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Is it all said for NSAIDs in Alzheimer's disease?

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Abstract: Epidemiological data suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) may protect against Alzheimer's disease (AD). Unfortunately, recent trials have failed in providing compelling evidence of neuroprotection. Discussion as to why NSAIDs effectivity is uncertain is ongoing. Possible explanations include the view that NSAIDs and other possible disease-modifying drugs should be provided preclinically. In addition, NSAID targets for neuroprotection are unclear. Both COX dependent and independent mechanisms have been put forward, including γ-secretase, the enzyme that cleaves the amyloid precursor protein (APP) and yields amyloid β peptide (Aβ). We have proposed a neuroprotection mechanism for NSAIDs based on inhibition of mitochondrial Ca^{2+} overload. Aβ oligomers promote Ca^{2+} influx and mitochondrial Ca^{2+} overload leading to neuron cell death. Several non-specific NSAIDs including ibuprofen, sulindac, indomethacin and R-flurbiprofen depolarize mitochondria in the low μM range and prevent mitochondrial Ca^{2+} overload induced by Aβ oligomers and/or N-methyl-D-aspartate (NMDA). However, at larger concentrations, NSAIDs may collapse mitochondrial potential (ΔΨ) leading to cell death. Accordingly, this mechanism may explain neuroprotection at low concentrations and damage at larger doses, thus providing clues on the failure of promising trials. Perhaps lower NSAID concentrations and/or alternative compounds with larger dynamic ranges should be considered for future trials to provide definitive evidence of neuroprotection against AD.

Keywords: Alzheimer's disease, non-steroidal anti-inflammatory drugs, calcium, mitochondria, amyloid β oligomers, N-methyl-D-aspartate.

1. INTRODUCTION

Alzheimer's disease (AD) is the most prevalent dementia worldwide, but at the moment no treatment can modify the course of the disease [1]. Cognitive decline and dementia are caused by a massive neuron dysfunction and loss in brain areas related to learning and memory acquisition, including hippocampus and cortex. Pathological hallmarks of AD include accumulation of extracellular senile plaques made mostly of amyloid β peptide (Aβ), a cleavage product of the amyloid precursor protein (APP), as well as intracellular neurofibrillary tangles made of hyperphosphorylated tau protein. Several hypotheses have been proposed to explain the physiopathology of AD. Although challenged recently, the most prevalent view is that excess of Aβ induced by either increased production and/or defective clearance induces synaptic and neuronal dysfunction, leading to cell death by a cascade of events known as the amyloid hypothesis of AD [1]. Among these events, a critical step is that the excess of soluble Aβ species accumulated in plaques may promote a damaging inflammation reaction [2]. This view is supported, among other evidence, by the relationship between familial forms of the disease and mutations in proteins involved in Aβ synthesis including APP and presenilins (PS) 1 and 2, critical enzymes involved in the γ-secretase complex. In addition, excess of inflammatory cytokines and activated astroglia are also found in AD patients. Thus, a prevalent, common view is that AD is related to a chronic inflammatory state in brain regions involved in cognitive functions [2]. Consistently with this view, a large number of epidemiological studies strongly suggest that non-steroidal anti-inflammatory drugs (NSAIDs) may provide protection against AD [3]. Altogether, these findings support the view that inflammation caused by excess of Aβ species plays a pivotal role in AD.

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The etiology of AD is far from being solved and this lack of knowledge is hampering advances in the prevention and/or treatment of the disease. Whereas in familial forms of AD the excess of Aβ relates to mutations in APP and PS, in sporadic AD (accounting for more than 95% of the cases), the mechanisms involved in Aβ accumulation remain obscure. Moreover, mechanisms for Aβ-induced neuron damage and neuron cell death remain uncertain as well. For instance, considerably controversy exists about the early effects of different Aβ species and forms, including monomers, soluble oligomers, large fibrils and plaques. A series of studies provide evidence that some Aβ species increase Ca^{2+} permeability in brain cells [4]. In addition, neurons from AD models show dishomeostasis of intracellular Ca^{2+} [5], a key second messenger involved in critical neuronal functions including neurotransmitter release, synaptic plasticity, neurogenesis, neuron metabolism and cell death. Defects in Ca^{2+} homeostasis induced by AD-related mutations and excess of Aβ species support the so-called Calcium hypothesis of AD [6]. In this review, we will make the case that NSAIDs may protect against AD acting on the disturbed intracellular Ca^{2+} induced by Aβ oligomers in AD.

2. NSAIDS AFFORD NEUROPROTECTION IN AD

A series of epidemiological studies suggest decreased prevalence of AD among long-term users of selected NSAIDs. A first report performed in more than 1,500 participants concluded that subjects taking NSAIDs for more than two years show a low relative risk for AD of 0.40 compared to 0.65 for those using NSAIDs for less than 2 years [7]. This finding has been confirmed later by several additional studies. For example, a population-based cohort study of 7,000 subjects found a relative AD risk of 0.20 in long-term users and decreased protection in short-term users [8]. A meta-analysis of nine related studies (six cohort and three case-control studies) confirmed the results [9]. A pooled review of seven prospective cohort studies concluded that, in patients reporting use of NSAIDs for more than two years, the combined risk estimated for developing AD was 0.42 compared to 0.74 in shorter uses [10]. Moreover, a large case-control study reported an odd ratio of 0.76 for patients reporting more than five years of NSAIDs use [11]. For users of ibuprofen, the odd ratio was even lower (0.56). As pointed out by Imbimbo et al. [3], neuroprotection afforded by NSAIDs may depend on the duration of treatment and age with the greater benefits the longer NSAIDs are taken. Effectiveness, on the other hand, may vary largely with APOE genotype. The finding that increasing duration of NSAIDs correlates with decreased AD risk may reflect that long-term users are taking NSAIDs earlier in time, when the disease is likely preclinical. In addition, NSAIDs could be more efficient in APOE-ε4 carriers just because these subjects are more prone to Aβ deposition and AD [3].

Interestingly, not all types of NSAIDs do actually show benefits. In general, effects are larger for users of non-aspirin compounds compared to those using aspirin. In addition, no association was found between AD risk and use of acetaminophen, neither a trend of decreasing risk with increasing duration of use [7]. Some reports suggest that decreased AD risk is restricted to the pool of NSAIDs that lower plaque associated inflammation but recent data indicate that neuroprotection is similar among NSAIDs with and without Aβ lowering capability [12]. Studies with other anti-inflammatory agents including steroidal, anti-inflammatory drugs and cyclooxygenase 2 (COX-2) specific inhibitors yielded negative or even detrimental results [13].

The epidemiological data summarized above prompted development of several clinical trials, and up to 16 of them have been conducted in the last few years with rather negative results [3]. The initial pilot study suggested that indomethacin promoted a slower cognitive decline. However, in this and other similar studies the withdrawal rate was very high, suggesting poor tolerability to NSAIDs by AD patients and compromising interpretation of the results. A series of trials with COX-2 specific inhibitors failed to demonstrate efficacy or even showed detrimental effects [14-17]. A placebo-controlled study in mild-to-moderate AD patients found no effects of ibuprofen, although patients carrying one or more ε4 allele showed lower decay [18]. Another placebo-controlled trial with tarenflurbil (R-flurbiprofen) in mild-to-moderate AD yielded overall negative results. However, patients with mild AD taking the largest tarenflurbil dose showed lower rates of decline than the ones with moderate AD [19]. Unfortunately, the phase III of this study came out negative or even showed some detrimental effects [20].

Considerable concern has grown on why trials failed to provide evidence of neuroprotection strongly suggested by epidemiological studies. Testing of wrong doses, biased assumptions about the role of neuroinflammation in AD and incomplete knowledge about NSAID targets may have contributed to failure [21,22]. The emerging view is that NSAIDs target should be clarified and disease-modifying drugs could be only effective preclinically, thus calling for new, earlier trials [21,22].

3. MECHANISMS OF ACTION PROPOSED FOR NSAID NEUROPROTECTION IN AD

One of the potential targets of NSAIDs in AD is believed to be the activated microglia that most often surrounds senile plaques [23]. In fact, both non-demented AD patients treated with NSAIDs and mouse models of the disease under a NSAID regime show decreased microglia and less inflammatory mediators as COX and cytokines [24,25]. However, in NSAID-treated AD patients, decreases in microglia and astroglia were not observed, suggesting either they are not relevant for neuroprotection or that effects are stage-specific [22]. Consistently, it is not so clear that canonical targets for NSAIDs, including COX1 and COX2, are responsible for neuroprotection afforded by NSAIDs [26]. This view is based in that some, but not all NSAIDs show neuroprotection, and that structural analogs of classic NSAIDs lacking anti-inflammatory activity like, for instance, R-flurbiprofen, do provide protection as well [27].

NSAIDs may influence inflammatory mediators by different pathways than COX. For example, NSAIDs may modulate the peroxisome proliferator activated receptor-γ (PPARγ) involved in transcription of pro-inflammatory genes [28]. These effects could be relevant as PPARγ activation may

decrease Aβ burden [29]. In addition, neuroprotection could be also mediated by BACE1 transcription or inhibition of RhoA [30]. Finally, another well established target of NSAIDs is the nuclear translocation of NFkB, a transcription factor involved in inflammatory responses. Some NSAIDs including R-flurbiprofen have been reported to target and inhibit either γ-secretase activity and/or APP, leading to a lower Aβ burden both in vitro and in vivo models [31,32]. Accordingly, NSAIDs may modulate inflammatory responses acting not only on COX or on proteins involved in expression of inflammatory mediators but also on Aβ levels. However, R-flurbiprofen and non-specific NSAIDs decrease Aβ levels only at very high concentrations, difficult to be achieved the brain. In addition, some studies concluded that the effects of NSAIDs seem to be independent of the $A\beta_{42}$ reducing activity [9,13]. Therefore, neuroprotection afforded by R-flurbiprofen and other NSAIDs may rely on alternative targets independently of their anti-inflammatory and $A\beta_{42}$ lowering activities. We have reported an unsuspected action mechanism of neuroprotection for NSAIDS against the neurotoxicity induced by Aβ oligomers in neural cultures based in the Calcium hypothesis of AD [4].

4. AMYLOID TOXICITY INVOLVES MITOCHONDRIAL CALCIUM OVERLOAD

We have shown that $A\beta_{42}$ oligomers, the assembly state correlating best with neuron damage and cognitive defects in AD [33,34], induce Ca^{2+} influx into neuronal cells including hippocampal, cortical, and cerebellar neurons [4,35,36] (Fig. 1). These effects are mimicked by Aβ surrogates as $A\beta_{25-35}$, but not by fibrils made of $A\beta_{42}$. This finding agrees with previous results showing that $A\beta_{42}$ oligomers, but not monomers or fibrils, increased cytosolic $[Ca^{2+}]$ in a human neuroblastoma cell line [37]. These results do not mean that fibrils are not toxic (in fact at large concentrations they are) but suggest that the mechanism of neurotoxicity by fibrils and oligomers differ from each other as previously proposed [38]. The pathway for enhanced Ca^{2+} influx is not solved yet but proposed candidates include plasma membrane permeabilization [37], the so-called amyloid channels [39,40] and/or activation of Ca^{2+} channels in plasma membrane, particularly Ca^{2+} permeable glutamate receptors activated by NMDA [41,42]. The Ca^{2+} entry pathway activated by Aβ has been controversial because oligomers were initially prepared in media containing glutamate receptor agonists [43]. However, we have reported recently that Aβ oligomers prepared in simple media devoid of glutamate receptor agonists promote Ca^{2+} entry into hippocampal neurons [35].

Regardless of the Ca^{2+} entry pathway, the large and sustained Ca^{2+} influx evoked by Aβ oligomers promotes mitochondrial Ca^{2+} overload as shown directly by mitochondrial Ca^{2+} measurements based on bioluminescence imaging of neurons expressing a low-affinity aequorin targeted to mitochondria [4,44]. We found that both $A\beta_{25-35}$ and $A\beta_{42}$ oligomers, but not fibrils, induce mitochondrial Ca^{2+} overload to concentration values close to the mM level (Fig. 1). In addition, this mitochondrial Ca^{2+} overload promoted a series of events initiated by ROS production, mitochondrial permeabilization, release of cytochrome c and finally, apoptosis and cell death (Fig. 1). Interestingly, the effects of Aβ oligomers on Ca^{2+} entry and on apoptosis are much larger in long-term cultures of rat hippocampal neurons that resemble aged neurons [36,45]. Therefore, Ca^{2+} entry and mitochondrial Ca^{2+} overload may play a critical role in neuron cell death induced by Aβ oligomers. This view is supported by the fact that mitochondrial depolarization induced by mitochondrial uncouplers inhibit both mitochondrial Ca^{2+} overload and all the subsequent steps leading to cell death, including ROS formation, permeability transition pore opening, cytochrome c release, apoptosis, and cell death (Fig. 1). These results strongly suggest that mitochondrial Ca^{2+} overload contributes largely to cell death induced by Aβ oligomers [46]. Additional mechanisms might contribute to the mitochondrial toxicity induced by Aβ oligomers. For example, intracellular Aβ species may also interact with mitochondria in AD mouse models and affected AD brains [47] and this interaction may promote apoptosis and cell death.

The action mechanism of Aβ oligomers proposed here resemble the mechanism of excitotoxicity reported previously for glutamate. Excitotoxicity (excess of the neurotransmitter glutamate that leads to overactivation of glutamate receptors, as NMDARs, causing an excess of cytoplasmic free Ca^{2+} concentration that eventually leads to neuronal death) is one of the most important mechanisms contributing to cognitive decline in Alzheimer's disease [48,49]. It is well established that glutamate-induced neuron death requires mitochondrial Ca^{2+} uptake [50] and low concentrations of the mitochondrial uncoupler FCCP prevent both mitochondrial Ca^{2+} overload, ROS production and cell death induced by NMDA [51]. We have shown that NMDA induces mitochondrial Ca^{2+} overload and neuron cell death [4,52] in rat hippocampal neurons. In addition, this effect is enhanced in long-term cultures of rat hippocampal neurons that resemble aged neurons [52] consistently with changes in the expression of NMDA receptor subunits. Finally, NMDA-induced cell death is prevented by mitochondrial depolarization that inhibits mitochondrial Ca^{2+} overload. Aβ oligomers may also promote synaptotoxicity and/or neurotoxicity by binding to G-protein coupled receptors in the plasma membrane (Fig. 1). For example, the metabotropic glutamate receptor 5 is considered a co-receptor for Alzheimer Aβ oligomer bound to cellular prion protein [53]. In addition, the Ca^{2+}-sensing receptor, a unique receptor involved in extracellular Ca^{2+} homeostasis with still unknown functions in multiple tissues and cells including neurons, has been reported to bind Aβ oligomers and has been also involved in AD [54].

The mechanism of neurotoxicity proposed for Aβ oligomers fits nicely in the emerging model of the Ca^{2+} hypothesis of AD. In familial AD, mutations in APP and PS induce early excess of Aβ species that enhance Ca^{2+} influx and intracellular Ca^{2+} dishomeostasis. In addition, PS may have additional physiological functions unrelated to γ-secretase and involved in intracellular Ca^{2+} homeostasis. For instance, mutations in PS may lead to disturbed Ca^{2+} responses due to a larger release of Ca^{2+} from intracellular stores [55]. The mechanism for this exaggerated release is still controversial and includes activation of intracellular Ca^{2+} channels as IP3

and ryanodine receptors [55] or, alternatively, inhibition of Ca^{2+} leak channels at the ER leading to ER Ca^{2+} overload [3].

Regardless of the mechanism, exaggerated Ca^{2+} responses may lead also to mitochondrial Ca^{2+} overload, particularly in the presence of an excess of Aβ species, clearance defects or deficiency in intracellular Ca^{2+} buffers. It is noteworth that deficits in the Ca^{2+} binding protein calbindin have been reported in aging and AD [56]. Lack of calbindin might be critical in a scenario of Aβ excess where mitochondrial Ca^{2+} overload cannot be counterbalanced by intracellular Ca^{2+} buffers. Taken together, these results suggest that mitochondrial Ca^{2+} overload seems to have an important role in a series of AD-related scenarios including excess of amyloid, mutations in APP and PS, age, as well as age-related deficits in Ca^{2+} binding proteins and excitotoxicity. Therefore, the ability of mitochondria to take up Ca^{2+} is an appealing target for the prevention of AD-related neurotoxicity. Therefore, all the above data indicate that mitochondria Ca^{2+} uptake can be considered a suitable target for neuroprotection. We have reported evidence that some NSAIDs may prevent Aβ oligomers-induced neuron cell death acting on mitochondrial Ca^{2+} uptake, thus providing a suitable mechanism of AD chemoprevention by NSAIDs.

5. NSAIDS MAY PROTECT AGAINST AD ACTING ON MITOCHONDRIAL CALCIUM UPTAKE

As concluded above, mitochondrial Ca^{2+} overload contributes to the neurotoxicity induced by $A\beta_{42}$ oligomers and likely plays a role in both familial and sporadic AD. Accordingly, any drug targeting mitochondrial Ca^{2+} uptake might be beneficial against Aβ toxicity and likely AD. As detailed above, overwhelming epidemiological data suggest that classic NSAIDs protects against AD. Although the action mechanism is controversial, NSAIDs are believed to act largely through mechanisms other than their anti-inflammatory activities. Some NSAIDs, including R-flurbiprofen, modulate and inhibit γ-secretase activity at rather large concentrations (100 μM) leading to a lower Aβ burden [31]. We have recently shown that, at very low concentrations around 1 μM, classic non-specific NSAIDs including indomethacin, sulindac, ibuprofen and R-flurbiprofen depolarize mitochondria to the same extent than low concentrations of the well established mitochondrial uncoupler FCCP (Fig. 1). As mitochondrial depolarization decreases the driving force for mitochondrial Ca^{2+} uptake, NSAIDs are able to inhibit mitochondrial Ca^{2+} overload induced by Aβ oligomers without preventing Ca^{2+} entry through the plasma membrane. Similar effects were achieved by salicylate, the most important aspirin metabolite, although at about 100-fold larger concentrations [4]. Consistently, we showed that NSAIDs also prevent all the downstream effects related to mitochondrial Ca^{2+} overload, including ROS production, cytochrome c release, apoptosis and cell death induced by Aβ oligomers (Fig. 1).

NSAIDs not only protect against excitotoxicity [57]. They may also prevent the cognitive decline associated with AD [58] and aging [59]. The action mechanisms remain unknown but reports suggest that they may well be unrelated to the classic anti-inflammatory activity of these drugs. We have reported that low concentrations of NSAIDs, similar to those producing partial mitochondrial depolarization, inhibit NMDA-induced mitochondrial Ca^{2+} uptake, and NMDA-induced apoptosis in aged cultures [52]. In addition, the increase in cytosolic and mitochondrial Ca^{2+} response induced by $A\beta_{42}$ oligomers in aged cultures is prevented by low concentrations of NSAIDs and R-flurbiprofen acting on mitochondrial Ca^{2+} overload [4,36,46]. Meanwhile R-flurbiprofen at higher concentrations promote apoptosis [36] by itself. Therefore, NSAIDs may inhibit neurotoxicity induced by Aβ oligomers based on the primary inhibition of mitochondrial Ca^{2+} overload and prevention of the ensuing mitochondrial permeability transition opening and downstream steps to cell death. A cartoon depicting the proposed mechanism of Aβ oligomers toxicity and neuroprotection by NSAIDs is shown in Fig. 1. It is worth noting that, at low concentrations, NSAIDs have no effect on γ-secretase activity whereas fitting the concentration range achieved in brains by human therapeutic dose [31].

The fact that NSAIDs depolarize mitochondria is not new. Their chemical structure of carboxylic acids bound to aromatic residues resembles the structure of mitochondrial uncouplers. As a matter of fact, this is considered a common characteristic of anti-inflammatory agents with an ionizable group [60]. For example, salicylate, the major aspirin metabolite, is taken by mitochondria as salicylic acid (driven by the concentration gradient) and leave as salicylate anion (driven by voltage and concentration gradients) thus producing a net proton entry [61]. At therapeutic concentrations, salicylate may cause a net H^+ influx into mitochondria that is considered enough to explain the reported "loose coupling" effect. Interestingly, triflusal, a salicylate derivative, did not alter the total brain Aβ accumulation but significantly rescued cognitive deficits in a mouse model of AD [62] and this effect was associated with a lower rate to conversion to dementia, in a trial on patients with mild cognitive impairment [63].

Aspirin, salicylate, salicylate derivatives and classic, non-specific NSAIDs have deleterious effects mostly related to damage to the gastrointestinal tract. Accordingly, chemical derivatives lacking anti-inflammatory activity (as R-flurbiprofen) were selected probably for trials since high concentrations are required to inhibit γ-secretase. However, at high concentrations, non-specific NSAIDs may collapse mitochondrial potential, thus compromising aerobic energy, and eventually leading to neuron cell death. Consequently, high concentrations of NSAIDs may be detrimental rather than protective. To test this hypothesis, we recently investigated the dose-dependent effects of R-flurbiprofen on cell death induced by Aβ oligomers. Interestingly, we found that R-flurbiprofen inhibits largely cell death induced by Aβ oligomers at very low concentrations (0.1 to 1 μM). However, at larger concentrations above 10 μM, and particularly at 100 μM, R-flurbiprofen fails to provide any protection against Aβ oligomers. In fact, when tested alone in the absence of Aβ oligomers, R-flurbiprofen does not induce cell death in cultured neurons at low concentrations, but at larger concentrations R-flurbiprofen is almost as efficient as Aβ oligomers in killing neurons [36]. These results suggest that neuroprotection afforded by R-

flurbiprofen and, likely, non-specific NSAIDs, is efficient only at low concentrations that prevent mitochondrial Ca^{2+} overload without compromising aerobic energy. However, at larger concentrations NSAIDs may operate as mitochondrial poisons. In this scenario, large concentrations of NSAIDs (such as those used to target secretase) do not only fail to protect but may be rather detrimental. These data may help to explain why R-flurbiprofen was not positive in the phase III trial of Tarenflurbil [20]. Nevertheless, the phase II trial did not show benefit when used at a lower concentration [19]. Perhaps the concentration of R-flurbiprofen was still high and the possible neuroprotection was counterbalanced by the neurotoxic effects related to strong mitochondrial uncoupling. Alternatively, it is possible that even very low concentrations have minor or no effect at all as well. This possibility is based in the dynamic model of AD [64] and the increasing realization that disease-modifying drugs could be effective only preclinically. According to the model, AD evolves continuously and stages are defined by biomarkers: There is a damaging starting phase (phase I) where Aβ and hyperphosphorylated tau accumulate, followed by a second phase characterized by synaptic and metabolic damage (phase II). The final phase (phase III) is characterized by the clinical symptoms [64]. According to this model, drugs targeting mitochondrial Ca^{2+} should be most efficient in the presymptomatic phase I, when Aβ oligomers start accumulating and damage neurons by promoting mitochondrial Ca^{2+} overload. Once the disease is installed and metabolic damage, synaptic loss and massive neurodegeneration destroys cognitive functions of the patient, NSAIDs may not provide neuroprotection but are rather detrimental. Perhaps it is not all said on NSAIDs and Alzheimer's disease and future early trials may give a second chance to over the counter NSAIDs and/or alternative drugs targeted to mitochondria in search for hope and the first disease-modifying drug in Alzheimer's disease.

CONFLICT OF INTEREST

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REFERENCES

[1] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297(5580): 353-356 (2002).

[2] Zhang F, Jiang L. Neuroinflammation in Alzheimer's disease. Neuropsychiatr Dis Treat 11: 243-256 (2015).

[3] Imbimbo BP, Solfrizzi V, Panza F. Are NSAIDs useful to treat Alzheimer's disease or mild cognitive impairment? Front Aging Neurosci 2: 1-14 (2010).

[4] Sanz-Blasco S, Valero RA, Rodríguez-Crespo I, Villalobos C, Núñez L. Mitochondrial Ca^{2+} overload underlies Aβ oligomers neurotoxicity providing an unexpected mechanism of neuroprotection by NSAIDs. PLoS One 3(7): e2718 (2008).

[5] Supnet C, Bezprozvanny I. The dysregulation of intracellular calcium in Alzheimer disease. Cell Calcium 47(2): 183-189 (2010).

[6] Berridge MJ. Calcium hypothesis of Alzheimer's disease. Pflugers Arch 459(3): 441-449 (2010).

[7] Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. Neurology 48(3): 626–632 (1997).

[8] in t' Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, Stijnen T, et al. Nonsteroidal anti-inflammatory drugs and the risk of Alzheimer's disease. N Engl J Med 345(21): 1515-1521 (2001).

[9] Etminan M, Gill S, Samii A. Effect of non-steroidal antiinflammatory drugs on risk of Alzheimer's disease: systematic review and meta-analysis of observational studies. BMJ 327(7407): 128–132 (2003).

[10] Szekely CA, Thorne JE, Zandi PP, Ek M, Messias E, Breitner JC, et al. Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review. Neuroepidemiology 23(4): 159–169 (2004).

[11] Vlad SC, Miller DR, Kowall NW, Felson DT. Protective effects of NSAIDs on the development of Alzheimer disease. Neurology 70(19): 1672–1677 (2008).

[12] Szekely CA, Green RC, Breitner JC, Østbye T, Beiser AS, Corrada MM, et al. No advantage of A beta 42-lowering NSAIDs for prevention of Alzheimer dementia in six pooled cohort studies. Neurology 70(24): 2291-2298 (2008).

[13] McGeer PL, Rogers J, McGeer EG. Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. J Alzheimers Dis 9 (3 Suppl): 271-276 (2006).

[14] Sainati SM, Ingram DM, Talwalker S, Geis G. Results of a double-blind, randomized, placebo-controlled study of celecoxib in the treatment of progression of Alzheimer's disease. 6th International Stockholm/Springfield Symposium on Advances in Alzheimer Therapy (Stockholm, Sweden) April 5–8, Abstract Book (p 180) (2000).

[15] Aisen PS, Schafer KA, Grundman M, Pfeiffer E, Sano M, Davis KL, . Effects of rofecoxib or naproxen vs. placebo on Alzheimer disease progression. JAMA 289(21): 2819–2826 (2003).

[16] Reines SA, Block GA, Morris JC, Liu G, Nessly ML, Lines CR, et al. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. Neurology 62(1): 66-71 (2004).

[17] Soininen H, West C, Robbins J, Niculescu L. Long-term efficacy and safety of celecoxib in Alzheimer's disease. Dement Geriatr Cogn Disord 23(1):8-21. (2007).

[18] Pasqualetti P, Bonomini C, Dal Forno G, Paulon L, Sinforiani E, Marra C, et al. A randomized controlled study on effects of ibuprofen on cognitive progression of Alzheimer's disease. Aging Clin Exp Res 21(2): 102–110 (2009).

[19] Wilcock GK, Black SE, Hendrix SB, Zavitz KH, Swabb EA, Laughlin MA. Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: a randomised phase II trial. Lancet Neurol 7(6): 483–493 (2008).

[20] Wilcock GK, Black SE, Balch AH, Amato DA, Beelen AP, Schneider LS, et al. Safety and efficacy of tarenflurbil in subjects with mild Alzheimer's disease: results from an 18-month international multi-center Phase 3 trial. Alzheimer's Dementia 5 (4): 86 (Abstract O1-04-07) (2009).

[21] Lichtenstein MP., Carriba P., Masgrau R., Pujol A., Galea E. Staging anti-inflammatory therapy in Alzheimer's disease. Front Aging Neurosci 2 (142) (2010).

[22] Sastre M, Gentleman SM. NSAIDs: How they Work and their Prospects as Therapeutics in Alzheimer's Disease. Front Aging Neurosci 2(20) (2010).

[23] Giulian D, Haverkamp LJ, Li J, Karshin WL, Yu J, Tom D, et al. Senile plaques stimulate microglia to release a neurotoxin found in Alzheimer brain. Neurochem Int 27(1): 119-37 (1995).

[24] Mackenzie IR, Muñoz DG. Nonsteroidal anti-inflammatory drug use and Alzheimer-type pathology in aging. Neurology 50: 986–990 (2008).

[25] Lim GP, Yang F, Chu T, Chen P, Beech W, Teter B, et al. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J Neurosci 20(15): 5709-5714 (2000).

[26] Lleo A, Galea E, Sastre M. Molecular targets of non-steroidal anti-inflammatory drugs in neurodegenerative diseases. Cell Mol Life Sci 64(11): 1403-1418 (2007).

[27] Townsend KP, Praticò D. Novel therapeutic opportunities for Alzheimer's disease: focus on non-steroidal anti-inflammatory drugs. FASEB J 19: 1592-1601 (2005).

[28] Jaradat MS, Wongsud B, Phornchirasilp S, Rangwala SM, Shams G, Sutton M, et al. Activation of peroxisome proliferator-activated receptor isoforms and inhibition of prostaglandin H(2) synthases by ibuprofen, naproxen, and indomethacin. Biochem Pharmacol 62(12): 1587-1595 (2001).

[29] Sastre M, Walter J, Gentleman SM. Interactions between APP secretases and inflammatory mediators. J Neuroinflammation 5(25) (2008).

[30] 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. Nonsteroidal anti-inflammatory drugs can lower amyloidogenic $A\beta_{42}$ by inhibiting Rho. Science 302(5648):1215-7 (2003)

[31] Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, et al. A subset of NSAIDs lower amyloidogenic $A\beta_{42}$ independently of cyclooxygenase activity. Nature 414(6860): 212-216 (2001).

[32] Eriksen JL, Sagi SA, Smith TE, Weggen S, Das P, McLendon DC, et al. NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Aβ42 in vivo. J Clin Invest 112: 440-449 (2003).

[33] Klein WL, Stine WB Jr, Teplow DB. Small assemblies of unmodified amyloid beta-protein are the proximate neurotoxin in Alzheimer's disease. Neurobiol Aging 25: 569-580 (2004).

[34] Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β peptide. Nat Rev Mol Cell Biol 8: 101-112 (2007).

[35] Caballero E, Calvo-Rodriguez M, Gonzalo-Ruiz A, Villalobos C, Núñez L. A new procedure for amyloid β oligomers preparation enables the unambiguous testing of their effects on cytosolic and mitochondrial Ca^{2+} entry and cell death in primary neurons. Neurosci Lett 612: 66–73 (2016).

[36] Calvo-Rodríguez M, García-Durillo M, Villalobos C, Núñez L. Aging enables Ca^{2+} overload and apoptosis induced by amyloid-β oligomers in rat hippocampal neurons: neuroprotection by non-steroidal anti-inflammatory drugs and R-flurbiprofen in aging neurons. J Alzheimers Dis 54(1): 207-221 (2016).

[37] Demuro A, Mina E, Kayed R, Milton SC, Parker I, Glabe CG. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. J Biol Chem 280: 17294-17300 (2005).

[38] Deshpande A, Mina E, Glabe C, Busciglio J. Different conformations of amyloid β induce neurotoxicity by distinct mechanisms in human cortical neurons. J Neurosci 26: 6011-6018 (2006).

[39] Arispe N, Diaz JC, Simakova O. Aβ ion channels: Prospects for treating Alzheimer's disease with Aβ channel blockers. Biochim Biophys Acta 1768: 1952-1965 (2007).

[40] Kawahara M, Ohtsuka I, Yokoyama S, Kato-Negishi M, Sadakane Y. Membrane incorporation, channel formation, and disruption of calcium homeostasis by Alzheimer's β-amyloid protein. Int J Alzheimers Dis 2011:304583 (2011).

[41] De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, et al. Aβ oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem 282: 11590-11601 (2007).

[42] Texidó L, Martín-Satué M, Alberdi E, Solsona C, Matute C. Amyloid β peptide oligomers directly activate NMDA receptors. Cell Calcium 49(3): 184-190 (2011).

[43] Zempel H, Thies E, Mandelkow E, Mandelkow EM. Aβ oligomers cause localized Ca^{2+} elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J. Neurosci 30: 11938–11950 (2010).

[44] Villalobos C, Núñez L, MonteroM, García AG, Alonso MT, García-Sancho J. Redistribution of Ca^{2+} among cytosol and organella during stimulation of bovine chromaffin cells. FASEB J 16: 343-353 (2002).

[45] Calvo-Rodríguez M, de la Fuente C, García-Durillo M, García-Rodríguez C, Villalobos C, Núñez L. Aging and amyloid β oligomers enhance TLR4 expression, LPS-induced Ca^{2+} responses and neuron cell death in cultured rat hippocampal neurons. J Neuroinflammation 14(24) (2017).

[46] Calvo-Rodríguez M, Núñez L, Villalobos C. Non-steroidal anti-inflammatory drugs (NSAIDs) and neuroprotection in the elderly: a view from the mitochondria. Neural Regen Res 10(9): 1371-1372 (2015).

[47] Reddy PH, Beal MF. Amyloid β, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. Trends Mol Med 14: 45-53 (2008).

[48] Greenamyre JT. Neuronal bioenergetic defects, excitotoxicity and Alzheimer's disease: "use it and lose it". Neurobiol Aging 12(4): 334-6 (1991).

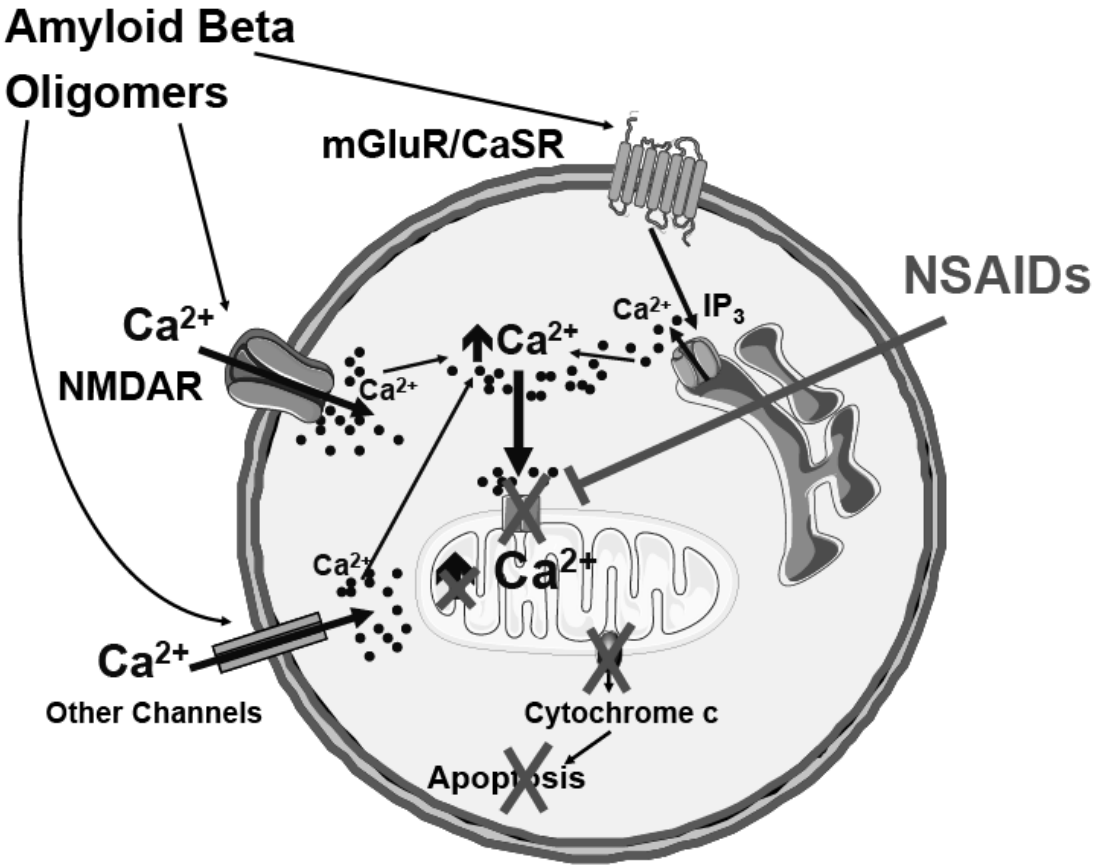
[55] Cheung KH, Shineman D, Müller M, Cárdenas C, Mei L, Yang J, et al. Mechanism of Ca^{2+} disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. Neuron 58(6): 871-883 (2008).

[56] Palop JJ, Jones B, Kekonius L, Chin J, Yu GQ, Raber J, et al. Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. Proc Natl Acad Sci U S A 100(16): 9572-7 (2003).

[57] Asanuma M, Nishibayashi-Asanuma S, Miyazaki I, Kohno M, Ogawa N. Neuroprotective effects of non-steroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals. J Neurochem 76(6): 1895-904 (2001).

[58] Van Dam D, Coen K, De Deyn PP. Ibuprofen modifies cognitive disease progression in an Alzheimer's mouse model. J Psychopharmacol. 24(3):383-8 (2010).

[59] Kern S, Skoog I, Ostling S, Kern J, Börjesson-Hanson A. Does low-dose acetylsalicylic acid prevent cognitive decline in women with high cardiovascular risk? A 5-year follow-up of a non-demented population-based cohort of Swedish elderly women. BMJ Open 2(5) (2012).



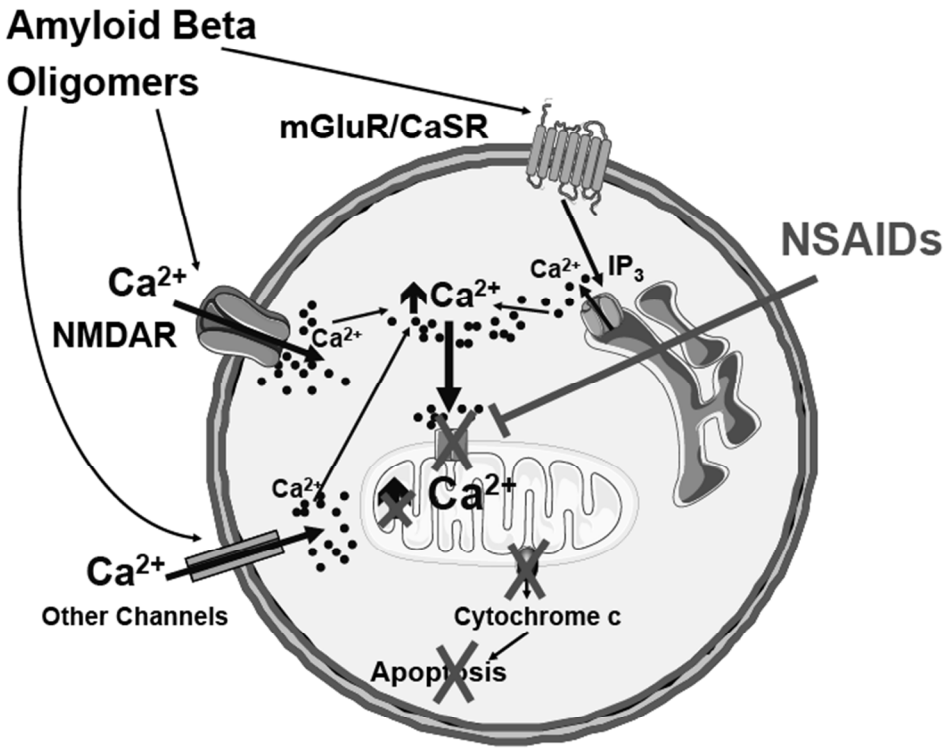


Figure 1. A model of Aβ-induced toxicity and neuroprotection by NSAIDs based on mitochondrial Ca^{2+}. Aβ oligomers induce Ca^{2+} influx through the plasma membrane likely mediated by formation of amyloid channels, activation of NMDA receptors or voltage-gated Ca^{2+} channels (VGCC). They may also activate metabotropic glutamate receptors (mGluR) and the Ca^{2+}-sensing receptor (CaSR). Rises in cytosolic Ca^{2+} concentration may promote mitochondrial Ca^{2+} overload, ROS production, mitochondrial permeability transition, cytochrome c release and apoptosis. Other factors related to AD may favor mitochondrial Ca^{2+} overload including exaggerated IP_3-induced release of Ca^{2+} from the ER in loss of function PS1 mutants related to familial AD and/or decreased abundance of endogenous Ca^{2+} buffers as calbindin-D28k during aging or sporadic AD. NSAIDs at low concentrations partially depolarize mitochondria and inhibit mitochondrial Ca^{2+} overload, thus preventing cytochrome c release and apoptosis induced by Aβ oligomers.