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Review

Molecular targets of non-steroidal anti-inflammatory drugs in neurodegenerative diseases

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Abstract. During the last decade, interest has grown in the beneficial effects of non-steroidal anti-inflammatory drugs (NSAIDs) in neurodegeneration, particularly in pathologies such as Alzheimer's (AD) and Parkinson's (PD) disease. Evidence from epidemiological studies has indicated a decreased risk for AD and PD in patients with a history of chronic NSAID use. However, clinical trials with NSAIDs in AD patients have yielded conflicting results, suggesting that these drugs may be beneficial only when used as preventive therapy or in early stages of the disease. NSAIDs may also have salutary effects in other neurodegenerative diseases with an inflammatory component, such as multiple sclerosis and amyotrophic lateral sclerosis. In this review we analyze the molecular (cyclooxygenases, secretases, NF-κB, PPAR, or Rho-GTPasas) and cellular (neurons, microglia, astrocytes or endothelial cells) targets of NSAIDs that may mediate the therapeutic function of these drugs in neurodegeneration.

Keywords. NSAIDs, PPARγ, Alzheimer's disease, inflammation, neurodegeneration.

1. Introduction

The discovery in postmortem analyses that innate immunity in the brain is activated in neurodegenerative diseases, together with the widely adopted belief that inflammation is deleterious for organs with reduced capacity of regeneration like the brain, have prompted numerous studies to investigate the therapeutic effects of non-steroidal inflammatory drugs (NSAIDs) in Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Although NSAIDs appear to be protective, present evidence is incomplete and conflicting, raising the controversy as to whether the inflammatory cascade is beneficial, is just a bystander or can contribute to disease progression. The notion has to be dispelled that brain inflammation encompasses a stereotypical set of all-purpose reactions. Complexity arises concerning the inflammatory elements activated, the type of disease, the time in the disease development and also whether the response is acute or chronic. These issues are taken into consideration in this review in which we discuss the effects of NSAIDs *in vitro* and *in vivo*, and their molecular and cellular targets. We also review the agreement of results obtained in cell culture and animal models with

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clinical evidence. The section related to the beneficial effects of NSAIDs in AD is more extensive than that of other illnesses as a greater number of studies have been performed on this topic.

2. Neuroprotective effects in Alzheimer's disease

AD is an age-dependent neurodegenerative disorder characterized by progressive cognitive loss and functional impairment. In this illness the brain shows extracellular accumulation of aggregated amyloid-β peptide (Aβ) in amyloid plaques, as well as the formation inside neurons of neurofibrillary tangles that contain abnormally phosphorylated forms of the microtubule-associated protein tau. These lesions are accompanied by profound neuronal and synaptic loss in the limbic system and in the neocortex, even in the early stages of the disease. Besides these typical hallmarks, inflammatory changes in the brain are an early and prominent feature. Activated microglia and astrocytes are, respectively, detected inside and surrounding the plaque, and presence of over 40 inflammatory mediators has been reported. The finding in epidemiological studies that treatment with NSAIDs was associated with a reduced risk of developing AD was initially taken as proof that inflammation – mostly due to the oxidative stress derived from activated microglia – is a pathogenic factor in AD. However, this interpretation has been shaken by evidence showing that: (i) NSAIDs appear to target molecules other than those that promote inflammation; and (ii) some of the inflammatory reactions may be protective rather than deleterious. In the following sections we review the molecular, cellular and functional targets of NSAIDs, to understand how they relate to clinical evidence.

2.1. Molecular targets

2.1.1. Cyclooxygenases. The canonical molecular target of classical NSAIDs, like aspirin, ibuprofen, naproxen, diclofenac and sulindac, are cyclooxygenases 1 and 2 (COX-1 and COX-2). COX-1 is constitutively expressed in most tissues, including microglia both in normal brain and AD [1]. COX-2 is the main isoform expressed in inflammatory processes, but also in normal brain, where it is present in discrete neuronal populations mainly distributed in the cortex and hippocampus [2]. The presence of a TATA box and multitude of binding sites for transcription factors in the COX-2 promoter, and the observation that astrocytes do not express COX [1], suggests that enzyme expression and activity are tightly regulated [3]. The hypothesis that the beneficial effect of NSAIDs could be mediated through COX-2 inhibition was based on observations that neuronal COX-2 is up-regulated early in AD [1, 4], that COX-2 is expressed in normal brain in regions affected early in AD [5], and that COX-2-overexpressing transgenic mice show age-dependent cognitive deficits and neuronal apoptosis [6]. However, studies of COX-2 expression in brains from AD patients have yielded conflicting results, and both increased and unchanged levels have been reported [7, 8]. Other studies have measured COX activity in the hippocampus from AD patients and have found no differences compared to age-matched controls [9]. An increase in prostaglandin E2 levels in cerebrospinal fluid (CSF) in AD has been reported, and the levels decline with the progression of the disease [9, 10]. However, elevated prostaglandin E2 levels in AD may reflect the known microglial activation found in this disease, rather than an increase in COX activity [9]. On the other hand, clinical trials in AD using COX-2 inhibitors, such as celecoxib, rofecoxib and nimesulide, have resulted in a negative outcome [11–13]. In the same line, the administration of celecoxib or nimesulide to animal models of AD produced no change [14, 15] or even an increase in Aβ levels [16]. Indeed, *in vitro* studies have demonstrated that COX-2 inhibitors do not decrease Aβ generation [17, 18] but raise Aβ42 production [16]. In summary, although evidence involves COX-2 in the pathogenesis of AD, COX-2 inhibition does not seem responsible for the protective effect of NSAIDs in AD. Considering the cytotoxic potential of activated microglia and the emerging evidence in prospective clinical trials using COX-1 selective inhibitors, additional studies are needed to explore the role of this COX in AD.

2.1.2. γ-secretase. A significant twist on the issue of NSAIDs occurred 6 years ago when Weggen et al. [17] described that some of the commonly used NSAIDs were able to selectively lower Aβ42 in cell culture and acute animal model experiments. Interestingly, the effect was independent of COX activity, the classical target of NSAIDs, and a subset of non-selective NSAIDs (*i.e.*, ibuprofen, sulindac sulfide, indomethacin) showed Aβ-lowering effects [19]. The reduction in Aβ42 was accompanied by a parallel increase in Aβ38 [16, 17]. Since then, many studies have replicated the original findings [20–22], which have been extended to animal models of AD [14, 15, 20, 21, 23–25].

Like other propionate derivates (*e.g.*, naproxen, flurbiprofen) ibuprofen, contains a chiral carbon in the α-position that can yield to different enantiomers, S- and R-, with different biological effects. It is the S-

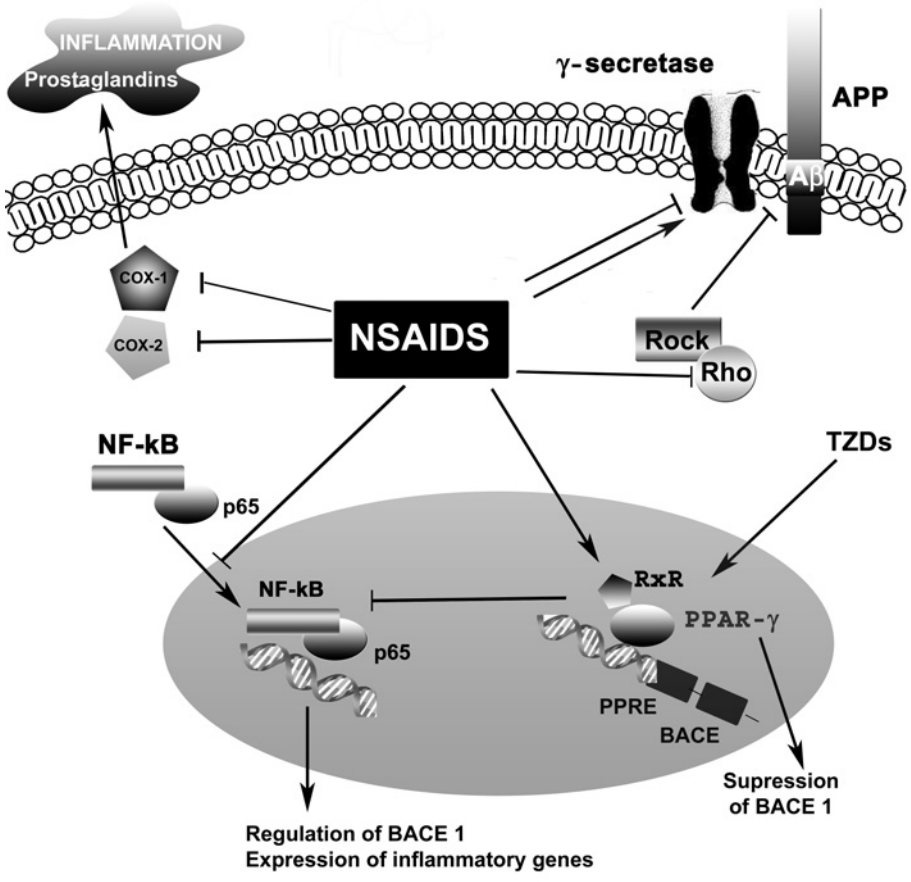

Figure 1. Molecular targets of non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease (AD). In addition to the classical cyclooxygenase (COX) inhibitory effect that leads to reduced levels of prostaglandins, some NSAIDs display other effects. S-Ibuprofen, indomethacin, flurbiprofen, sulindac sulfide and meclofen also are able to lower selectively amyloid-β42 (Aβ42) by modulating γ-secretase. Sulindac sulfide, indomethacin and ibuprofen also inhibit the small GTP-binding protein Rho and its associated kinase ROCK that leads to reduced Aβ40 and Aβ42. Ibuprofen, indomethacin and naproxen, fenoprofen and flufenamic acid are potent agonists of the peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear transcriptional regulator that is able to repress BACE1 gene expression. Finally, flurbiprofen and indomethacin are potent inhibitors of the nuclear translocation of NF-κB, which activates the transcription of many inflammatory genes.

form that inhibits COX, whereas both S- and R-enantiomers equally lower Aβ production in cell-free assays [17, 26]. Yet another unexpected finding about R-derivatives is that, despite the fact that they do not have COX-inhibiting activity, they have been shown to inhibit NF-κB translocation and to reduce expression of the COX-2 and many other proinflammatory genes in a NF-κB-dependent manner (Fig. 1) [27].

However, despite the number of studies, how NSAIDs alter the metabolism of the amyloid precursor protein (APP) is still debated. Some authors have suggested that NSAIDs may modulate amyloid-β (Aβ) generation by directly targeting the γ-secretase complex [21, 28]. γ-secretase is a multimember protein complex that contains presenilin 1 (PS1), whose gene is responsible for most cases of autosomal-dominant AD cases, as a key component [29, 30]. Mutation of either one of two conserved transmembrane aspartates (Asp257 or Asp385) in PS1 abolishes γ-secretase activity, suggesting that PS1 is an aspartyl protease whose catalytic center lies at the interface of two PS1 subunits [29, 31, 32]. At least three other members, nicastrin, aph-1 and pen-2, have been identified as a part of this complex [30, 33]. In addition to APP, γ-secretase cleaves more than 25 type I membrane proteins with a variety of biological functions including the Notch receptor [34].

The argument that NSAIDs target directly the γ-secretase complex is based on three lines of evidence. First, active NSAIDs appear to change the conformation of PS1 and lower Aβ in a cell-free assay of γ-secretase activity [17, 21]. Second, similar to APP processing, NSAIDs subtly shift the cleavage of the Notch receptor [35], and last, NSAIDs are also able to modulate the presenilin homologue signal peptide peptidase (SPP) [36]. Although direct evidence that NSAIDs bind to γ-secretase is still lacking, these results suggest that NSAIDs, at least at the doses that lower Aβ42 production, target PS1 at a conserved site between SPP and PS1. It is worth noting that NSAIDs do not impair the generation of the APP intracellular domain (AICD) at any doses, and alter Notch cleavage at a dose much higher than that which effectively blocks Aβ production [37]. This is clinically relevant because it suggests that there is a window for modulation in which a selective Aβ42-lowering effect can be observed. The question then arises as to whether NSAIDs, which have low brain penetration,

accumulate in brain at a concentration high enough to modulate γ-secretase. In support of this scenario, APP mice treated with a battery of NSAIDs at different doses showed drug plasma levels that were within the therapeutic range for humans, and that highly correlated with brain Aβ42 levels [20]. However, the fact that most *in vivo* and *in vitro* studies have used doses of NSAIDs above the tolerated doses in humans may question the role of γ-secretase as the sole mechanism by which NSAIDs lower Aβ and mediate the putative protective effect in AD. As discussed below, it is possible that targets other than γ-secretase contribute to the beneficial effects of NSAIDs (Fig. 1) or that the effects depend on systemic responses rather than actions inside the brain.

2.1.3. Peroxisome proliferator-activated receptor-γ. Peroxisome proliferator-activated receptor-γ (PPARγ) are ligand-activated transcription factors that belong to a nuclear receptor superfamily, and the two isoforms, *i.e.*, PPARγ1 [38] and PPARγ2 [39] are encoded by the same gene by alternative mRNA splicing. The endogenous ligand for PPARγ is 15-Deoxy-Δ12,14-protaglandin J2 (15dPGJ2)33. Potent synthetic antidiabetics such as the thiazolidinediones (TDZs) troziglitazone, rosiglitazone and pioglitazone are effective agonists for PPARγ [40, 41]. In addition, certain NSAIDs such as ibuprofen, indomethacin and naproxen are also PPARγ agonists [42].

Apart from playing a key regulatory role in the metabolism of lipids and glucids, PPARγ have been shown to inhibit the expression of a wide range of proinflammatory genes [43–45], thereby protecting neurons from cell death [46]. It is therefore not surprising that TZDs and 15dPGJ2 mimic the capacity of classical NSAIDs to inhibit the Aβ-stimulated secretion of proinflammatory products by microglia and monocytes, which are responsible for neurotoxicity and astrocyte activation [47]. Unexpectedly, PPARγ activation also appears to regulate APP processing, since the Aβ-lowering effects of NSAIDs are reversed by the use of PPARγ antagonists, and PPARγ agonists such as pioglitazone or the transfection of PPARγ cDNA display the same effects as ibuprofen [18]. The possibility that presenilins are a target of PPARs can be discarded because TZDs do not affect γ-secretase cleavage at position ε, as they do not inhibit generation of AICD or Notch intracellular domain [18, 28]. Rather, PPARs appear to regulate APP processing through the other secretase, BACE1. Unlike γ-secretase, BACE1 is up-regulated in neurons or reactive astrocytes in AD, and neuronal cultures upon exposure to pro-inflammatory cytokines [18]. Accordingly, PPARγ activation results in the decrease of BACE1 transcription, expression and activity [18]. Furthermore, active concentrations of NSAIDs found in human CSF are in the same order as concentrations of NSAIDs required to start the activation of PPARγ and to affect BACE1 transcription [48, 49]. Moreover, it was recently shown that BACE1 gene promoter contains a consensus binding site for PPARγ [50]. Reporter gene assays demonstrated that lack of endogenous PPARγ facilitates BACE1 promoter activity, indicating that PPARγ is possibly a repressor of BACE1 [50].

PPARγ levels are decreased in AD brains, which suggests that inflammatory events may decrease the PPARγ gene transcription. On the other hand, NSAIDs have been shown to increase the levels and transcription of PPARγ in adipocytes and neurons [50–52]. Taken together, this evidence points to a scenario where the pathological reduction of PPARγ may result in increased BACE1 activity and production of excess Aβ. NSAIDs may then be beneficial by restoring PPARγ expression thereby reducing the expression of BACE1 [50]. Other mechanisms to explain the protective effect of PPARγ agonists are regulation of Aβ clearance [53] or APP ubiquitination [54].

Animal studies using NSAIDs that typically activate PPARγ such as ibuprofen or indomethacin displayed different effects depending on the dose and the length of the treatment. In general, animals receiving long-term treatment with ibuprofen or indomethacin had a significant reduction in Aβ plaques and activated microglia, as well as a reduction in inflammatory markers [14, 15, 23–25, 55]. In the APP tg2576 model, the administration of TZDs such as pioglitazone was also able to reduce Aβ42 levels and the number of activated microglia and astrocytes. However, this occurred only when the drugs were given at high doses as lower doses showed only modest effects on plaque burden or microglia activation [24, 55].

It is worth noting that TZDs may have PPAR-independent actions. Pioglitazone reduces mitochondrial respiration in astrocytic cultures by directly acting at state III [56]. This causes increased glucose uptake and lactate production, suggesting activation of anaerobic metabolism to produce ATP to compensate for the impaired respiration [56]. It has recently been confirmed that pioglitazone has an effect on brain metabolism in mice [57]. This is of importance because, apart from reducing inflammation and Aβ production, TZDs may be beneficial by modulating brain metabolism, which can be compromised in AD. Other members of the PPAR family are the PPARα. However, their relevance to AD appears to be minor so far. Fenofibrate, an agonist of this receptor, increases Aβ42 levels only when incubated at high concentrations *in vitro* [16], whereas short-term dos-

ing of fenofibrate in Tg2576 at doses up to 100 mg/kg/day did not significantly alter Aβ42 levels [16].

2.1.4. NF-κB. Another target for some NSAIDs such as flurbiprofen and its enantiomers is NF-κB. Findings that link NF-κB to Aβ production are, first, that there is a consensus site for NF-κB in the BACE1 gene promoter [58]. In neurons exposed to soluble Aβ peptides and in TNFα-activated glial cells, mutations of the NF-κB site in the BACE1 promoter lead to significant decreases in promoter activity, indicating an activating role for NF-κB in BACE1 expression under inflammatory conditions [59]. Second, PPARγ agonists can antagonize the activity of transcription factors such as NF-κB [60], and the endogenous PPARγ ligand 15dPGJ2 can reduce NF-κB activity via inhibition of IκB kinase [61].

Of particular interest due to the clinical implications, Jantzen and colleagues [14] found that the 4-nitroxy butyl ester of flurbiprofen (NCX-2216) decreased Aβ load in APP/PS1 transgenic mice, suggesting that the presence of the NO group in this molecule was important for this lowering effect as well as for the decreased gastrointestinal side effects. The effects of another nitrate ester of flurbiprofen were tested by van Groen and Kadish [62] and they found that high doses of this drug reduced amyloid load by 43 %.

2.1.5. Rho-GTPases. Several lines of evidence implicate the activities of specific GTPases in the pathophysiology of AD [63]. In particular, recent reports suggest that GTPases can modulate APP processing, glial activation, tau phosphorylation and synaptic plasticity. One recent study demonstrated that signal transduction through the small GTPase Rho is able to modulate the secretion of Aβ42, and that the ability of NSAIDs to lower Aβ42 correlated with their ability to inhibit Rho activity [64]. Treatment with Y-27632, a well-characterized inhibitor of the Rho effector Rho kinase (ROCK), mimicked the effect of NSAIDs and lowered Aβ42 and increased Aβ38 [64]. However, another report found that most ROCK inhibitors lowered Aβ secretion but lacked selectivity for Aβ42, and did not affect Aβ production in a cell-free assay of γ-secretase [65]. In summary, current evidence supports the notion that Rho-GTPases can modulate Aβ production, although it is unlikely that they mediate the specific reduction of Aβ42 production by certain NSAIDs. Nevertheless, ROCK inhibitors may represent a useful therapeutic strategy in AD.

2.2. Cell targets

2.2.1. Neurons. Neurons have been the focus of attention of the Alzheimer field because they are a major site of production of Aβ, as well as a major target for the neurotoxic actions of Aβ. The protective effect of NSAIDs on neurons has been associated to decreased secretion of Aβ peptides and soluble APP (APPs), although the findings have been controversial (Fig. 2). Different hypothesis have been raised, including a decrease in full-length APP [66], an increase in α-APPs [67], a reduction in the generation of Aβ42 levels, which is the most aggressive form of the amyloid peptide [17], or a decrease in BACE1 transcription [18, 50]. In addition, a recent study revealed that NSAIDs such as acetylsalicylic acid would be able to decrease tau phosphorylation at position Ser422 [68].

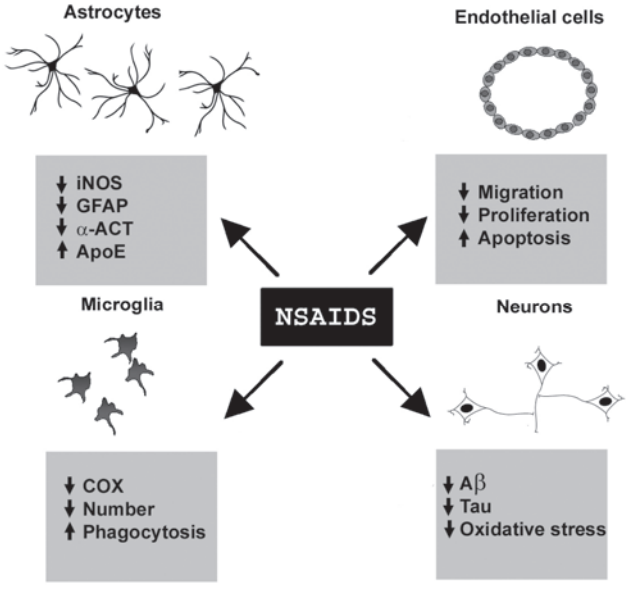

Figure 2. Cell targets of NSAIDs. NSAIDs mediate their effects through preferentially targeting different cellular populations. These include microglia, astrocytes, endothelial cells and neurons (iNOS: inducible nitric oxide synthase, GFAP: glial fibrillary acidic protein, α-ACT: α-chymotrypsin, ApoE: apolipoprotein E).

Other neuroprotective actions of NSAIDs include their effect of decreasing oxidative stress [69], the modulation of the firing of dopaminergic neurons by affecting kynurenic acid formation [70], and the inhibition of Aβ fibril formation [71] and Aβ aggregation [72, 73].

2.2.2. Microglia. It is accepted that Aβ peptides and their precursor protein are potent glial activators [74, 75]. Activated microglia is characteristically found in senile plaques, and it can be detected *in vivo* at early

stages of AD using PET with PK11195, a specific ligand that binds to activated microglia or brain macrophages [76]. It was also shown in animal models of AD that microglia activation may precede Aβ deposition [77], perhaps indicating that soluble oligomeric Aβ can activate glial cells.

Microglia can play a double role in AD, deleterious and protective. On one hand, they release a wide array of pro-inflammatory cytokines and reactive oxygen species that are toxic for the surrounding neurons, particularly when they are released in a chronic fashion [78]. On the other hand, microglia phagocytosis may play an important role in plaque removal [79]. However, microglia may display an activated proinflammatorty phenotype yet be unable to initiate a phagocytic response [79]. *In vitro*, proinflammatory cytokines attenuate microglial phagocytosis, and this may contribute to the accumulation of amyloid plaques in the AD brain by suppression of the endogenous Aβ clearance mechanisms [79].

In vitro neurotoxicity assays have demonstrated that preincubation of cells with NSAIDs effectively decreases inflammation-induced microglial neurotoxicity [80], demonstrating reduced secretion of inflammatory cytokines and neurotoxic agents. This could be explained because microglia express COX-1, and inhibition of this COX isoform has been shown to decrease microglial neurotoxicity [81]. Also, *in vitro*, NSAIDs can reverse the decreased phagocytosis induced by pro-inflammatory cytokines [79] and, ibuprofen may actually increase phagocytosis (E. Galea, unpublished observations). In contrast, there are other reports that support phagocytosis being inhibited by indomethacin [80].

Postmortem pathological analysis of the brain of patients on long-term medication with NSAIDs showed a 65% reduction in plaque-associated reactive microglia [82, 83]. In this line, several other NSAIDs with and without a COX-dependent mechanism have been effective in reducing microglial activation *in vivo* in different animal models [14, 23, 24, 55, 62]. It is also difficult to ascertain whether the reduced microglia activation *in vivo* results from a direct action of NSAIDs, or is secondary to reduced Aβ production caused by modulation of the secretases, although it is plausibly attributable to both. No evidence exists, however, of increased phagocytosis induced by NSAIDs *in vivo*.

2.2.3. Astrocytes. Recruited astrocytes, which assemble at the Aβ plaque site, have been described to prolong neuroinflammation and to contribute to NO-mediated neurotoxicity by expressing the inducible nitric oxide synthase (iNOS), and the L-arginine-supplying enzyme argininosuccinate synthetase [84]. iNOS seems to be a major instigator of Aβ deposition and disease progression [85]. Additionally, it has been suggested that astrocytes could be a source for Aβ because they overexpress BACE1 in response to chronic stress [86]. Astrocytes therefore represent an interesting target for AD therapy.

NSAIDs have been found to be strong astroglial inhibitors, decreasing GFAP and iNOS expression both *in vivo* and *in vitro* [23, 55, 87]. Inflammation-related astrocytic molecules like ApoE and α1-antichymotrypsin, which regulate amyloid pathology in APP transgenic mice, are also modulated by NSAIDs. Treatment of primary rat astroglial cell cultures with indomethacin and aspirin induced significant increases in extracellular ApoE protein levels [88]. On the other hand, chronic ibuprofen treatment in APP mice suppressed IL-1β induction of α1-antichymotrypsin by astrocytes [89], but did not affect ApoE levels. Conversely, the synthesis of NO by astrocytes could be neuroprotective in other situations because genetic removal of iNOs in mice expressing mutant APP resulted in pathological hyperphosphorylation of mouse tau, its redistribution to the somatodendritic compartment in cortical and hippocampal neurons, and aggregate formation [90].

2.2.4. Endothelial cells. Functional vascular abnormalities are one of the earliest clinical manifestations in both sporadic and familial forms of AD and other dementias [91]. Vessels are structurally and functionally damaged by the deposition of Aβ, primarily the Aβ40 form, which constitutes the cerebral amyloid angiopathy (CAA). CAA is a common neuropathological feature in sporadic AD and, in a few families, it has been associated with mutations in the APP gene [92]. Moreover, pathologies such as stroke, hypertension, diabetes, atherosclerosis, and hypercholesterolemia, akin to AD, are associated with chronic inflammation and altered blood vessel responsiveness [93, 94]. Brain microvessels from AD patients express high levels of inflammatory proteins and these proteins evoke the release of the neurotoxic protease thrombin from brain endothelial cells [95].

Surprisingly, it is not clear whether NSAIDs will be therapeutically appropriate for the treatment of CAA, because no NSAID treatment has been performed in mouse models of CAA, nor has specific attention been paid to vascular deposition in APP mice bearing mostly a parenchymal plaque pathology. On the other hand, it has been demonstrated that NSAIDs are able to inhibit angiogenesis [96] and several mechanisms are proposed, including alterations in mediators of vascular endothelial growth factor, induction of endothelial cell apoptosis and inhibition of endothelial cell migration, spreading and

Table 1. Characteristics of the studies that examined the role of non-steroidal anti-inflammatory drug (NSAID) use in the prevention of Alzheimer's disease (AD).

Study	Type of study	N	Diagnosis of AD	NSAIDs assessment	RR or OR (95 % CI)[a]
In'Veld [101]	Cohort	6989	Clinical investigation	Prescription database	0.86 (0.66–1.09)
Zandi [102]	Cohort	3227	Interviews and clinical investigation	Patient interview	0.67 (0.4–1.06)
Stewart [106]	Cohort	1686	Clinical investigation	Patient interview	0.46 (0.24–0.86)
Fourrier [107]	Cohort	516	MMSE score	Patient interview	2.84 (0.99–8.1)
Henderson [108]	Cohort	588	Interviews and clinical investigation	Patient interview	1.66 (0.64–4.32)
Breitner 1995 [105]	Cohort	205	Interviews and autopsy	Patient interview	0.37 (0.17–0.79)
Breitner 1994 [104]	Case-control	46	Telephone interview	Questionnaire	0.5 (0.1–2.23)
CSHA [109]	Case-control	793	Clinical investigation	Questionnaire	0.55 (0.37–0.82)
Beard [103]	Case-control	604	Medical records	Medical records	0.79 (0.2–1.38)
Pooled risk. (all studies)					0.72 (0.56–0.94)

[a] Adjusted relative risk (RR) or odds ratio (OR) (CI, confidence interval).

proliferation [97, 98]. Therefore, treatments that affect brain vessel remodeling and associated alterations may be useful as a therapy for AD cerebrovascular dysfunction.

2.3. Clinical evidence

The recognition of a prominent inflammatory response in the AD brain prompted the investigation of the potential use of NSAIDs in patients with AD. The studies can be classified under two categories: those that evaluate the role of NSAIDs in the prevention of AD, and those that evaluate their role in the treatment of AD.

NSAIDs for the prevention of AD. The first evidence for an excessive inflammatory process in AD came from the epidemiological observation that subjects with arthritis have a reduced incidence of AD [99]. Since then, retrospective observational studies have shown that NSAIDs may have a protective effect against the development of AD, as seen in Table 1 [100–112]. A meta-analysis of nine studies revealed that the use of NSAIDs was associated with a lower risk of developing AD, and that the benefit was greater with long-term use than with intermediate use (pooled relative risk 0.27 and 0.83, respectively) [100]. In the same study, aspirin had a protective effect, although the result was not significant. Interestingly, in the Rotterdam study [113], the benefit of NSAIDs was associated to those with Aβ-lowering properties. All in all, these reports indicated that chronic use of NSAIDs may delay the onset and progression of AD, reduce symptomatic severity and slow the cognitive impairment [114]. Whether the protective effects of NSAIDs will hold up in prospective studies is an open question. A large NIH-sponsored prospective prevention trial (ADAPT) with naproxen and celecoxib in a population at high risk of developing dementia was suspended in 2004 due to concerns over increased cardiovascular risk associated with both drugs. The recent publication of the full data showed that there were no significant increases in cardiovascular events in individuals taking celecoxib or naproxen, but looking at the composite score for death, infarction and stroke the hazard ratio was slightly increased in naproxen-users (HR 1.63 CI 1.04–2.55) (PLOS Clinical trials, November 2006). However, it should be noted that this study was not designed to evaluate cardiovascular events and this is in contrast with recent safety data about the cardiovascular risk of naproxen use [115].

NSAIDs for the treatment of AD. The possible preventive effect of NSAIDs has not been confirmed once AD has begun. Many trials (Table 2) have analyzed the efficacy of different NSAIDs in patients with established AD with no [12, 13, 116–118] or small benefit [119–121]. These results have been replicated in patients with mild cognitive impairment (MCI), in which rofecoxib, a selective COX-2 inhibitor, showed negative results [122]. A recent trial with triflusal, a COX-1 and NF-κB inhibitor used as a platelet antiaggregant, suggested a decreased rate of conversion to AD in patients with MCI [123].

The failure of the trials may be attributed to the facts that the benefit of NSAIDs may only be observed in early phases of the disease, that is, they are preventive and not curative, and also to the choice of NSAID, mostly COX-2-specific inhibitors because: (i) only a subset of NSAIDs are able to lower Aβ production, and (ii) this capacity appears to be COX-2 independent [17, 18]. Interest has therefore shifted to those

Table 2. Clinical trials with NSAIDs other than aspirin in AD or mild cognitive impairment (MCI).

Study	Population	Drug	Type of NSAID	Patients enrolled/ completed	Duration (weeks)	Outcome
Rogers [119]	AD	Indomethacin	COX-1 mainly. PPARγ agonist. NF-κB inhibitor	24/14	26	Small benefit
Scharf [117]	AD	Diclofenac/ Misoprostyl	COX-1/COX-2	24/12	25	No benefit
Aisen [13]	AD	Naproxen	COX-1/COX-2. PPARγ agonist	118/90	52	No benefit
Aisen [12]	AD	Nimesulide	COX-2 selective	26/18	12+12	No benefit
Sainati [11]	AD	Celecoxib	COX-2 selective	285/278	52	No benefit
Aisen [13]	AD	Rofecoxib	COX-2 selective	122/89	52	No benefit
Reines [118]	AD	Rofecoxib	COX-2 selective	348/253	52	No benefit
Zanetti [120]	AD	Ibuprofen	COX-1/COX-2. PPARγ agonist	132/95	54+27	Nonsignificant slower decline at 18 months
Black [121]	AD	R-flurbiprofen	Aβ-lowering NF-κB inhibitor	207/163	54	Slower decline in mild AD
Thal [122]	MCI	Rofecoxib	COX-2 selective	1457/588	4 years	No benefit
Gómez-Isla [123]	MCI	Triflusal	COX-1 mainly. NF-κB inhibitor	257/152	58	Lower rate of progression

Table 3. Clinical trials with peroxisome proliferator-activated receptor-γ (PPARγ) agonists in AD.

Study	Population	Drug	Patients enrolled/ completed	Duration (weeks)	Outcome
Watson [127]	MCI/AD	Rosiglitazone	36/30	26	Small cognitive benefit
Geldmacher [128]	AD	Pioglitazone	29/25	81	No benefit
Risner [129]	AD	Rosiglitazone	511	24	No overall benefit. Improvement in ADAS-Cog in the ApoEε4-treated group.

NSAIDs with Aβ42-lowering properties like R-flurbiprofen, which as explained above, lacks COX activity and in addition inhibits NF-κB. A recent phase 2 trial sponsored by Myriad showed promising results with Flurizan® (R-flurbiprofen) in cases with mild AD [121, 124, 125]. As a result, a large Phase III placebo-controlled trial with Flurizan® has recently been launched in Europe and the USA. In addition, many derivatives of the R-flurbiprofen molecule with greater Aβ-lowering potency and minimal anti-inflammatory effects have already been developed, and their potential clinical use is being evaluated [126].

Clinical trials with NSAIDs that are PPARγ activators have yielded conflicting results (Table 1). A small 6-month, double-blind, placebo-controlled study of 100–150 mg/day indomethacin showed little benefit in AD patients [119]. Another trial with naproxen at 400 mg/day for 52 weeks did not show any beneficial effect [13]. Recently, preliminary results of a clinical trial in patients with mild-to-moderate AD exposed to ibuprofen for 12–18 months showed a slight, non-significant slower decline at 18 months [120]. Clinical trials with classical PPRAγ agonists have shown no or minimal benefit (Table 3). A small pilot study with the classical PPARγ agonist rosiglitazone in AD and MCI has revealed a small change in verbal memory and selective attention in subjects receiving rosiglitazone compared with placebo [127, 128]. A recent clinical trial with pioglitazone has shown no benefit [129].

In summary, there is some epidemiological evidence from retrospective studies that the long-term use of NSAIDs may have a protective effect for AD, although this has to be confirmed in prospective trials. On the other hand, as clinical trials with NSAIDs in AD have mainly been negative, NSAIDs are not currently recommended as treatment for patients with AD. Clinical trials in MCI or mild AD may yield more positive results. The use of inappropriate doses, the lack of potency or the need to apply these treatments early in the disease process may explain the failure of these trials. Many NSAIDs derivatives have already been developed, and their potential clinical use is under evaluation [126].

3. Neuroprotective effects in Parkinson's disease

3.1. Clinical evidence

PD is a neurodegenerative condition characterized by muscle rigidity, tremor, a slowing of physical movement (bradykinesia), and gait difficulties. The neuropathological hallmarks of PD are α-synuclein aggregates in the brain in the form of Lewy bodies and Lewy neurites. The primary symptoms are the result of the degeneration of the dopaminergic neurons of the pars compacta region of the substantia nigra. Indeed, it was recently demonstrated that nigral degeneration is only part of a more extended brain degeneration that begins in the medulla oblongata and then spreads to the mesencephalon and cerebral cortex [130].

Like other neurodegenerative diseases, PD has an important inflammatory component. Studies that fully explore the difference in inflammatory cascades among different neurodegenerative diseases are lacking, and could provide hints regarding the role of inflammation and the therapeutic value of NSAIDs. This caveat notwithstanding, the use of nonaspirin NSAIDs has been associated with a 26% lower risk for PD [131, 132]. Particularly, ibuprofen users had reduced risk of PD compared with non-users [132]. However, other studies have indicated that this lower incidence of PD among non-aspirin NSAID users was applicable to men but not to women [133].

Concerning clinical trials in PD patients, only one study has evaluated the role of indomethacin on orthostatic hypotension and the authors found some benefit against postural hypotension and orthostatic symptoms [134]. Few clinical trials have been performed to date and further studies are required to confirm whether NSAIDs may prevent or delay the onset of PD.

3.2. Molecular targets

3.2.1. Cyclooxygenases. Some of the most-used animal models for PD are induced by environmental toxins, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The neurotoxin MPTP produces selective dopaminergic neuronal death and parkinsonism in animals via the inhibition of mitochondrial complex I activity [135]. COX-2 has been found to be up-regulated in microglia in the midbrain of MPTP-treated parkinsonian models and in patients with PD, and selective COX-2 inhibition has been shown to prevent MPTP-induced dopaminergic neuronal loss in the substantia nigra [136]. It is intriguing that COX-2 is up-regulated in microglia in experimental and clinical PD, but not in AD.

Pretreatment with sodium salicylate, aspirin or meloxicam, a preferential COX-2 inhibitor, significantly prevented MPTP-induced striatal dopaminergic depletion, locomotor activity and loss of nigral dopaminergic neurons [137–140], although other nonselective NSAIDs (for instance, ibuprofen, diclofenac and indomethacin) did not show protective effects [137]. Moreover, the reduction of nigral dopaminergic neurons by MPTP injection was prevented in a COX-2 knockout mice [141]. However, the protective effects of aspirin and sodium salicylate may be independent of COX inhibition, as indicated by a study demonstrating that dopaminergic neurotoxicity of 1-methyl-4-phenylpyridium ion (MPP^+) is COX independent [142] and that COX-2 inhibition with rofecoxib had no neuroprotective effect [143].

In vitro, selective NSAIDs can exert protective effects against neurotoxin-induced neurodegeneration of dopaminergic neurons [144, 145]. However, it is still unclear whether these effects are related to the COX-inhibiting activity or simply to the capability to directly reduce toxicity.

3.2.2. PPAR-γ. Administration of the classical PPARγ agonist pioglitazone attenuates the MPTP-induced decrease in striatal dopamine levels and the dopaminergic cell loss in the substantia nigra [146] but not in the striatum. Furthermore, as described in another report, mice treated with pioglitazone showed reduced activation of microglia, reduced induction of iNOS-positive cells and less glial fibrillary acidic protein-positive cells in both striatum and substantia nigra pars compacta [147]. The protective effects of pioglitazone in this last study correlated with an increase in inhibitory protein-κ-Bα (IκBα) expression, and the inhibition of the translocation of the NF-κB subunit p65 to the nucleus in dopaminergic neurons, glial cells and astrocytes, suggesting that pioglitazone sequentially acts through PPARγ activation, IκBα induction, blockade of NF-κB activation, iNOS induction and NO-mediated toxicity [147]. As commented earlier, some of these effects may be mediated by PPAR-independent protective mechanisms.

4. NSAIDs in other neuroinflammatory diseases

4.1. Multiple sclerosis

MS is a chronic inflammatory disease of the central nervous system and represents the most common disabling neurological disease in young adults. Although it has long been regarded as a demyelinating disease, evidence now suggests widespread damage to axons as well.

The most widely used animal model for multiple sclerosis is the experimental autoimmune encephalomyelitis (EAE) model, which induces the disease by

immunization with myelin antigens such as myelin oligodendrocytes glycoprotein. In rats with EAE, it has been demonstrated that COX-2 is up-regulated in endothelial cells in inflammatory lesions. Furthermore, treatment with COX-2 inhibitors can ameliorate the symptoms of EAE pathology [148–150]. In a recent study, celecoxib but not other inhibitors were able to inhibit EAE, reducing interferon-γ production, adhesion molecules and chemokines in a COX-independent manner [151].

The PPAR-γ1 isoform is expressed in human T lymphocytes. MS patients exhibit decreased PPARγ levels in T cells compared with healthy controls, and treatment of T cells with pioglitazone induced an increase in PPARγ DNA-binding activity [152]. PPARγ-activating drugs are able to regulate T cell responsiveness [153, 154]. Particularly, pioglitazone, ciglitazone and GW347845 suppressed the phytohemaglutinin-induced T cell proliferation and induced apoptosis in activated T lymphocytes obtained from MS patients [154].

On the other hand, oral administration of PPAR-γ agonists has been found to ameliorate the clinical course and histopathological features in EAE, suggesting a potential role for PPARγ agonists in the treatment of autoimmune diseases [155]. Preliminary treatments with PPARγ agonists in MS patients have been carried out and clinical trials are currently being performed. Interestingly, in a patient with secondary progressive MS, daily treatment with 45 mg p.o. pioglitazone for 3 years induced clinical improvement without adverse events [156]. Further testing of the therapeutic potential of PPARγ agonists in MS appears indicated.

4.2. Amyotrophic lateral sclerosis

ALS is a neurodegenerative disease characterized by loss of motor neurons in the motor cortex, brainstem and spinal cord. Unable to function, the muscles gradually weaken, waste away and twitch, causing atrophy and fasciculations. The ultimate cause of the disease is unknown, but missense mutations in the gene encoding for the Cu/Zn superoxide dismutase (SOD-1) were identified in a subset of patients with autosomal dominant inherited ALS [157]. The pathogenesis of cell death in ALS may involve glutamate-mediated excitotoxicity, oxidative damage, and/or apoptosis. COX-2 is increased in the mutant SOD1 transgenic mouse model of ALS and in postmortem spinal cord samples and CSF from sporadic ALS patients [158–160]. Treatment with COX inhibitors, such us nimesulide, celecoxib, rofecoxib and sulindac, in G93A SOD1 transgenic mouse model resulted in delayed onset of motor impairment and extended survival [161–165]. However, a recent clinical trial with celecoxib in ALS patients did not show any beneficial effect [166].

On the other hand, oral administration of the PPARγ agonist pioglitazone in G93A SOD1 transgenic mouse has shown to be effective in improving motor performance, delaying weight loss, attenuating motor neuron loss, reducing microglial activation and extending survival [167, 168]. Treatment with drugs that are PPARγ activators is therefore promising as a potential therapy for neurodegenerative diseases, such as ALS.

4.3. Huntington's disease

HD is a genetically based late-onset neurodegenerative disorder in which a loss of neostriatal neurons is the main feature. The degeneration causes uncontrolled movements, loss of intellectual faculties, and emotional disturbances. From the etiopathogenic perspective, HD is characterized by CAG trinucleotide repeat expansion encoding polyglutamine. Proposed mechanisms of neurodegeneration include intranuclear or intracellular protein aggregates, proteolytic cleavage of huntingtin (*cf.* caspase, calpain), altered transcription and other neurotransmitter signaling deficits. The HD-N171–82Q mouse model of HD expresses an N-terminal fragment of mutant huntingtin, displays loss of motor function and dies prematurely. Anti-inflammatory treatments could benefit HD patients by improving huntingtin folding, reducing cell destruction by inflammatory pathways, and decreasing proapoptotic signaling mediated by NF-κB or other transcription factors.

Unfortunately, treatment with either acetylsalicylate or rofecoxib in the R6/2 and N171–82Q transgenic mouse models of HD have shown no benefit [169]. Celecoxib treatment in N171–82Q mice even reduced life expectancy [170], and more studies are needed to address the role of NSAIDs in HD.

5. Conclusions and future directions

The issue of NSAID treatment in neurodegenerative diseases revolves around three main questions: whether it works in patients, how it works, and whether it is beneficial in diseases that are markedly different in their etiology and symptoms, such as AD, PD or MS. In AD, although a wealth of epidemiological studies unequivocally link intake of some NSAIDs – but not aspirin- with a reduced risk of developing the disease, prospective studies have not yet convincingly confirmed the protective effects. The reasons may be twofold. First, prospective studies have been performed mainly with patients, while it is plausible that, to be protective, NSAIDs should be

administered long before clinical symptoms appear. Unfortunately, the only prospective prevention trial in AD was interrupted because of concerns over adverse effects of NSAIDs users. Second, as most prospective studies have used new selective COX-2 inhibitors, attention may have been drawn to the wrong molecular target. Accumulated evidence in cell culture and mice points to γ-secretase, PPARγ, and COX-1 as targets that underlie the protective actions of NSAIDs by reducing inflammation and the synthesis of Aβ42. Ongoing clinical trials with NSAIDs such as R-flurbiprofen, which can modulate γ-secretase and inhibit NF-κB, and with PPARγ agonists (*i.e.*, pioglitazone and rosiglitazone) will possibly provide final confirmation of the therapeutic interest of targeting COX-2-independent pathways. Further, it is worth considering that the key to the success of NSAIDs like ibuprofen is the capacity to simultaneously target several pathogenic cascades, which stresses the importance of testing multifunctional drugs.

The potential of anti-inflammatory treatment in PD is far from being established. There is a shortage of epidemiological data, and research in the MPTP animal model has provided conflicting results, although evidence mostly indicates that COX-2 inhibition is neuroprotective. However, the lack of an animal model that better mimics the symptoms observed in PD makes it difficult to assign a specific target for NSAIDs in this disease. Finally, treatment with PPARγ agonists shows clear protective effects in animal models of MS and ALS, which in the case of MS, appear to be confirmed in patients using pioglitazone, thus reinforcing the therapeutic value of PPARγ-related therapy in neurodegenerative diseases.

In summary, although there are few data demonstrating that inflammation can trigger or promote neurodegeneration, growing evidence suggests that anti-inflammatory drugs may prove effective in the treatment of neurodegenerative diseases. The challenge is to develop therapeutic strategies that will promote the beneficial aspects of the inflammatory response in the brain, while avoiding the deleterious effects related to certain inflammatory mediators. A more complete understanding of the role of inflammation in brain diseases will open new therapeutic avenues for the use of anti-inflammatory treatments.

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