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Possible Mechanisms of Action of NSAIDs and Related Compounds that Modulate γ -Secretase Cleavage

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Abstract

Genetic and biochemical evidence continues to implicate the production and accumulation of the A β 42 peptide as the causative factor in Alzheimer's disease (AD). Thus, a variety of strategies have been developed to decrease the production and/or aggregation of this peptide, which may be clinically useful for the treatment of this devastating disorder. Recently, the discovery that some non-steroidal anti-inflammatory drugs (NSAIDs) appear to selectively decrease the production of A β 42 has opened a novel therapeutic avenue for AD treatment that may circumvent potential toxicity associated with long-term global inhibition of γ -secretase activity. One drug from this class of compounds, R-flurbiprofen, has advanced to phase 3 clinical trials and may soon provide insight into the viability of this strategy for the prevention or treatment of AD. Delineating the target and mechanism of these compounds is essential for developing new agents with increased potency and optimized pharmacologic properties. The evidence indicating that these chemicals modulate the production of A β peptides by directly interacting with the γ -secretase complex is summarized.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and is estimated to currently affect 4.5 million Americans [1]. This number is projected to grow to 16 million by 2050 unless new effective therapies are developed. Understanding the factors responsible for AD is critical for the development of effective preventive and therapeutic strategies for this debilitating neuro-degenerative disease. Clinically, AD is characterized by a progressive decline in memory and in other cognitive functions, often accompanied by behavioral changes [2]. Neuropathologically, AD is diagnosed by the presence of senile plaques in the brain parenchyma and by neurofibrillary tangles inside neurons. Plaques are predominantly composed of fibrillar aggregates of the amyloid beta (A β) peptide, while tangles contain abnormally phosphorylated tau. A long-standing question in the AD field is whether or not A β and/or tau proteins are markers of disease progression or if they are causally related to pathogenesis of the disease.

A β 42 AS A THERAPEUTIC TARGET IN AD

A great deal of evidence supports the hypothesis that the overproduction and accumulation of the A β peptide in the brain is responsible for the development of AD [3]. A β is a secreted peptide of normal cellular metabolism produced through the sequential cleavage of the β -

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CONFLICT OF INTEREST

T.E.G. is a co-inventor on a patent with claims pertaining to the use of A β 42 lowering NSAIDs and NSAID derivatives in Alzheimer disease.

Amyloid Precursor Protein (APP) by β and γ -secretases [4]. A β peptides have a heterogeneous carboxyl-terminus with the majority (~60–90%) composed of 40 amino acids (A β 40), while a minor product (~5–20%) contains a two amino acid extension (A β 42). Additional minor A β peptides are also normally produced (e.g., A β 1–34, 1–37, 1–38, 1–39). A β peptides have been shown to be prone to aggregation, amyloid fibril formation and are the primary constituent of the senile plaques that are a post-mortem diagnostic feature in the brains of patients with AD [5]. In particular, it has been demonstrated that A β 42 is much more prone to aggregate into amyloid and is more toxic in cultured cell and animal models than A β 40. Additionally, A β 42 is deposited earlier and more consistently than A β 40 in the brain parenchyma of individuals affected by AD [6]. Genetic studies of familial forms of AD (FAD), animal modeling of APP and presenilin mutants, as well as biophysical and toxicology studies have all established a pathogenic role in AD for A β 42. Mutations which cause AD that are found in the genes encoding presenilin (PS1, encoded by PSEN1), presenilin 2 (PS2, encoded by PSEN2) and APP, alter A β peptide levels or in rare cases directly alter the A β sequence in a way that increases the propensity of the mutant A β to form fibrils. The vast majority of these mutations linked to AD selectively increase the relative levels of A β 42 peptides compared to control levels (reviewed in [7]). Furthermore, it has been recently discovered that duplications in the APP locus can lead to autosomal dominant early-onset AD [8].

Small shifts in A β 42 production have a tremendous impact on the development of AD. In humans, AD-causing mutations elevate plasma levels of A β 42 by ~30–100% [9]. Even mutations showing small increases in A β 42 levels are associated with the onset of dementia in the fourth or fifth decade of life. In transgenic mice, these same mutations produce small increases in A β 42 levels and markedly accelerate A β deposition [10]. More recent studies in transgenic mice and *Drosophila* selectively expressing A β 40 and A β 42 in the secretory pathway show that A β 42 but not A β 40 is sufficient to drive A β deposition, and, at least in *Drosophila*, neurodegeneration [11,12]. Thus, it has been suggested that an ideal therapeutic approach for the treatment or prevention of AD may be the selective targeting of A β 42 production.

SOME NSAIDS SELECTIVELY LOWER A β 42 PRODUCTION

Numerous epidemiologic studies have demonstrated an association of long-term (> 2 year) non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) with a decreased risk of developing AD. To date, such epidemiologic studies have not been confirmed by appropriately controlled, prospective clinical studies. Nevertheless, given the strength of the epidemiologic data, there is currently a great deal of research focused on understanding how NSAIDs might confer protection from AD [13]. Until recently, the dominate hypothesis was that NSAIDs protect from AD due to cyclooxygenase (COX) mediated inhibition of the extensive inflammatory process prevalent in the AD brain [14], [15]. This led to a number of clinical trials, most recently the AD Anti-inflammatory Prevention Trial (ADAPT), which was halted prior to producing any meaningful clinical data due to safety concerns over an increased risk of cardiovascular complications [13,16].

An additional explanation for the protective effect of NSAIDs was identified when it was discovered that certain NSAIDs lower the secretion of A β 42 *in vitro* and *in vivo* [17,18]. The initial report identified three commonly used NSAIDs- ibuprofen, indomethacin, and sulindac sulfide- as prototypical compounds that displayed this property. Using cultured cells over-expressing APP and peptide-specific sandwich ELISAs it was demonstrated that some NSAIDs could selectively decrease A β 42 levels, with varying potency, while maintaining A β 40 production (Fig. 1A). Two of the most widely utilized NSAIDs, aspirin and naproxen, do not exhibit this activity, suggesting that this effect is not mediated by inhibition of the COX enzymes, the primary target of this family of drugs (Fig. 1B). Further support for this hypothesis

was provided by the observation that sulindac sulfide was still able to decrease the production of A β 42 in fibroblasts deficient in both COX-1 and COX-2 enzymes. Additional experiments also demonstrated that NSAID treatment did not affect APP or A β metabolism [18].

The apparent selective reduction in one species of A β peptides by some NSAIDs suggested a mechanism distinct from well known γ -secretase inhibitors that typically target all A β species. Immunoprecipitation followed by mass-spectrometry or bicine-urea electrophoresis was used to provide a more global view of changes in A β peptide production. Interestingly, these complementary techniques both showed that, in cell culture, sulindac sulfide mediated A β 42 reductions were accompanied by increases in the production of A β 38, which is typically a minor species. These results suggested that A β 42-lowering NSAIDs were not acting as classic protease inhibitors and may be better characterized as γ -secretase cleavage modulators. The identification of chemicals that could selectively decrease A β 42 generated excitement that new compounds could be identified that target the putative toxic species and thereby modify disease progression without globally inhibiting COX or γ -secretase activity, reducing potential side-effects.

NSAIDs ARE ACTIVE *IN VIVO*

The initial study describing the A β 42 lowering effect of certain NSAIDs only examined a small group of this diverse class of compounds. Eriksen et al. expanded on these initial studies and examined the effect of 20 commonly used NSAIDs on A β levels in cell culture and the Tg2576 transgenic AD mouse model [19], [20]. Of the NSAIDs examined, only a few selectively lowered A β 42, including diclofenac, fenpropfen, meclofenamate and flurbiprofen (Fig. 2). Importantly, both the S- and R-enantiomers of flurbiprofen were active with similar potencies. Since R-flurbiprofen has greatly reduced COX-inhibitory activity this compound provides proof of principle that drugs lacking COX activity but retaining A β modulating ability can be identified. Similar results have been published showing that the R-enantiomer of ibuprofen also reduces A β 42 in cell culture [21].

Acute, three-day administration of these compounds to Tg2576 mice demonstrated that they were able to decrease A β 42 levels *in vivo* following short-term dosing [17]. However, an apparent inconsistency between drug concentrations seen in the CNS of mice (1–2 μ M) and concentrations that were effective in cell culture (25–200 μ M) was observed. A few possible explanations were advanced including 1) specific partitioning and accumulation of the drugs with the target 2) differences between the γ -secretase and/or other target that is modulated in these two systems and 3) a higher potency active metabolite. The reason for this discordance between *in vitro* and *in vivo* potencies is still unknown.

The ability of other researchers to replicate this finding has been mixed. One study by a group at Pfizer attempted to replicate the short-term, acute dosing paradigm with flurbiprofen but did not detect robust reductions in A β 42 levels in the same mouse model [22]. One possibility for this discrepancy is that this study used both different extraction conditions and ELISA systems to measure A β . Reliable detection of A β 42 changes in complex systems such as animal tissue is sensitive to assay conditions, ELISA strategies, and extraction buffer [22,23]. In particular, some denaturing extraction conditions for Tg2576 brain samples such as the guanidinium buffer used in the Pfizer study have been observed to mask reductions in A β 42 levels by some NSAIDs and γ -secretase inhibitors (unpublished observation, T.K. and [22]). The reason for this is unclear but potentially could be explained by assay interference, high background due to APP-CTFs, or exposure of distinct pools of A β that are resistant to modulation or inhibition [24]. Another study reported that acute 7 day treatment of 10-month-old APPV717I mice with ibuprofen (375 ppm; ~62.5 mg/kg/day) in food reduced soluble A β levels; however this effect did not reach statistical significance [25].

More recent work with synthetic analogues of flurbiprofen have further documented short-term A β reductions *in vivo*. Three-day dosing of ibuprofen and a dimethyl derivative of flurbiprofen (21 mg/kg/day) reduced soluble A β 42 levels ~20% [26]. Further work from another group reported that oral administration of R-flurbiprofen and three novel derivatives over a 7 day period (12.5 mg/day twice a day) reduced plasma levels of A β 42 with varying efficacy (20–40%), while A β levels in the brain were not significantly affected [27]. Long term studies with NSAIDs (ibuprofen, indomethacin, flurbiprofen) or derivatives capable of lowering A β 42 more consistently show reductions in A β loads, whereas non-A β 42 lowering NSAIDs (celecoxib, nimeluside) typically show no effect on A β deposition [28–32].

OTHER KNOWN NSAID TARGETS DO NOT REDUCE A β 42 PRODUCTION

Although the primary mode of action of NSAIDs is mediated by their inhibition of COX enzymes, these drugs are notoriously promiscuous and can influence a number of other pharmacologically relevant pathways [33]. In an effort to further characterize the A β 42 modulating mechanism, a study by Sagi et al. investigated the influence of other known targets of NSAIDs on A β secretion [34]. The general anti-inflammatory activity of these compounds did not appear to be a key requirement since other known anti-inflammatory drugs such as glucocorticoids, curcumin, and histamine receptor antagonists did not replicate the effect seen with some NSAIDs. Furthermore, other pathways influenced by NSAIDs- including arachidonic acid metabolism, lipoygenase enzymes, peroxisome proliferator-activated receptors as well as nuclear factor kappaB- did not appear to selectively influence the release of A β 42 from cultured cells. These results do not exclude that these or other pathways of NSAIDs may have a positive impact on AD pathology. However, they did suggest that this specific effect of NSAIDs on modulation of A β 42 production is most likely mediated through a novel target.

NSAIDs SELECTIVELY MODULATE APP CLEAVAGE

An important question to address is whether or not NSAIDs can interfere with the processing of other γ -secretase substrates, as is seen with many γ -secretase inhibitors. The initial report describing the ability of some NSAIDs to lower A β 42 production also examined the impact of these drugs on the cleavage of Notch, an important substrate of γ -secretase [18]. Activity was assayed in HEK293 cells by monitoring the intramembrane cleavage of an artificial Notch construct to produce the Notch intracellular cytoplasmic domain (NICD), which is essential for the signaling activity of the Notch pathway. In this assay system, treatment with doses that significantly decreased A β 42 did not inhibit Notch cleavage. These results suggest that these compounds exhibited a preference, at least at the doses tested, for modulation of APP processing. These observations were followed up by examining the release of these fragments in more detail for both APP and an additional γ -secretase substrate, ErbB-4 [35]. Treatment of cells with either sulindac sulfide or ibuprofen had no detectable effect on the production of the soluble intracellular cytoplasmic domain of both substrates. Assays using higher doses (0–400 μ M) and membrane fractions from cells overexpressing APP showed that NSAIDs did not inhibit release of the APP intracellular domain (AICD).

These results were in contrast to work by Takahashi et al. that reported stimulation of AICD and NICD production at lower doses and inhibition at the highest doses tested [36]. A potential explanation for this difference may be the use of recombinant substrates in the Takahashi study as opposed to cell membranes that contain properly processed APP-CTFs. Using a more sensitive cell-based heterologous reporter system, Weggen and colleagues were also not able to detect any inhibition of AICD-mediated signaling by sulindac sulfide or ibuprofen, providing further evidence that CTF cleavage was not compromised [35]. Collectively, these experiments support a novel action of NSAIDs on γ -secretase activity, which is specific to APP processing

and does not involve inhibition of the ϵ -cleavage activity that is required for the physiological roles of γ -secretase substrates. Additionally, a recent report also identified compounds that target a putative nucleotide-binding site on γ -secretase that inhibits APP processing over Notch, providing additional evidence that specific substrates of γ -secretase can be targeted without globally inhibiting γ -secretase activity [37].

NSAIDs APPEAR TO TARGET THE γ -SECRETASE COMPLEX DIRECTLY

A number of lines of evidence indicate that NSAIDs are working directly through the γ -secretase complex. This complex consists of cleavable substrate (i.e. APP-CTF), either presenilin 1 or presenilin-2 (which have been proposed to contain the active site) and three essential accessory proteins: nicastrin, APh-1 and PEN-2 [38],[39]. First, NSAIDs retain their ability to reduce A β 42 in cell-free *in vitro* γ -secretase assays [17,40]. This is similar to known γ -secretase inhibitors, which have been shown to bind presenilin, providing evidence that presenilins are the active site of γ -secretase [41]. NSAIDs displayed IC₅₀ values in both membrane fractionated and detergent solubilized γ -secretase assays that were similar to those observed in cell based assays [35].

Additional evidence for this hypothesis comes from studies looking at mutations in presenilin or APP known to cause FAD [35]. Overexpression of the PS1-M146L mutation in CHO cells expressing wild-type (wt) APP enhanced the A β 42 reduction mediated by sulindac sulfide in this cell line by almost 20%. Interestingly, another FAD associated mutation, PS1- Δ Exon9, had the opposite effect and attenuated the reduction in A β 42 after NSAID treatment. Mutations in APP also had variable effects. The “Swedish” mutation, which enhances β -secretase cleavage to generate more APP CTF-99 fragments, did not change the response to NSAID treatment. However, the “Indiana” mutation, APPV717F, caused a significant increase in the degree of A β 42 reduction induced by sulindac sulfide. Based on these findings it was concluded that NSAIDs lower A β 42 by a direct, possibly allosteric, modulation of γ -secretase activity or the substrate for enzymatic activity (APP) and that conclusive proof would have to await the development of NSAID photoaffinity reagents [35].

A recent study utilizing an assay based on fluorescence resonance energy transfer (FRET) called fluorescence lifetime imaging (FLIM) provided additional support for an allosteric mechanism of action by showing that A β 42- lowering NSAIDs affected the proximity of APP and PS1 and altered PS1 conformation [42]. In this technique, when two fluorophores are in close proximity (less than 10nm), the lifetime of the donor is shortened proportionally to the distance that separates the two fluorophores. Using FLIM, it was shown that the lifetime between donors on the PS1 loop region and the APP C-terminus was increased by compounds known to decrease A β 42 (ibuprofen and flurbiprofen), while this effect was not seen with aspirin, which does not lower A β 42 levels. This was interpreted to suggest that active NSAIDs changed the conformation in such a way to increase the distance between these two epitopes. Additional FLIM studies with these compounds demonstrated an increase in fluorescence lifetime when studying FRET between the presenilin C and N termini. Furthermore, treatment with the γ -secretase inhibitors DAPT or WPE-III-31C did not change fluorescence lifetime, providing additional evidence that the effect of NSAIDs on γ -secretase is distinct from global γ -secretase inhibitors. FLIM studies on sections of brain tissue from Tg2576 that had received 15 days of S-ibuprofen diet (375 p.p.m.) replicated the results that were seen with PS C and N termini in cell culture, suggesting that the conformation of PS is also altered *in vivo*.

Studies investigating the enzyme kinetics of γ -secretase activity also demonstrate that R-flurbiprofen and sulindac sulfide display selective non-competitive inhibition of A β 42 production at physiological levels where modulation of A β is observed [43]. At high doses (mM range), evidence for global inhibition of γ -secretase was observed. Furthermore,

radioligand binding experiments showed that these compounds act as non-competitive antagonists of γ -secretase inhibitors, suggesting an allosteric mechanism. Based on this evidence it was concluded that NSAIDs that inhibit production of A β 42 do so by altering the conformation of the γ -secretase complex by binding at a site that is distinct from γ -secretase inhibitors, which are transition state analogs that target the active site [43].

Further support for this hypothesis was reported recently in a study showing that γ -secretase cleavage of Notch also releases an A β -like peptide (N β) [44]. Two main species of N β were identified, N β 21 and N β 25, which were produced in cell culture at a ratio of ~5:1. Similar to their effects on A β , FAD mutations in PS were shown to increase the longer form of N β . Additionally, some NSAIDs were shown to modulate the levels of A β and N β in cells overexpressing APP and a constitutively cleaved Notch construct [44]. The similar effect of NSAIDs on A β and N β production provides further support that these compounds are acting through a component of the γ -secretase complex.

IDENTIFICATION OF COMPOUNDS THAT RAISE A β 42

Most of the NSAIDs described previously either lowered A β 42 or had no effect on A β production. We have recently published our efforts to identify other agents with reduced COX activity, structural diversity and increased potency that lower A β 42 by screening hundreds of compounds including natural products, NSAIDs, NSAID derivatives, and structurally related compounds [40]. This assay system analyzed the effect of these compounds on secreted A β from human neuroglioma (H4) cells overexpressing APP using sandwich ELISAs that were selective for A β 42, A β 40 and A β 38. Interestingly, the majority of molecules that we identified had the opposite effect of the original NSAIDs and actually selectively increased the production of A β 42, much like FAD mutations in PS and APP.

One of the more potent A β 42 elevating compounds, fenofibrate, is not an NSAID but a peroxisome proliferator-activated receptor alpha (PPAR α) agonist that is used as a lipid-regulating agent in humans (Fig. 3). We also identified a novel compound from an in-house library, FT1, that has some structural similarity to A β 42-lowering agents. Another A β 42-raising agent identified in the screen was the COX-2-selective NSAID celecoxib, which was being tested alongside naproxen in the ADAPT trial [45]. A more extended examination of a series of COX-2-selective NSAIDs showed that some COX-2 inhibitors raise A β 42 with varying potency (tilmacoxib, S247, L745337, and valdecoxib), whereas rofecoxib, etoricoxib, and lumiracoxib showed only small nonselective increases in A β levels at higher doses. Furthermore, no COX-2 inhibitors were identified that could lower A β 42 selectively. The general characteristics of these compounds are similar to previously described NSAIDs; they typically display an inverse relationship in modulating A β levels (in this case increasing A β 42 while lowering shorter fragments), and they appear to directly target γ -secretase and can increase A β levels in animal models [40].

The identification of COX-2 inhibitors that increase A β 42 levels raises a number of questions regarding the use of these compounds for the treatment of AD [46]. A 1-year double-blind clinical trial designed to examine the clinical efficacy of celecoxib (200 mg twice daily) in AD versus placebo detected no differences in cognitive decline between the two groups [47]. Another placebo-controlled study compared rofecoxib and naproxen in mild to moderate AD patients and found no statistical difference in cognitive decline between groups after 1 year, although there was a trend towards increased decline with the rofecoxib group [48]. A more recent 4-year randomized double blind study of 1,457 patients with mild cognitive impairment (MCI) found no evidence that rofecoxib slowed the conversion rate to AD [49]. The annual rate of conversion from MCI to AD was slightly higher in the rofecoxib group compared to control group (6.4% versus 4.5%, $p = 0.011$), although there was no evidence of differences

in cognition or global function between the treatment groups. The association between NSAIDs that raise A β 42 (celecoxib) or have no effect (rofecoxib, naproxen) and their lack of efficacy in clinical trials treating AD or MCI is intriguing but should be interpreted cautiously. Further studies are needed to fully understand if the long-term consumption of NSAIDs can provide protection from, increase the risk of or have no effect on the development of AD.

Interesting insight into the structure-activity relationships of these compounds can be gained by considering the results of amide and ester derivatives of the A β 42-lowering agent indomethacin. Conversion of the carboxylic acid into an amide, such as LM4114, converts the parent compound into a preferential COX-2 inhibitor (Fig. 4) [40,50]. However, screening a library of similar indomethacin derivatives revealed that none of these modifications produced compounds that lowered A β 42 and the majority selectively increased A β 42 and lowered A β 38. Methyl and *tert*-butyl esters of flurbiprofen (compound 25 and 26) derivatives have been reported recently to also raise A β 42 in culture, suggesting that the carboxylate is important for retaining A β 42 lowering activity (Fig. 5) [27]. However, this study also demonstrated that a tetrazole and other isosteres of carboxylic acids could replace this functionality and decrease A β 42 secretion, although the potency was significantly reduced compared to the parent compounds.

Similar increases in A β 42 secretion from cells and in the plasma of Tg2576 have recently been reported with nimodipine, a Ca²⁺ channel antagonist [51]. No definitive explanation for this activity was identified, but characteristics of nimodipine's effects on A β are reminiscent of the NSAID-like A β 42 modulating compounds, suggesting they may have a common site of action. Structurally, nimodipine is derived from a 1,4-dihydropyridine core and contains two alkyl ester functional groups which are tantalizingly similar to modifications in the NSAIDs just mentioned that are associated with conversion from A β 42-lowering to -raising agents (Fig. 5). Clearly further research is needed to clarify this association and to identify the definitive mechanism. However, taken together these results suggest that a free carboxylic acid, or similar functionality, is necessary to retain the ability to selectively lower A β 42. The mechanistic reason for this structural requirement is still unclear and necessitates a much more detailed understanding of the target of this class of chemicals.

THE Rho PATHWAY DOES NOT APPEAR TO BE THE TARGET OF NSAIDS THAT SELECTIVELY LOWER A β 42

An alternative explanation for the activity of A β -lowering NSAIDs has also been published. Work by Zhou et al. hypothesized that the small GTPase RhoA and its effector, Rho-associated kinase (ROCK), were the target of A β 42-lowering NSAIDs [52]. This was supported by the demonstration that activation of Rho and ROCK lead to selective increases in A β 42. Inhibition of Rho and ROCK through biological and pharmacological means had the opposite effect. Administration of Y-27632, a ROCK inhibitor, to PDAPP transgenic mice also decreased brain levels of A β 42. An association was also demonstrated between the ability of NSAIDs to lower A β 42 levels and their inhibition of ROCK activation in cell culture, suggesting that the two effects were related.

A subsequent study was published to further examine the effect of the Rho pathway on APP processing and A β 42 levels [53]. Two commercially available ROCK inhibitors, Y-27632 and HA-1077, were shown to dose-dependently reduce total A β secretion, but no selective effect on A β 42 was seen via ELISA or IP/MS. Additionally, these compounds were not active in a γ -secretase *in vitro* assay in contrast to sulindac sulfide, suggesting a different mode of action. Furthermore, ROCK inhibitors did not increase A β 38 levels concomitantly with reductions in A β 42 levels as is seen with NSAIDs that lower A β 42. Overexpression of wild-type, dominant negative, or constitutively active ROCK constructs had no effect on secreted A β levels.

Although different constructs were used, it is not immediately clear why such dissimilar effects were observed via modulation of ROCK activity compared to the original report. Interestingly, it has been reported that the isoprenoid geranylgeranyl diphosphate (GGPP), which activates Rho other small GTPases, also selectively increases A β 42 levels in cells and *in vitro* assays, suggesting it also directly targets γ -secretase like NSAIDs [40]. The effect of GGPP was not associated with GTPase activation, and, moreover, A β 42-raising compounds celecoxib and fenofibrate were shown not to activate RhoA. It is still unknown how ROCK inhibitors, which showed no activity in γ -secretase assays, function to lower total A β levels in cells. Since doses above the reported IC₅₀ values of these drugs for ROCK were required to see A β reduction and kinase inhibitors are notoriously unselective, perhaps these drugs are targeting an alternative kinase or pathway. Taken together these results seem to rule out a link between the ability of NSAIDs to selectively lower A β 42 production and the Rho-ROCK pathway.

CONCLUSION

Epidemiologic studies have provided an intriguing association between the long-term use of NSAIDs and a reduced risk of developing AD. Although these findings are provocative, they have not been replicated in prospective clinical trials. There are a number of theoretical mechanisms whereby NSAIDs could provide protection from the development of AD or even other neurodegenerative disorders [33]. A number of these pathways have shown positive effects on AD pathology in various models including effects on A β aggregation [54,55], nuclear factor-kappa B levels [29], COX [56,57], α 1 antichymotrypsin [58], and PPAR γ activation [15,59]. Clearly, further studies are needed to dissect the contribution of these various effects on AD pathology and their relative contribution to potential protection from AD.

The observation that a subset of some NSAIDs can selectively lower the production of A β 42 has led to the identification of a new potential mechanism for the protective actions of NSAIDs and has opened a new therapeutic strategy that targets the putative toxic species in AD. Since the initial discovery of this novel activity of certain NSAIDs, considerable progress has been made in deciphering the molecular mechanism of this effect. These compounds do not appear to function as classical protease inhibitors and instead appear to modulate the cleavage preference of γ -secretase; most likely, they target the enzyme directly, possibly through allosteric modulation. Two general types of modulators have been identified, A β 42 lowering and raising compounds. Evidence indicates that they share the same target but induce opposite effects on cleavage specificity. Crosslinking studies using photoaffinity versions of A β -modulating compounds are one strategy that should be able to provide further insight into their mechanism by providing information about their binding site in the γ -secretase complex or perhaps some other unknown target or regulatory factor [41].

One member of this class of compounds, R-flurbiprofen (MPC-7869; Flurizan, Myriad Pharmaceuticals, Inc.), has advanced to Phase 3 clinical trials for the treatment of mild AD. Initial results released from a 1 year randomized, placebo-controlled, double-blind Phase 2 trial in 207 subjects with mild to moderate AD (MMSE 15–26) with MPC-7869 demonstrated that it was well tolerated, even up to 800 mg/kg/day [60]. Mild AD (MMSE of 20–26) subjects that received the highest dose of MPC-7869 showed statistically significant benefit in measures of activities of daily living (ADCS-ADL; $P=0.033$) and global function (CDR-sb; $P=0.042$), with positive trends in cognition (ADAS-cog). No benefit was observed in moderate AD patients or those on the 400 mg/kg/day dose. The ongoing phase 3 clinical trial should provide critical information about the therapeutic utility of selectively targeting A β 42 production using modulators of γ -secretase activity.

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References

1. Szekely CA, Thorne JE, Zandi PP, Ek M, Messias E, Breitner JCS, Goodman SN. *Neuroepidemiology* 2004;23:159–169. [PubMed: 15279021]
2. Desai AK, Grossberg GT. *Neurology* 2005;64:S34–39. [PubMed: 15994222]
3. Hardy J, Selkoe DJ. *Science* 2002;297:353–356. [PubMed: 12130773]
4. Reinhard C, Hebert SS, De Strooper B. *Embo J* 2005;24:3996–4006. [PubMed: 16252002]
5. Golde TE, Eckman CB, Younkin SG. *Biochim Biophys Acta* 2000;1502:172–87. [PubMed: 10899442]
6. Mann DM, Iwatsubo T, Ihara Y, Cairns NJ, Lantos PL, Bogdanovic N, Lannfelt L, Winblad B, Maat-Schieman ML, Rossor MN. *Am J Pathol* 1996;148:1257–66. [PubMed: 8644866]
7. Selkoe DJ, Podlisny MB. *Annual Review of Genomics and Human Genetics* 2002;3:67–99.
8. Rovelet-Lecrux A, Hannequin D, Raux G, Meur NL, Laquerriere A, Vital A, Dumanchin C, Feuillette S, Brice A, Vercelletto M, Dubas F, Frebourg T, Campion D. *Nat Genet* 2006;38:24–26. [PubMed: 16369530]
9. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad L, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. *Nat Med* 1996;2:864–870. [PubMed: 8705854]
10. Duff K, Eckman C, Zehr C, Yu X, Prada CM, Pereztur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S. *Nature* 1996;383:710–713. [PubMed: 8878479]
11. McGowan E, Pickford F, Kim J, Onstead L, Eriksen J, Yu C, Skipper L, Murphy MP, Beard J, Das P. *Neuron* 2005;47:191–199. [PubMed: 16039562]
12. Iijima K, Liu HP, Chiang AS, Hearn SA, Konsolaki M, Zhong Y. *PNAS* 2004;101:6623–6628. [PubMed: 15069204]
13. McGeer PL, McGeer EG. *Neurobiology of Aging*. In Press, Corrected Proof
14. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T. *Neurobiol Aging* 2000;21:383–421. [PubMed: 10858586]
15. Sastre M, Klockgether T, Heneka MT. *International Journal of Developmental Neuroscience* 2006;24:167–176. [PubMed: 16472958]
16. van Gool WA, Aisen PS, Eikelenboom P. *Journal of Neurology* 2003;250:788–792. [PubMed: 12883918]
17. Eriksen JL, Sagi SA, Smith TE, Weggen S, Das P, McLendon DC, Ozols VV, Jessing KW, Zavitz KH, Koo EH, Golde TE. *J Clin Invest* 2003;112:440–449. [PubMed: 12897211]
18. Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, Findlay KA, Smith TE, Murphy MP, Bulter T, Kang DE, Marquez-Sterling N, Golde TE, Koo EH. 2001;414:212–216.
19. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. *Science* 1996;274:99–103. [PubMed: 8810256]
20. Janus C, Westaway D. *Physiology & Behavior* 2001;73:873–886. [PubMed: 11566220]
21. Morihara T, Chu T, Ubeda O, Beech W, Cole GM. *Journal of Neurochemistry* 2002;83:1009–1012. [PubMed: 12421374]
22. Lanz TA, Fici GJ, Merchant KM. *J Pharmacol Exp Ther* 2005;312:399–406. [PubMed: 15340006]
23. Lanz TA, Schachter JB. *Journal of Neuroscience Methods*. In Press, Corrected Proof
24. Best JD, Jay MT, Otu F, Ma J, Nadin A, Ellis S, Lewis HD, Pattison C, Reilly M, Harrison T, Shearman MS, Williamson TL, Atack JR. *J Pharmacol Exp Ther* 2005;313:902–908. [PubMed: 15743924]

25. Heneka MT, Sastre M, Dumitrescu-Ozimek L, Hanke A, Dewachter I, Kuiperi C, O'Banion K, Klockgether T, Van Leuven F, Landreth GE. *Brain* 2005;128:1442–1453. [PubMed: 15817521]
26. Stock N, Munoz B, Wrigley JDJ, Shearman MS, Beher D, Peachey J, Williamson TL, Bain G, Chen W, Jiang X. *Bioorganic & Medicinal Chemistry Letters* 2006;16:2219–2223. [PubMed: 16455248]
27. Peretto I, Radaelli S, Parini C, Zandi M, Raveglia LF, Dondio G, Fontanella L, Misiano P, Bigogno C, Rizzi A, Riccardi B, Biscailoli M, Marchetti S, Puccini P, Catinella S, Rondelli I, Cenacchi V, Bolzoni PT, Caruso P, Villetti G, Facchinetti F, DelGiudice E, Moretto N, Imbimbo BP. *J Med Chem* 2005;48:5705–5720. [PubMed: 16134939]
28. Lim GP, Yang F, Chu T, Chen P, Beech W, Teter B, Tran T, Ubeda O, Ashe KH, Frautschy SA, Cole GM. *J Neurosci* 2000;20:5709–5714. [PubMed: 10908610]
29. Sung S, Yang H, Uryu K, Lee EB, Zhao L, Shineman D, Trojanowski JQ, Lee VMY, Pratico D. *Am J Pathol* 2004;165:2197–2206. [PubMed: 15579461]
30. Jantzen PT, Connor KE, DiCarlo G, Wenk GL, Wallace JL, Rojiani AM, Coppola D, Morgan D, Gordon MN. *J Neurosci* 2002;22:2246–2254. [PubMed: 11896164]
31. Quinn J, Montine T, Morrow J, Woodward WR, Kulhanek D, Eckenstein F. *Journal of Neuroimmunology* 2003;137:32–41. [PubMed: 12667645]
32. Yan Q, Zhang J, Liu H, Babu-Khan S, Vassar R, Biere AL, Citron M, Landreth G. *J Neurosci* 2003;23:7504–7509. [PubMed: 12930788]
33. Townsend KP, Pratico D. *FASEB J* 2005;19:1592–1601. [PubMed: 16195368]
34. Sagi SA, Weggen S, Eriksen J, Golde TE, Koo EH. *J Biol Chem* 2003;278:31825–31830. [PubMed: 12805355]
35. Weggen S, Eriksen JL, Sagi SA, Pietrzik CU, Ozols V, Fauq A, Golde TE, Koo EH. *J Biol Chem* 2003;278:31831–31837. [PubMed: 12805356]
36. Takahashi Y, Hayashi I, Tominari Y, Rikimaru K, Morohashi Y, Kan T, Natsugari H, Fukuyama T, Tomita T, Iwatsubo T. *J Biol Chem* 2003;278:18664–18670. [PubMed: 12637581]
37. Fraering PC, Ye W, LaVoie MJ, Ostaszewski BL, Selkoe DJ, Wolfe MS. *J Biol Chem* 2005;M501368200.
38. Brunkan AL, Goate AM. *Journal of Neurochemistry* 2005;93:769–792. [PubMed: 15857382]
39. Wolfe MS. *Biochemistry* 2006;45:7931–9. [PubMed: 16800619]
40. Kukar T, Murphy MP, Eriksen JL, Sagi SA, Weggen S, Smith TE, Ladd T, Khan MA, Kache R, Beard J, Dodson M, Merit S, Ozols VV, Anastasiadis PZ, Das P, Fauq A, Koo EH, Golde TE. *Nat Med* 2005;11:545–50. [PubMed: 15834426]
41. Li YM, Xu M, Lai MT, Huang Q, Castro JL, DiMuzio-Mower J, Harrison T, Lellis C, Nadin A, Neduvilil JG, Register RB, Sardana MK, Shearman MS, Smith AL, Shi XP, Yin KC, Shafer JA, Gardell SJ. *Nature* 2000;405:689–94. [PubMed: 10864326]
42. Lleo A, Berezovska O, Herl L, Raju S, Deng A, Bacskai BJ, Frosch MP, Irizarry M, Hyman BT. 2004;10:1065–1066.
43. Beher D, Clarke EE, Wrigley JDJ, Martin ACL, Nadin A, Churcher I, Shearman MS. *J Biol Chem* 2004;279:43419–43426. [PubMed: 15304503]
44. Okochi M, Fukumori A, Jiang J, Itoh N, Kimura R, Steiner H, Haass C, Tagami S, Takeda M. *J Biol Chem* 2006;281:7890–7898. [PubMed: 16434391]
45. Martin BK, Meinert CL, Breitner JCS, The ARG. *Controlled Clinical Trials* 2002;23:93–99. [PubMed: 11852171]
46. Secko D. *CMAJ* 2005;172:1677. [PubMed: 15967966]
47. Sainati, SM.; Ingram, D.; Talwalker, S.; Geis, G. 6th International Stockholm-Springfield Symposium of Advances in Alzheimer's Therapy; Stockholm, Sweden. 2000. Abstract p. 180
48. Aisen PS, Schafer KA, Grundman M, Pfeiffer E, Sano M, Davis KL, Farlow MR, Jin S, Thomas RG, Thal LJ. *Jama* 2003;289:2819–26. [PubMed: 12783912]
49. Thal LJ, Ferris SH, Kirby L, Block GA, Lines CR, Yuen E, Assaid C, Nessly ML, Norman BA, Baranak CC, Reines SA. *Neuropsychopharmacology* 2005;30:1204–15. [PubMed: 15742005]
50. Kalgutkar AS, Crews BC, Rowlinson SW, Marnett AB, Kozak KR, Remmel RP, Marnett LJ. *PNAS* 2000;97:925–930. [PubMed: 10639181]

51. Facchinetti F, Fasolato C, Del Giudice E, Burgo A, Furegato S, Fusco M, Basso E, Seraglia R, D'Arrigo A, Leon A. *Neurobiology of Aging* 2006;27:218–227. [PubMed: 16399208]
52. Zhou Y, Su Y, Li B, Liu F, Ryder JW, Wu X, Gonzalez-DeWhitt PA, Gelfanova V, Hale JE, May PC, Paul SM, Ni B. *Science* 2003;302:1215–7. [PubMed: 14615541]
53. Leuchtenberger S, Kummer MP, Kukar T, Czirr E, Teusch N, Sagi SA, Berdeaux R, Pietrzik CU, Ladd TB, Golde TE, Koo EH, Weggen S. *J Neurochem*. 2005
54. Thomas T, Nadackal TG, Thomas K. *Neuroreport* 2001;12:3263–7. [PubMed: 11711868]
55. Hirohata M, Ono K, Naiki H, Yamada M. *Neuropharmacology* 2005;49:1088–1099. [PubMed: 16125740]
56. Pasinetti GM. *Neurosignals* 2002;11:293–7. [PubMed: 12566930]
57. Qin W, Ho L, Pompl PN, Peng Y, Zhao Z, Xiang Z, Robakis NK, Shioi J, Suh J, Pasinetti GM. *J Biol Chem* 2003;278:50970–7. [PubMed: 14507922]
58. Morihara T, Teter B, Yang F, Lim GP, Boudinot S, Boudinot FD, Frautschy SA, Cole GM. *Neuropsychopharmacology* 2005;30:1111–1120. [PubMed: 15688088]
59. Sastre M, Dewachter I, Rossner S, Bogdanovic N, Rosen E, Borghgraef P, Evert BO, Dumitrescu-Ozimek L, Thal DR, Landreth G, Walter J, Klockgether T, van Leuven F, Heneka MT. *PNAS* 2006;103:443–448. [PubMed: 16407166]
60. Black S, Wilcock GK, Hawworth J, Hendrix S, Zavitz K, Christensen DbM-H, Bass S, Laughlin M, Swabb E. *Neurology* 2006;66(Suppl2)

Fig. 1. Some NSAIDs lower the secretion Ab42 from cell culture (A) or have no effect (B).

Fig. 2.

Other NSAIDs that decrease A β 42 *in vitro* and *in vivo*. The R- enantiomer of flurbiprofen is currently being tested in Phase III clinical trials for Alzheimer's disease due to its ability to reduce A β 42 and greatly reduced inhibition of COX.

Fig. 3. Compounds identified that mimic Familial-Alzheimer's disease (FAD) mutations and elevate the production of A β 42.

Fig. 4.

Structural modification of idomethacin into a COX-2 selective inhibitor also creates a compound (LM4114) that now selectively raises A β 42.

Fig. 5.
Additional compounds identified that elevate A β 42.