NSAID and Antioxidant Prevention of Alzheimer's Disease

Lessons from *In Vitro* and Animal Models

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ABSTRACT: **Both oxidative damage and inflammation are elevated in brains of Alzheimer's disease (AD) patients, but their pathogenic significance remains unclear. The reduced AD risk associated with high intake of both nonsteroidal anti-inflammatory drugs (NSAIDs) and antioxidants suggests causal roles, but clinical trials in AD patients have yielded only limited or negative results. To test the potential efficacy and mechanisms of candidate approaches, we have explored conventional and unconventional NSAIDs, antioxidants, and combined NSAID/antioxidants in cell culture and animal models for AD (including aging APPsw transgenic mice and soluble Aβ rodent infusion models). The conventional NSAID ibuprofen has the strongest epidemiological support. At sustainable doses designed to mimic protective consumption in the epidemiology, ibuprofen reduces amyloid accumulation but suppresses a surprisingly limited subset of inflammatory markers in APPsw transgenic mice. Both Aβ production (APP, β- and γ-secretases) and post-production pathways (those affecting Aβ aggregation or clearance: e.g., IL-1 or α1ACT) are potentially involved in ibuprofen and other NSAID anti-AD activities. The post-production pathways are predictably shared with other seemingly protective NSAIDs, including naproxen that do not lower Aβ42 *in vitro*. Using clinically feasible dosing, brain levels of NSAIDs appear too low to implicate a number of pharmacological dose targets that have been demonstrated *in vitro*. Ibuprofen did not suppress microglial markers related to phagocytosis. The putative anti-inflammatory omega-3 fatty acid DHA had a profound impact on pathogenesis but did not lower inflammation, while vitamin E was surprisingly ineffective in reducing oxidative damage or amyloid in the aged APPsw mouse. In contrast, the unconventional NSAID/antioxidant curcumin was effective, lowering oxidative damage, cognitive deficits, synaptic marker loss, and amyloid deposition. Curcumin proved to be immunomodulatory, simultaneously inhibiting cytokine and microglial activation indices related to neurotoxicity, but increasing an index of phagocytosis. Curcumin directly targeted Aβ and was also effective in other models, warranting further preclinical and clinical exploration.**

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INTRODUCTION

Acquittal in Clinical Trials and the Case against Oxidative Damage and Inflammation

There is no doubt that the usual suspects of oxidative damage[1–4] and inflammation[5–7] are present early and throughout Alzheimer's disease (AD) pathogenesis. Both have long rap sheets as toxic effectors in chronic degenerative diseases and are implicated by thick dossiers on AD versus control brains. Further evidence, suggesting possible causal roles, comes from epidemiological studies that repeatedly show reduced AD risk with higher intake of antioxidants[8–10] or non-steroidal anti-inflammatory drugs (NSAIDs).[11–13] However, one requires more than a circumstantial case to prove whether either or both of these suspected perpetrators are minor accomplices or actually guilty of playing important causal roles in AD pathogenesis. Strong DNA (genetic) evidence may add strong credence to causality, so despite holes in the amyloid cascade hypothesis, data on autosomal dominant familial AD mutations influencing β-amyloid would maintain that amyloid β peptide 1-42 (Aβ42) causes the disease. Although there is no genetic evidence for oxidation as causal process, there are suggestions, some controversial, of increased association of multiple inflammatory gene alleles (α1ACT, IL-1β, TNFα, IL-6, IL-10)[14–20] and α2-macroglobulin[21] with increased sporadic AD risk. This suggests that, like ApoE alleles, inflammatory processes may modulate age of onset and risk in sporadic AD, but will not exert a powerful enough drive to cause autosomal dominant AD. Nevertheless, factors delaying the onset of sporadic AD may have profound impact because it is estimated that a 10-year delay would reduce the number of cases by 75%. Overall, the evidence from pathology and epidemiology, implicating oxidative damage and inflammation in AD has been strong enough to support clinical trials in AD patients using antioxidant, vitamin E, and several NSAIDs (naproxen, COX-2 inhibitors). Unfortunately, despite some promising data from several small trials, the results from large clinical trials have failed to show that either vitamin E or NSAIDs can slow cognitive decline and AD progression. On the grounds of these negative trial data alone, one might be tempted to dismiss the case against oxidative damage and inflammation as mediators of the disease.

Convicted by Preclinical Data?

Before we conclude that oxidative damage and inflammation should simply be acquitted, we need to explore the evidence provided by preclinical *in vitro* and *in vivo* studies. Do these studies provide support for the conviction that oxidative damage and inflammation play important causal roles in the AD? Will their effective treatment help prevent or delay disease onset?

CNS INFLAMMATION INDUCES OXIDATIVE DAMAGE, AN IMPORTANT MEDIATOR OF Aβ AGGREGATES AND OLIGOMER TOXICITY

While cytotoxic T cells, autoimmune antibodies, and high inflammatory cytokine levels may be important effectors in peripheral inflammatory conditions, the CNS inflammation in AD is primarily characterized by reactive microglia, IL-1, and complement factors.[7] Apart from the role of activation of the complement pathway, which should be considered separately, reactive oxygen species (ROS) produced by activated microglia are perhaps the most obvious toxic effectors with central nervous system (CNS) inflammation. In principle, activated macrophages and microglia induce NADPH oxidase and iNOS to produce a respiratory burst of relatively diffusible superoxide and NO that combine focally and at some distance, to produce the highly reactive effector germicidal, peroxynitrite. Other ROS, for example, myeloperoxidase-derived hypochlorite may also contribute to toxicity, but these toxins are less clearly implicated. Consistent with this, the principal neurotoxins induced by Aβ aggregates stimulate microglia activation *in vitro*, require iNOS stimulation and appear to be peroxynitrite radicals,[22,23] responsible for extensive damage in AD.[3] Although more controversial, Aβ aggregates may also promote oxidative damage via direct effects including induction of hydrogen peroxide production in neuronal cell lines and primary neurons,[2] catalysis via iron or copper binding,[24] or even peptidyl radicals.[25] Overall, these results argue for some potential for treatments that target the specific inflammatory factors and Aβ species, causing oxidative damage in AD.

WHAT ARE THE RELEVANT TARGETS OF OXIDATIVE DAMAGE AND INFLAMMATION?

Synaptic and Postsynaptic Targets

Neuron death is not the only index of damage induced by Aβ-induced inflammatory ROS. One relevant very recent example comes from Montine's group who finds that intracerebroventricular administration of a bolus of LPS to mice results in marked dendritic regression within 24 h that is dependent on CD14, iNOS, COX-2, PGE2, and EP-1 receptors.[26,27] Remarkably, the dendritic deficits occur in the absence of any neuron loss and are transient, as demonstrated by a full reversal one week later. Another example is provided by administration of small Aβ oligomers that produce very rapid deficits in long-term potentiation (LTP) *in vitro*[28] or *in vivo*.[29] Knockout mice and pharmacological data show that these Aβ aggregate–induced deficits are dependent on iNOS and NADPH oxidase induction and can be inhibited by catalase and superoxide dismutase, consistent with a microglia-mediated peroxynitrite attack.[30] These results are also entirely consistent with experiments from our group showing that chronic intracerebroventricular infusion of Aβ can induce selective loss of postsynaptic markers, including drebrin, NR2B, and PSD-95, which can be blocked by the combined NSAID–antioxidant curcumin.[31] Similarly, microarray analysis shows small but significant alterations in postsynaptic mRNAs involved in synaptic plasticity in amyloid-laden aging APPsw × PS1 mutant transgenic mice that have Aβ-dependent cognitive deficits.[32] Our own recent data, discussed below, also

support synaptic and largely postsynaptic attacks in APPsw mice mediated by amyloid and oxidative damage, which can be modulated by dietary factors.

Unsaturated Fatty Acids

The CNS is a fatty tissue loaded with polyunsaturated fatty acids that are extremely vulnerable to oxidative attack by lipid peroxidation. While hydroxyl radicals have a plethora of targets, the danger of lipid peroxidation is that it is an autocatalytic feed-forward process, generating lipid peroxides that initiate more lipid peroxidation. Because of this, lipid peroxidation is likely to occur in localized bursts. Global measures of lipid peroxidation that include relatively uninvolved sites may obscure or dilute the severity of focal damage. In addition to being sites of Aβ generation and accumulation, neurons and synapses are highly enriched in long chain unsaturated fatty acids, and one of the most vulnerable is the omega-3 fatty acid, docosahexaenoic acid (DHA, C22:6 (n-3)) because of its six double bonds. DHA is primarily neuronal. The large increases in oxidized DHA (F4-isoprostanes or neuroprostanes) in AD[33] are a good index of the oxidative attack on neurons that may result in focal DHA depletion.

Reduced DHA intake (from fish) is a risk factor for AD that could be easily remedied with supplements. Most laboratory chow is enriched in DHA and other omega-3 fatty acids that may limit the effects of familial AD genes in mouse models. APPsw mice on a DHA-depleting diet showed elevated oxidative damage in conjunction with transgene-dependent reductions in brain DHA levels. These changes were accompanied by large (≥80%) losses of postsynaptic proteins like the actin-regulatory protein drebrin, which is known to be reduced in AD.[34] DHA-depleting chow not only exaggerated APPsw transgene-dependent focal postsynaptic caspase-cleaved actin and postsynaptic deficits, but also induced presumably Aβ-dependent defects in the neuroprotective PI3-kinase pathway in mice. The insulin-signaling PI3-kinase pathway appears to be induced by APP transgene overexpression and has been invoked as one explanation for limited neurodegeneration in APP transgenics.[35]

Because free radicals are notoriously reactive, their targets are typically proximal neighbors including proteins, lipids, DNA, RNA, and small molecule targets. While all of these targets show evidence of increased oxidative modification in AD,[25,36–38] low-level damage is either repairable or circumvented by redundancy. For example, unlike dividing cells where unrepaired nuclear DNA damage can lead to replicating carcinogenic mutations, neurons in AD may be able to sustain and accumulate high levels of unrepaired DNA damage in redundant or non-coding regions (including strand breaks detectable by TUNEL[39]) without rapidly dying. This DNA damage accumulates prior to tangles and is associated with protein nitration.[40] Most RNA or protein damage is either effectively repaired or managed by normal turnover or surveillance and targeting by defenses like the heat shock system. Proteasomal degradation, which is very effective, can fail when overwhelmed by protein aggregates. Not surprisingly, most age-related neurodegenerative diseases involve protein aggregates that circumvent the degradation processes and accumulate.

Protein Aggregation Is Seeded by Oxidative Damage–Driven Dimerization

In addition to the prominent extracellular Aβ deposits, AD has intraneuronal aggregates of phosphorylated tau (tangles/curly fibers), α-synuclein (Lewy pathol-

ogy), and actin/ cofilin (Hirano pathology), as well as intraneuronal Aβ. The initial dimerization step in the protein aggregation process appears likely to be driven by an oxidative damage step. For example, synuclein pathology is heavily nitrated[41] and promoted by tyrosine dimerization[42] and by peroxynitrite generation *in vitro*.[43] Similarly, prior to autophosphorylation, the initial tau dimerization is promoted by cysteine oxidation and disulfide crosslinking[44,45] or fatty acid oxidation.[46] Another aging and AD pathology, Hirano body accumulation, appears to involve cofilin dimerization with analogous disulfide mediated dimer or oligomer formation.[47] Finally, even Aβ dimerization occurs early during aging[48] and can be promoted or stabilized by metals with oxidation and dityrosine crosslinking.[49,50] In all of these cases, the rate-limiting early seeding events in intracellular aggregate formation would theoretically be promoted by oxidative damage related to inflammation, aging, and/or environmental risk factors, favoring the formation of rare seeding events. For example, synuclein aggregates may be initiated after short-term exposure to MPTP, rotenone, or other mitochondrial toxins, while tau aggregates may be initiated by head injury earlier in life. After oxidant-mediated aggregate seeding has been fully initiated and stabilized, one can hypothesize that monomer will deposit onto existing seeds in the presence of basal levels of monomer. Seeding may occur even in the absence of continued exposure to initiating pathological stimuli or pathologically elevated levels of monomer production. One corollary hypothesis is that antioxidant or NSAID intervention should occur early and will be relatively ineffective in reducing subsequent monomer addition steps.

Signal Transduction Pathways

Toxic forms of aggregated Aβ applied to neurons and neuronal cell lines in primary culture are reported to stimulate signal transduction pathways including FAK, Fyn, PK-C, PI3-K, JNK, GSK3β, and ERK.[51–57] Similarly, toxic soluble Aβ oligomer species are reported to bind to synaptic and postsynaptic sites coincident with PSD-95[58] and block the induction of LTP.[59] COX-2 on the postsynaptic side appears to play some important role in synaptic plasticity through pathways that are not fully understood,[60] suggesting that low doses of COX inhibitors may be able to limit prooxidant or Aβ oligomer effects on LTP and cognitive deficits, while high doses of COX inhibitors will actually block LTP. Recent data from Ashe and collaborators suggest that NSAIDs can effectively reduce cognitive deficits in Tg2576 mice through COX-2 inhibitory activity in the absence of direct effects on Aβ accumulation.[61] This property could be shared by other NSAID cyclooxygenase inhibitors, like naproxen, to the extent that they are capable of entering the brain and suppressing prostaglandin production.

IBUPROFEN AND OTHER CONVENTIONAL CYCLOOXYGENASE-INHIBITING NSAIDs AND AMYLOID REDUCTION

The first over-the-counter NSAID, ibuprofen, was most widely used and has the strongest epidemiological support of any single NSAID for reducing AD risk. How does it work? Ibuprofen may have multiple targets (FIG. 1). (Tau pathology has not yet been tested as a target for NSAIDs.) Strong data identify glial-mediated neurodegeneration, COX-2 in neurons, and excitotoxic neurodegeneration as NSAID

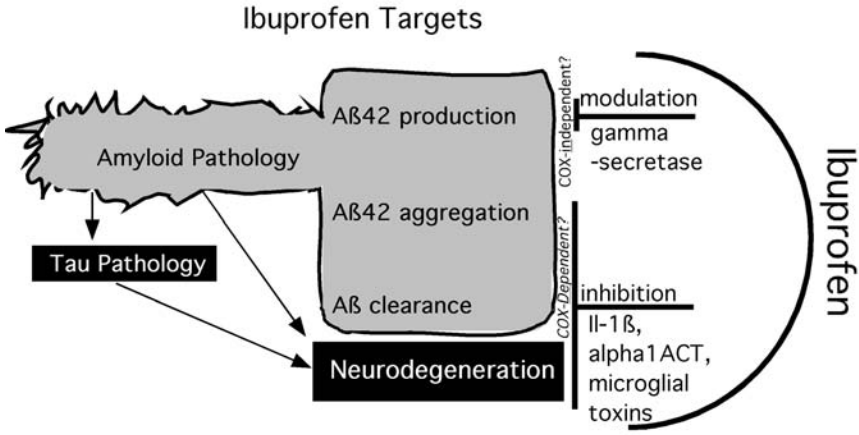

FIGURE 1. Ibuprofen may influence more than one target in AD pathogenesis. While tau remains an untested target, experimental data support neurodegeneration mediated by COX-2-sensitive excitotoxicity and microglial toxin production as plausible neuroprotective pathways. In addition, ibuprofen limits amyloid accumulation (TABLE 1), which may be due to reduced production of Aβ42 via modulation of γ-secretase or reduced production of IL-1β and downstream targets including pro-amyloidogenic α1ACT. Inhibition of pro-oxidants could also reduce amyloid aggregation, but in our models ibuprofen did not reduce oxidative damage or iNOS.

targets. These theories would predict that the neurodegeneration and synapse loss occurring throughout the clinical decline phase of AD should be limited by anti-inflammatory treatment, resulting in slowed progression. However, to date, the clinical trial evidence is against a strong NSAID effect on clinical progression, but consistent with far greater NSAID efficacy in reducing AD risk when NSAIDs were used longer than two years before assessment. Instead, the epidemiology from the twin sibling studies suggests that, like ApoE genotype, the primary NSAID effect is on age of onset leading to a 10-year delay in onset and risk reduction in 75% of NSAID users.[62] Age of onset is known to be accelerated by APP and presenilin mutations that increase Aβ42 production and by ApoE4 genotype, which is known to influence Aβ clearance and deposition. Therefore it was reasonable to wonder whether NSAIDs could also delay onset and reduce risk by influencing Aβ accumulation.

At sustainable doses designed to mimic apparently protective NSAID consumption in the epidemiology, ibuprofen reduces amyloid accumulation[63] but suppresses a surprisingly limited subset of inflammatory markers in APPsw transgenic mice, notably IL-1β and downstream murine ACT mRNA, but not iNOS, macrosialin, C1q, CD11b, or CD11c mRNA (Morihara and colleagues, submitted for publication). The latter two markers are upregulated on phagocytic microglia suggesting that at this dose, ibuprofen does not suppress microglial phagocytosis and amyloid clearance, although ibuprofen dosing was high enough to be anti-inflammatory. Several groups have been able to observe the amyloid-lowering impact of ibuprofen or related NSAIDs *in vivo*, but not all NSAID experiments have produced significant reductions in amyloid (TABLE 1).

TABLE 1. Results from various research groups

Research group	Mice	Drug and dose	Paradigm (start to finish, age in months)	Reported effects on Aβ42 *in vitro*	Amyloid pathology *in vivo*	Other pathology
Lim *et al.*,[63] 2001	Tg2576	Ibuprofen 375 ppm	10–16	Reduce	Plaque 56%, SDS-insoluble Aβ 39%	
Lim *et al.*,[64] 2002	Tg2576	Ibuprofen 375 ppm	13–16	Reduce	Ent cortex plaque	
Jantzen *et al.*,[65] 2002	Tg2576, PS1, M146L	Ibuprofen 375 ppm	7–12	Reduce	Aβ load (IHC) 20–25%	
		NCX-2216 (nitro-NSAIDs) 375 ppm	7–12	Probably reduce?	Aβ load (IHC) 40–45%, Congo red 35–40%	Microglial activation
		Celecoxib (COX-2) 175 ppm	7–12	Increase	NS trend, Aβ load (Aβ-ir plaques 15–20%)	
Staufenbiel *et al.*,[66] 2002	APP23	Ibuprofen 60 mg/kg	10.7–13.7	Reduce	NS effect	
Dedeoglu *et al.*,[67] 2002	Tg2576	Ibuprofen 100 mg/kg	16–19	Reduce	Total Aβ plaque area, Aβ42 plaque area	
Yan *et al.*,[68] 2003	Tg2576	Ibuprofen 375 ppm	11–15	Reduce	SDS-sol Aβ42 & 40, formic acid–sol Aβ42 & 40, NS trend, plaques 60%	
Volmar *et al.*,[69] 2002	PSAPP	NS-398 (COX-2) 20 mg/kg ip	3–6	Increase	Thioflavin S, 4G8-ir plaque	GFAP
Quinn *et al.*,[70] 2003	Tg2576	Indomethacin 56 μg/day or 2.2 mg/kg/day	12–20	Reduce	Plaque, only in hippo	PGE2
Eriksen *et al.*,[71] 2003	Tg2576	Ibuprofen 375 ppm	8–16	Reduce	Insoluble Aβ40 & Aβ42	
		R-Flurbiprofen 67 ppm	8–16	Reduce	Insoluble Aβ40 & Aβ42	
Pratico *et al.*,[72] 2003	Tg2576	Indomethacin 10 mg/L	8–15	Reduce	Aβ40, Aβ42	PGE2
		Nimesulide (COX-2) 40 mg/L	8–15	?	Aβ	PGE2
Kotilinek *et al.*,[61] 2001	Tg2576	Ibuprofen 375 ppm	11.5–13	Reduce	NS trend, Aβ40	

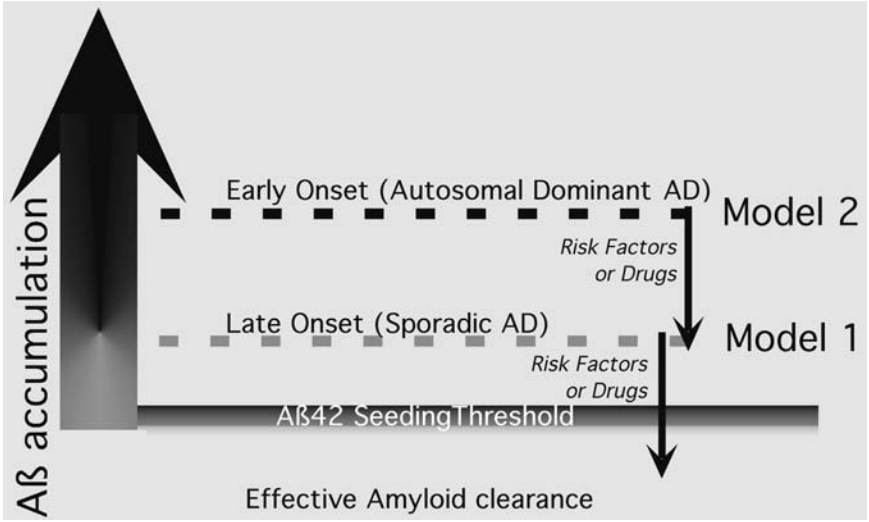

FIGURE 2. Seeding threshold model for amyloid reduction. A threshold for Aβ initial aggregation and onset limiting seeding is hypothesized to be defined by the balance of Aβ1-42 production and clearance mechanisms. If the Aβ42 production level is close to this seeding threshold (as is likely the case with late onset sporadic AD) an AD risk factor, drug, or other treatment that modestly lowers or raises Aβ42 production or clearance may have a profound effect on the onset of amyloid deposition. If the production level is significantly above the seeding threshold (as is the case with one or more powerful autosomal dominant AD mutations), the onset will be earlier and relatively impervious to the same small changes in Aβ production or clearance that influence AD risk in sporadic AD.

Among the possible explanations for the differing amyloid reduction results are the variable timing of the interventions and the more aggressive nature of some of the models. In general, as illustrated in FIGURE 2, one can assume that Aβ42 production rates need to reach some threshold set by clearance and aggregation rates before amyloid will accumulate. If the higher Aβ42 is over this hypothetical threshold, the earlier rate-limiting seeding occurs. The kinetics of aggregation argues that seeding is the rate-limiting event and thus, early intervention to prevent seeding is most likely to be effective. Aβ burden could be much less sensitive to treatments occurring after the exponential growth phase (~10–16 months in Tg2576 mice) when amyloid deposition is presumably fully seeded. In transgenic mice, very high levels of APP expression and autosomal dominant mutations that increase total Aβ (Swedish) or Aβ42 (London or PS1) are needed to drive Aβ42 over the threshold for deposit formation. Early APP transgenic models failed because APP expression was less than two times the expression of endogenous murine APP and insufficient for aggregation. Aβ42 aggregate seeding determines the age of onset and is supported by acceleration of deposition with injection of preformed aggregates.[73] In the newer generations of transgenic models employing multiple autosomal dominant AD

mutations, high levels of Aβ42 production result in deposit accumulation in the absence of aging. These mice are better models for autosomal dominant early onset AD than late onset sporadic AD. More or less by definition, prevalent environmental protective factors will not dramatically influence the outcome in autosomal dominant AD where you get the disease if you inherit the mutation. Thus, models that drive Aβ, presumably 42, far above the threshold needed for aggregation may not respond to factors that are efficacious in less aggressive sporadic AD models. Further, while quite useful for testing potent secretase inhibitors or anti-aggregation agents, the aggressive models are less useful to study drugs that target the age-dependent changes in clearance or aggregation that may pertain to the majority of sporadic AD cases.

Both Aβ production (APP, β- and γ-secretases) and post-production pathways (IL-1>α1ACT are potentially involved in ibuprofen and other NSAID anti-AD activities.

γ-Secretase Modulation

A subset of NSAIDs including ibuprofen (but not naproxen and COX-2 inhibitors) can selectively lower Aβ42 production without inhibiting total Aβ or NOTCH *in vitro* or *in vivo*.[74,75] This important result has led to the discovery of selective Aβ42 lowering agents that do not suppress total γ-secretase activity and other substrate pathways, such as notch signaling. While this is undoubtedly a highly significant result relevant to new drug discovery, it remains unclear that conventional NSAID dosing can be maintained at high enough levels to limit Aβ42 production *in vivo*. We confirmed that selective Aβ42 reduction can be achieved *in vitro* with high (>100 μM) doses of R-enantiomers of ibuprofen and flurbiprofen, which have limited COX inhibitory activity.[76] Whereas in the rodent models, R-flurbiprofen can be converted to the more toxic COX inhibiting S-flurbiprofen, this conversion is limited in humans and high doses are better tolerated. Using clinically feasible dosing, brain levels of NSAIDs are typically in the range predicted to inhibit COX, but at less than 5 μM, are too low to implicate a number of pharmacological dose targets like NFκB and PPARγ and possibly γ-secretase. Not all groups have reported success in lowering Aβ42 with ibuprofen or similar NSAIDs *in vivo*. Whether or not modulation of γ-secretase with sustainable NSAID treatment results in lower Aβ42 and increased Aβ1-38 in relevant *in vivo* models is difficult to determine solely on the basis of measuring levels of Aβ42. This is because the Aβ42 peptide is prone to aggregate and aggregate clearance mechanisms involving astrocytes and microglia may also be influenced by NSAIDs. Additional measurements of Aβ1-38 by mass spectroscopy, ELISA or other methods after acute and chronic *in vivo* NSAID treatments in multiple models should help resolve this currently controversial issue.

Inhibition of BACE1 Expression

There is a strong argument for increased cytokines, notably IL-1 in AD.[77] IL-1 may impact amyloid production because the principal β-secretase, BACE1, can be induced in IFN-γ–primed neuronal cells by the pro-inflammatory cytokines, IL-1β or TNFα, and the BACE1 induction can be blocked by treatment with ibuprofen or PPARγ agonists.[78] We can confirm these *in vitro* results but have not observed

in vivo reductions in BACE1 with chronic ibuprofen (Morihara and colleagues, unpublished observations). Similarly, oxidative damage can induce BACE1 in human neuronal NT2 cells by activating JNK and p38,[79] suggesting that both oxidative damage and inflammation may increase focal BACE1 expression in AD. Based on that hypothesis, we have tested the antioxidant and NSAID curcumin and found it to be a very effective inhibitor of pro-inflammatory cytokine-induced BACE expression *in vitro* and *in vivo* (Morihara *et al*., in preparation).

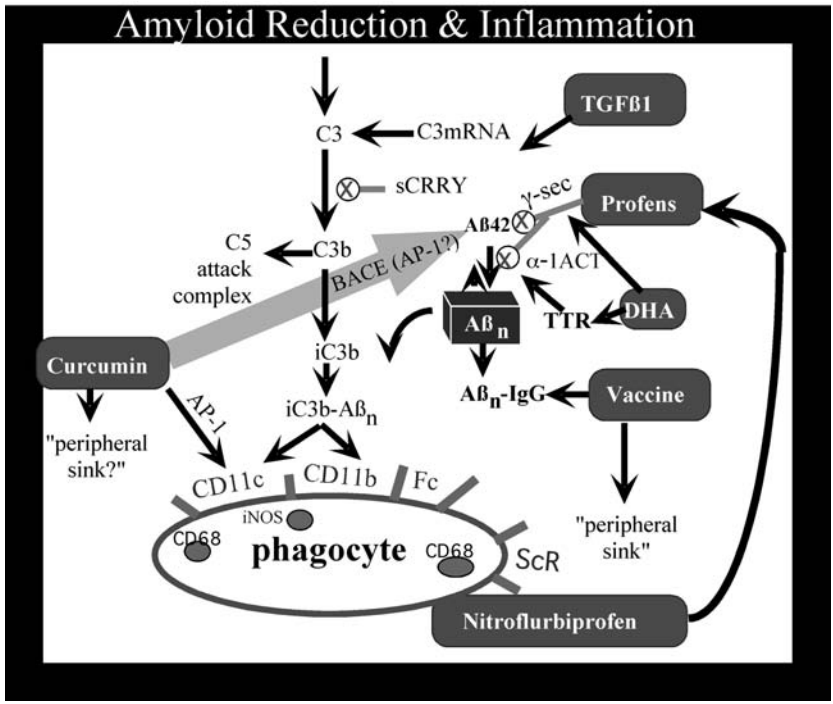

FIGURE 3. Amyloid reduction related to inflammation. As demonstrated by Wyss-Coray and collaborators, TGFβ1 stimulation of complement C3 can promote Aβ aggregate ($A\beta_n$) opsonization by C3B and iC3b fragments and their clearance by CD11b and CD11c receptors on microglia or other monocytic lineage phagocytes. The amyloid vaccine antibodies reduce Aβ aggregates by directly binding to them, promoting Fc-mediated amyloid clearance and functioning as a "peripheral sink." NSAID profens (ibuprofen, flurbiprofen, etc.) can act on γ-secretase to reduce Aβ42 production and also reduce IL-1–mediated pro-amyloidogenic α1ACT. Nitroflurbiprofen has the additional property of activating microglia and apparent clearance. Curcumin can bind directly to Aβ aggregates, limit their production and possibly act as a "peripheral sink". Curcumin can also suppress JNK activation and potentially suppress inflammation or oxidative damage induced BACE1 and γ-secretase activity. Curcumin also suppresses iNOS/pro-oxidant production and CD11b while promoting phagocytic markers CD11c and CD68 (macrosialin).

THE OMEGA-3 FATTY ACID DHA HAS BOTH NEUROPROTECTIVE AND ANTI-AMYLOID EFFECTS

Depletion or replacement of the putative anti-inflammatory omega-3 fatty acid DHA had a profound impact on AD pathogenesis from 17–22 months in aged Tg2576 mice. DHA depletion increased drebrin and other postsynaptic marker losses (PI3-K p85a subunit, and phosphoBAD), oxidative damage, and caspase activation; while DHA repletion rescued these in aged Tg2576 mice.[34] DHA also reduced amyloid burden and Aβ levels.[80] In contrast, dietary omega-3 fatty acids and DHA did not lower the presynaptic synaptophysin or inflammatory cytokines or influence ApoE or GFAP levels in our Tg2576 experiments. Brain DHA loss in the model was APP-transgene–dependent and presumably driven by oxidation of DHA by Aβ aggregates. Our results support a DHA role in modulating insulin signaling via PI3-kinase and levels of its p85α subunit. DHA depletion aggravated cognitive deficits in the Morris water maze in Tg2576 mice, while DHA supplementation was protective.[34] Relevant DHA and fish oil mechanisms may include the observed effects on PI3-K and downstream pathways, modulation of secretase activities via membrane fluidity changes, induction of anti-amyloidogenic transthyretin,[81] and metabolism to potent neuroprotective compounds.[82] Like fish oil, DHA can be taken as a supplement at high doses with few side effects and both are likely protective against vascular disease, one of the major contributors to age-related dementia. Collectively, these data suggest that fish oil and more specifically DHA, may be clinically useful for AD treatment or prevention and, like the amyloid vaccine, profen NSAIDs, and curcumin may reduce amyloid by influencing more than one target (FIG. 3).

COMBINED ANTIOXIDANT NSAID AND CURCUMIN TARGETS Aβ PATHOGENESIS AT MULTIPLE SITES

If oxidative damage and damage to neuroprotective molecules like DHA are relevant to AD pathogenesis, antioxidants would be expected to provide some protection. In our hands, neither ibuprofen nor vitamin E supplements were effective in controlling protein carbonyls as an index of oxidative damage, but other groups have some evidence for protection with these agents. In contrast, the unconventional NSAID–antioxidant curcumin combination was remarkably effective—lowering oxidized proteins, inflammatory cytokines, activated microglial markers iNOS and CD11b, cognitive deficits, postsynaptic marker loss, and amyloid accumulation.[83,84]

As shown in FIGURE 4, curcumin has the potential to suppress the AD pathogenic cascade at multiple sites. Curcumin is not only a potent antioxidant,[85] but an effective inhibitor of the inflammatory cytokines COX-2 and iNOS, via inhibition of JNK kinase–mediated AP-1 transcription.[86] As reviewed above, cytokines, JNK, COX-2, and iNOS are all implicated in Aβ toxicity or Aβ-induced AD pathogenesis. Curcumin can block JNK *in vitro* and may also limit inflammation and oxidative damage induction of BACE1[79] or γ-secretase activity.[87] Curcumin proved to be immunomodulatory, simultaneously inhibiting cytokine and microglial activation indices related to neurotoxicity, but increasing mRNA and immunostaining for several markers of phagocytic microglia (Morihara, unpublished data). As previously reviewed, curcumin resembles the amyloid binding dye Congo Red, binds and labels

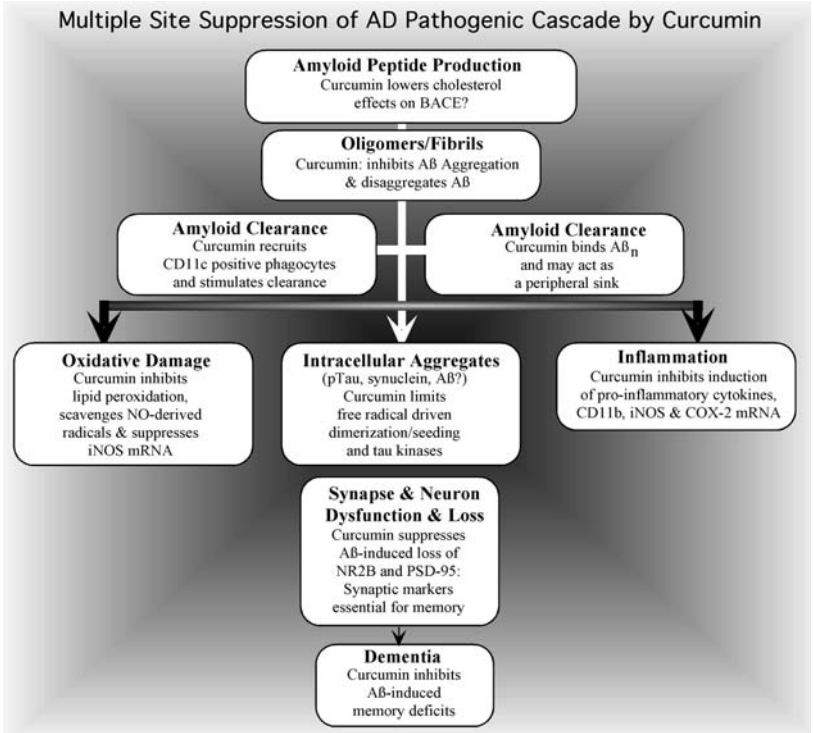

FIGURE 4. Multiple site suppression of the AD pathogenic cascade by curcumin. Curcumin's cholesterol-lowering ability and JNK inhibition may reduce Aβ production and inhibit its toxicity. Like Aβ antibodies, curcumin directly inhibits Aβ aggregate formation and promotes aggregate dissolution. The compound can also bind to $A\beta_n$ and promote clearance by microglia or possibly as a peripheral sink. Unlike antibodies, curcumin directly suppresses lipid peroxidation and COX-2, CD11b, iNOS, and pro-inflammatory cytokine expression. By limiting oxidative damage directly or from inflammation, curcumin should block intracellular protein aggregate seeding at the initial dimerization step. In our models, this multi-step interference in AD pathogenic mechanisms protects against synaptic marker loss and memory deficits.

plaques *in vitro* and *in vivo*, and inhibits amyloid aggregation *in vitro* and *in vivo*.[84] More recent data show that curcumin also inhibits oligomer formation and oligomer-dependent Aβ toxicity *in vitro* (Yang and colleagues, submitted for publication). Whether part of curcumin's anti-amyloid activity *in vivo* is due to its anti-aggregation activity is difficult to establish since the compound has multiple anti-amyloid actions including antioxidant and cholesterol lowering activity[83,84] and possible suppression of BACE1 induction. Curcumin is an amyloid-binding compound whose peripheral blood and tissue levels are higher in the gastrointestinal tract than in the brain.[88] Thus, it is conceivable that, like Congo Red, gelsolin, and anti-Aβ antibodies, curcumin may also act as a "peripheral sink." Because curcumin effectively inhibits iNOS expression *in vitro,*[89,90] *in vivo*,[91,92] and in the CNS (our data), it

should be able to suppress key aspects of the Aβ response that are iNOS-dependent, including microglial neurotoxin production[26] and LTP inhibition.[30]

CONCLUDING REMARKS

Based on its strong anti-carcinogenic activity, curcumin has undergone extensive preclinical toxicology and clinical testing and has a very favorable safety profile.[86,93] Based on its potential for intervention at multiple targets in AD pathogenesis, its ready availability and inexpensive cost, clinical trials in mild to moderate AD patients under an FDA-approved Investigational New Drug (IND) study have been initiated by our colleagues at the UCLA Alzheimer Center (Drs. J. Cummings and J. Ringman). Clinical trials are the final judge and jury for any drug, but the case for a successful intervention based on the epidemiology of risk reduction will inevitably be better for prevention Epidemiology tells us who got the disease, not what affects the rate of progression or efficacy in any given symptomatic stage. The evidence from epidemiology and preclinical trials argues strongly for a combined and synergistic role of inflammation and oxidative damage in driving AD pathogenesis at early stages that should be addressed with combined antioxidant and NSAID treatments.

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