AD in humans. [58-83] Many of the early studies

from the 1990's examined inflammatory conditions such as arthritis, with some [59, 60, 62, 63, 66, 67] but not all [58, 61, 64] finding an inverse association with AD. In a meta-analysis of these earlier studies, McGeer and colleagues reported that a history of arthritis was associated with a 44% reduction in risk of AD. [1]

It was suggested that a history of arthritis was a surrogate for NSAID exposure [63], and that the chronic use of NSAIDs among those with arthritis was responsible for the observed reduction in risk of AD. Thus, later studies began to focus specifically on the use of NSAIDs. Of twelve such studies that examined the use of non-aspirin NSAIDs using a non-prospective study design (i.e., case-control or cross-sectional) [68–71, 73–76, 79, 81, 84, 85], ten concluded that AD cases were less likely to have been using these agents whereas two concluded there was no association. [73, 79] The odds ratios (ORs) reported in these studies ranged from 0.19 (95% CI = 0.06 to 0.64) in a sibling study conducted by Breitner and colleagues [70] to 0.79 (95% CI = 0.45 to 1.38) in a retrospective case-control study conducted as part of the Rochester Epidemiology Project. [73] Eight of the twelve non-prospective studies are summarized in a meta-analysis in Figure 1a. To be included in the meta-analysis the studies had to have an outcome of AD that was diagnosed by formal criteria (e.g., the Diagnostic and Statistical Manual of Mental Disorders [87] or the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [88]), the original data must have been reported for both the cases and the controls, and the criteria for exposure to non-aspirin NSAIDs must have been well-documented. The combined OR from these studies, which is based on a total of 1,833 AD cases and 13,780 controls, indicates a 53% risk reduction of AD in those participants who reported using a non-aspirin NSAID (combined OR = 0.47, 95% CI = 0.36 to 0.62).

In two studies conducted by Breitner and colleagues, an effort was made to tease apart the separate effects of NSAID use and arthritis on AD risk. [70, 85] When this was done, the OR for individuals who had a history of arthritis but did not use NSAIDs was 0.60 (95% CI = 0.10 to 3.50) in a study of twins and 0.68 (95% CI = 0.38 to 1.22) in a study of siblings. For individuals who used NSAIDs but did not have a history of arthritis the OR was 0.08 (95% CI = 0.01 to 0.69) in the twin study and indeterminate in the other study due to limited sample size. Although not definitive, these results provided evidence that the previously observed reduction in AD risk was due to the use of NSAIDs and not arthritis.

Seven of the non-prospective studies also had data available on aspirin use. [70, 73, 75, 76, 81, 83, 85] As seen in Figure 1b, which is based on data from 1,509 AD cases and 7,923 controls, aspirin use was also associated with a reduced risk of AD (combined OR = 0.55, 95% CI = 0.44 to 0.70), but in nearly half of the studies the confidence interval included the null. As discussed above, aspirin is like other NSAIDs in that it inhibits the COX enzyme, but it does so in a different manner by irreversibly blocking the active site. Another consideration with aspirin is that it is typically taken by the elderly for cardioprophylaxis at lower doses that will not have a potent anti-inflammatory effect.

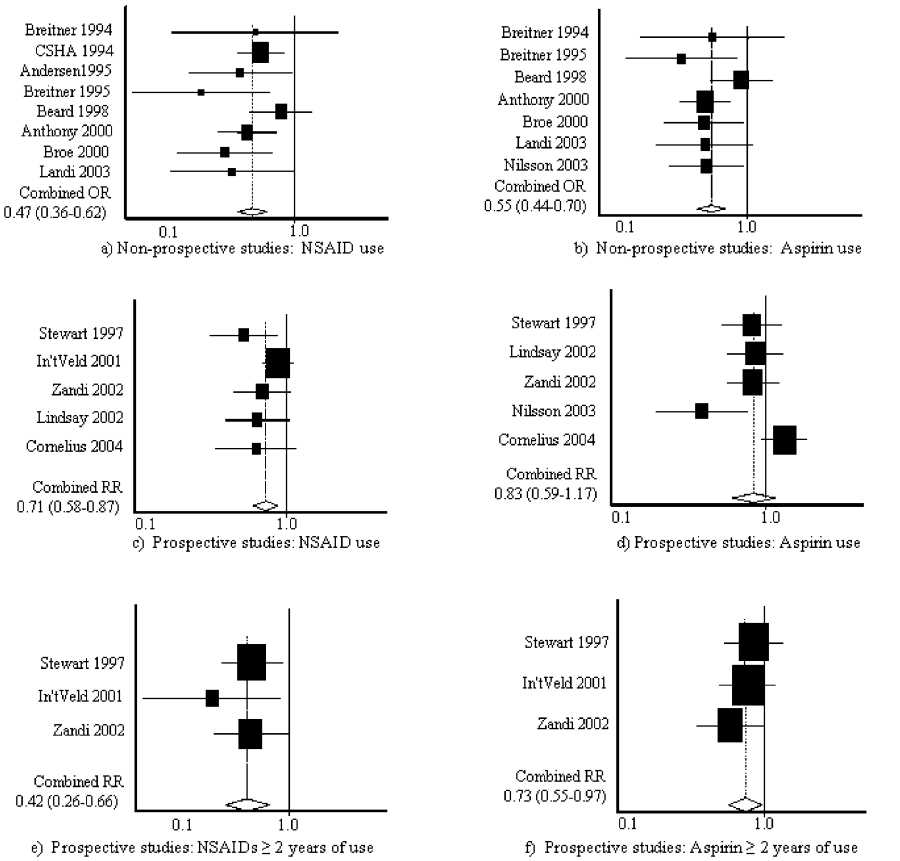

Figure 1. Meta-analyses showing the relationship between 1a) non-aspirin NSAID use and AD in non-prospective studies, 1b) aspirin use and AD in non-prospective studies, 1c) non-aspirin NSAID use and AD in prospective studies, 1d) aspirin use and AD in prospective studies, 1e) two or more years of non-aspirin NSAID use and AD in prospective studies, and 1f) two or more years of aspirin use and AD in prospective studies. (OR = odds ratio; RR = risk ratio). Figures 1a, c, and e modified from Szekely et al: Neuroepidemiology 2004;23:159–169 with permission from S. Karger AG, Basel; Statistical analysis performed using Stata 8.0 [86]

A major limitation of non-prospective observational studies like the ones discussed above is that they are unable to establish the temporality between NSAID use and AD, making it difficult to draw firm conclusions about the causal relationship between the two. Furthermore, such studies can be biased by differential recall of NSAID exposure in patient and control groups. This recall may be especially problematic in studies of diseases affecting memory such as AD. As a result, prospective studies in which information on exposure to NSAIDs is collected prior to diagnosis of AD can provide more conclusive evidence about their putative association.

Five prospective studies including a total of 836 AD cases and 16,294 controls have been published on the relation between NSAID use and incidence of AD. The findings from these studies are summarized in Figure 1c. [72, 77, 78, 80, 82] The combined risk ratio (RR) for lifetime use of non-aspirin NSAIDs and AD was 0.71 (95% CI 0.58 to 0.87). All five studies showed a trend for an inverse association between NSAIDs and AD, but it should be noted that all but one risk estimate included the null. In three of the studies in which data on duration was available, [72, 77, 80] the combined RR for two or more years of NSAID use was 0.42 (95% CI 0.26 to 0.66) (Figure 1e), suggesting a greater reduction in risk of AD with longer user. Interestingly, these three studies were also able to examine lag effects between NSAID use and onset of AD. In particular, two of these studies [77, 80] reported evidence suggesting that NSAID use was effective in reducing the risk of AD only if taken several years before the clinical onset of disease. By contrast, NSAID use within several years of the onset of disease did not appear to have any protective effect. These latter findings appear to be consistent with those from the study by Jankowsky and colleagues described above in which they showed using a transgene mouse model that it was not possible to reverse the AD pathology by simulating γ-secretase inhibition once the pathology was established. [57]

Five prospective studies also reported data on aspirin use. Figure 1d. shows a marginal reduction in risk of AD with lifetime use (RR = 0.83, 95% CI 0.59 to 1.17). However, as shown in Figure 1f, the reduction in risk became more apparent for use of aspirin greater than two years (RR = 0.73, 95% CI 0.55 to 0.97), again suggesting a duration effect similar to what is found with the other non-aspirin NSAIDs.

Other than aspirin, the effects of individual NSAIDs on AD risk have not been systematically examined in the published observational studies. The problem is that the sample sizes in these studies have typically been too small to allow for such investigations. Aspirin is an exception because it is used much more widely among the elderly for cardioprophylaxis. Consequently, it remains an open question whether those NSAIDs that have been shown to lower $A\beta_{42}$ in cell cultures and in animal models are associated with lower risk of AD in observational studies compared to NSAIDs that do not possess this property. An abstract from the Rotterdam Study [89], one of the five prospective studies that found a reduced risk of AD with NSAIDs, attempted to address this question and was reported at a research conference. The authors suggested that the observed reduction in AD risk was attributable to the use of $A\beta_{42}$-lowering NSAIDs such as ibuprofen and flurbiprofen, but the findings were inconclusive because of very small sample sizes. To overcome this limitation, we have pooled data from prospective studies of AD with the goal of assembling a sample that has sufficient power to adequately assess the neuroprotective effects of the different types of NSAIDs. Preliminary findings from this individual-patient-data meta-analysis, based on three published studies, suggested that NSAID exposure was associated with decreased risk of AD, but the reduction was not dependent on the $A\beta_{42}$-lowering capability of the NSAID. [90] We are currently nearing completion of this project and hope that the results from

the larger pooled sample will provide more definitive conclusions regarding the role of type of NSAID on AD risk.

It should be noted that even though data from observational studies provide invaluable information about the relationship between NSAID use and AD, there are confounders and biases inherent in the design of these studies that must be considered when interpreting their results. One type of confounding that is a particular problem in studies of pharmacologic treatments is confounding by indication, in which a drug under investigation is used as a treatment for a disease which is, in itself, associated with the outcome of interest. [91, 92] For example, the apparent risk reduction with NSAID use and AD could be due to the presence of arthritic disease, for which NSAIDs are routinely taken. However, the studies that attempted to address this issue provided some evidence that NSAID use, in the absence of arthritis, still reduced risk for AD. Results from studies of medication can also be influenced by the healthy drug user bias in which individuals who use medications may be more health conscious or may have greater access to health care compared to non-users. [93] This is probably not a substantive bias for studies on NSAID use, as compared to hormone replacement therapy or vitamin use, as NSAIDs are often taken for chronic pain relief and not as preventive therapies. Also, many of the observational studies found null results when looking at a control medication, acetaminophen (also used for pain management), suggesting that this is not an important source of bias. Another potential source of bias in prospective studies could result from differences in mortality between exposed groups, or mortality bias. If participants exposed to NSAIDs have a higher rate of mortality due to NSAID-related complications compared to those unexposed to NSAIDs, the relationship between NSAID use and mortality could then result in an apparent inverse relationship between NSAID use and AD because the NSAID users are removed from the risk set (they die) before they have a chance to develop dementia. This issue was addressed in both the Rotterdam [94] and Cache County (unpublished data) cohorts where it was found that NSAID use was associated with a reduced risk of AD but not with all-cause mortality. Despite these (and other) limitations of observational studies, the consistency of findings across many studies does support the notion that NSAIDs might, in theory, be efficacious in reducing the risk of AD.

3.2. Randomized Trials

The encouraging findings from the observational studies have provided a rationale for carrying out randomized controlled trials (RCT) to formally test the effects of NSAIDs on AD. To date, seven RCTs have been carried out to test whether NSAIDs can slow the progression of clinically established AD (see Table 1). Such trials are often referred to as tertiary prevention or treatment trials. The NSAIDs tested thus far include indomethacin [95], diclofenac [96], nimesulide [97], naproxen [98] and the more recently developed selective COX-2 inhibitors celecoxib and rofecoxib. [98–100] Sample sizes of these RCTs ranged from 40 to 692 subjects, and the

Table 1. Randomized placebo-controlled trials using NSAIDs (or related compounds) for treatment, secondary prevention, and primary prevention

PI and year	Drug and dose per day	Sample size	Duration (months)	Main outcome measures	Main findings or trial status
Treatment trials					
Rogers 1993 [95]	Indomethacin (100 to 150 mg)	44	6	Cognitive measures	Indomethacin group improved, placebo group declined; significant difference between groups on composite cognitive score
Scharf 1999 [96]	Diclofenac (50 mg)	41	6	Cognitive measures, clinical rating, ADL	No significant differences
Sainati 2000 [100]	Celecoxib (400 mg)	425	6	Cognitive measures, clinical rating, ADL, psychiatric symptoms	No significant differences
Aisen 2002 [97]	Nimesulide (200 mg)	40	6	Cognitive measures, clinical rating, ADL, psychiatric symptoms	No significant differences
Aisen 2003 [98]	Rofecoxib (25 mg) Naproxen (440 mg)	351	12	Cognitive measures, clinical rating, psychiatric symptoms, quality of life	No significant differences; trend for rofecoxib group to deteriorate more on cognitive measure and ADL vs placebo
Reines 2004 [99]	Rofecoxib (25 mg)	692	12	Cognitive measures, clinical rating	No significant differences
Black 2005 [101]	R-flurbiprofen (800 mg) R-flurbiprofen (1600 mg)	207	12	Cognitive measures, clinical rating, ADL	In mild AD with 1600 mg/day there was a trend for benefit on all three measures

(Continued)

Table 1. (Continued)

PI and year	Drug and dose per day	Sample size	Duration (months)	Main outcome measures	Main findings or trial status
Treatment trials					
Ringman [102]	Curcumin (2 doses)	33 exp	12	Cognitive and behavioral measures, Aβ and tau levels	Recruiting
Laughlin [103]	R-flurbiprofen (800 mg)	1600 exp	18	Cognitive measures, ADL	Recruiting
Baum [104]	Curcumin (1 g) Curcumin (4 g)	30 exp	6	Cognitive measures, Aβ and isoprostane levels	Recruiting
Secondary prevention trials					
Thal 2005 [105]	Rofecoxib (25 mg)	1457	48	Time to clinically diagnosed AD, cognitive measures, clinical rating	Risk to convert to AD higher in rofecoxib vs. placebo group; No significant differences on other measures
Small [106]	Celecoxib (400 mg)	135 exp	18	Further cognitive decline, neuroimaging changes	Recently completed
Primary prevention trial					
Breitner [107]	Celecoxib (400 mg) Naproxen (440 mg)	2528	20	Time to clinically diagnosed AD, cognitive measures, clinical rating	Recently completed/ treatment suspended

PI=principal investigator/author; mg=milligrams; g=grams; exp=expected; ADL=activities of daily living; AD=Alzheimer's disease; Aβ =amyloid-beta

duration of follow-up was typically 6 to 12 months. Only one of these trials showed a mild benefit in slowing cognitive decline [95], while the others did not offer any evidence of a therapeutic effect. More recently, preliminary results were reported from a RCT of 207 subjects testing an enantiomer of flurbiprofen which was selected specifically because it modulates γ-secretase but has little activity against COX. This trial showed slightly less decline in cognitive ability in patients with mild AD over a 12 month period among subjects taking 1600 mg of R-flurbiprofen compared to placebo. [101] While the results do not show a striking improvement, they are promising and are currently being followed-up by a RCT with a larger sample and longer period of follow-up. [103, 108] Two other RCTs for the treatment of AD are also currently recruiting subjects to test curcumin. [102, 104] This compound, derived from the spice turmeric, may be of interest as it has strong anti-inflammatory and anti-oxidant properties and has been shown in animal models of AD to decrease levels of plaque burden, circulating amyloid, and proinflammatory cytokines. [47, 109]

Only one RCT has been carried out to test whether NSAIDs can delay the progression of prodromal mild cognitive impairment (MCI) to AD. [105] Such trials are often referred to as secondary prevention trials. In this secondary prevention trial, a total of 1,457 subjects were randomized to receive either 25 mg of rofecoxib or placebo and followed for up to four years. Surprisingly, subjects on rofecoxib converted to AD at a faster rate than those on placebo, but there were no significant differences in other functional measures. Another secondary prevention trial using 400 mg of celecoxib with 135 subjects followed for 18 months was recently completed, but the results have not yet been reported. [106]

Only one RCT has been initiated to test whether an NSAID can delay the progression to AD among cognitively normal elderly individuals. Such trials are often referred to as primary prevention trials. The Alzheimer's Disease Anti-inflammatory Prevention Trial [110], known as ADAPT, began recruiting participants in 2001 with an expected follow-up of up to seven years. The trial was designed to test two NSAIDs, naproxen and celecoxib, for the prevention of AD in approximately 2,625 elderly individuals who were cognitively normal with no evidence of MCI but who were at higher risk of AD than the general population because they had at least one first-degree relative with a history of dementia. Other exclusion criteria were based on a number of safety measures related to potential toxic side effects of NSAIDs. Participants were asked to travel to the clinics for routine biannual safety monitoring and annual cognitive assessments as well as additional safety-related visits. As ADAPT progressed, evidence from other studies emerged raising concerns about the cardiovascular safety of COX-2 inhibitors. [111, 112] More definitive evidence that these drugs, particularly rofecoxib, increased the risk of cardiovascular events was later reported. [113, 114] This led to withdrawal of rofecoxib from the market and to suspension of two cancer trials using celecoxib. In December of 2004, ADAPT investigators decided to suspend treatment in ADAPT due to the findings from outside trials and also because preliminary data suggested an increased risk of cardiovascular events in the

naproxen group. [107, 115] ADAPT participants were asked to stop taking study drug, but were asked to continue followup visits for safety and outcome monitoring. The results pertaining to cardiovascular events, AD, or cognitive decline are not available at the time of this writing.

4. SUMMARY AND CONCLUSIONS

The evidence is growing that NSAIDs may be useful in combating AD. Laboratory studies indicate there are compelling, biologically plausible reasons to believe that NSAIDs have a potent neuroprotective effect. By inhibiting COX, NSAIDs may reduce inflammatory responses in the brain thought to be associated with AD pathogenesis. Furthermore, certain NSAIDs may also modulate the activity of γ-secretase to reduce amyloid plaque production by shifting APP metabolism away from the more toxic $A\beta_{42}$ species. In line with this, epidemiologic studies carried out since the early 1990's have consistently shown that NSAIDs are associated with a lower risk of AD. A meta-analysis of the most rigorously conducted observational studies suggested that the use of non-aspirin NSAIDs for more than two years is associated with a 60% reduction in risk of AD, while the use of aspirin for more than two years is associated with a 30% reduction. The less apparent effect with aspirin may be due to the fact that it is typically taken by the elderly at low doses for cardioprophylaxis. Although observational studies have not had sufficient sample size to examine whether the reduction in risk is different for specific NSAIDs shown to selectively reduce $A\beta_{42}$, preliminary findings from a pooled analysis that we are carrying out suggest this may not be the case. Results from randomized trials have not been as encouraging. Seven trials with NSAIDs have been reported in the literature. With the possible exception of the most recent trial with R-flurbiprofen, the results from these trials suggest that NSAIDs (or related agents) may not be effective for the tertiary or secondary prevention of AD. These results are not surprising, and in fact appear to be consistent with observational studies that have shown that NSAIDs need to be taken several years prior to the clinical onset of disease in order to have any effect on lowering AD risk. Thus, it is reasonable to conclude that NSAIDs may be particularly effective for the primary prevention of AD before the course of disease has progressed to a point beyond remediation.

The best way to formally demonstrate the efficacy of NSAIDs on primary prevention is through randomized trials. Unfortunately, the one existing trial that was appropriately designed to address this important question, ADAPT, suspended treatment early due to safety concerns. Primary prevention trials of AD, like ADAPT, present significant challenges. These challenges include attaining an adequate sample size, allowing for a sufficient follow-up time, maintaining compliance, and implementing a cost-effective data collection schedule. [116] Participants willing to enroll in trials may have lower disease rates and mortality compared to others, and therefore either the sample size must be increased or the follow-up lengthened to ensure accrual of sufficient disease endpoints. [117]

Additionally, a longer trial may be needed to accommodate agents that require a certain amount of time to fully exert a protective effect. However, as trials become bigger and longer, the threat of non-compliance increases [116] and the costs quickly become prohibitive. Finally, there are considerable ethical concerns in giving drugs like NSAIDs, which can have significant toxic side effects, to elderly subjects who do not have the disease.

Despite these challenges, it is important that efforts continue to further clarify the neuroprotective role of NSAIDs. Because many elderly take NSAIDs regularly for a variety of indications such as arthritis, it is crucial to establish the risks of taking NSAIDs in regards to potential gastrointestinal and cardiovascular adverse effects relative to the benefits including any concomitant salutary effects on cognition. Furthermore, by elucidating the mechanisms by which NSAIDs may protect against AD, more rational interventions can be adapted that maximize the benefits while minimizing the risks, and certain groups of participants who might tolerate the new treatments better than others can be more successfully identified. Thus, future studies should continue to investigate the relative contribution of COX and γ-secretase mediated effects of NSAIDs and how these different effects translate into a reduced risk of AD in human populations. Additionally, it will be important for investigators to focus on the issue of how the timing of NSAID exposure and the duration of use influences the underlying progression of AD. With 2.5 to 4.5 million prevalent cases in the United States alone and projections that these numbers will likely quadruple in the United States over the coming 50 years [12], AD is a major public health problem that threatens to worsen. Thus, there is considerable motivation to develop effective strategies for delaying or even preventing the disease.

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