

Journal Club

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Potential Effects of Aspirin on Lysosomal Biogenesis and Amyloid- β Clearance: An Old Drug and Novel Insights in Alzheimer's Disease Therapy

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Review of Chandra et al.

Alzheimer's disease (AD) is a complex, multifactorial, progressive neurodegenerative disorder and the most common form of dementia in the elderly, affecting >47 million patients worldwide and expected to affect 131 million by 2050 (Prince et al., 2016). Unfortunately, even with numerous advances in understanding AD pathophysiology over the years, there is currently no available cure or drug that prevents disease progression.

Like other neurodegenerative disorders, AD is associated with the presence of aberrant protein aggregates in the brain. The two major histopathological hallmarks of AD are amyloid plaques, which are extracellular deposits of insoluble forms of amyloid- β peptide ($A\beta$), and neurofibrillary tangles, which are intracellular aggregates of hyperphosphorylated microtubule-associated tau protein (Masters et al., 2015).

Since the original description by Alois Alzheimer, and reinforced by the "amyloid hypothesis" of AD, increasing amounts of soluble and insoluble $A\beta$ peptide in several brain areas has been considered central to the pathogenesis of AD. In particular, $A\beta$

deposition affects areas of the cerebral cortex and hippocampus, resulting in cognitive symptoms, such as learning and memory deficits, as well as some noncognitive effects (Selkoe and Hardy, 2016). Although rare, dominantly inherited genetic mutations promote $A\beta$ accumulation by accelerating cleavage of β -amyloid precursor protein (APP) and thus increasing $A\beta$ production, sporadic risk factors are thought to promote accumulation of toxic $A\beta$ monomers, oligomers, insoluble fibrils, and plaques by impairing $A\beta$ clearance mechanisms (Selkoe and Hardy, 2016).

The autophagy-lysosome system is an essential and highly regulated intracellular degradation pathway by which abnormal protein aggregates are removed from cells. Autophagosomes sequester these aggregates and transport them to lysosomes for degradation (He and Klionsky, 2009). In AD, dysfunction in autophagy and lysosomal function compromises $A\beta$ clearance. Importantly, the brain of AD patients and transgenic mice expressing AD-linked mutations (APP/PS1) exhibit accumulation of autophagic vacuoles in dystrophic neurites and a reduction in lysosomal degradation capacity (Yu et al., 2004; Nixon et al., 2005). Moreover, amyloid plaque deposition and accumulation impair the retrograde axonal transport of lysosome precursors, inducing local accumulation of immature lysosomes containing low levels of lysosomal pro-

teases, and thus reduced proteolytic capacity (Gowrishankar et al., 2015). Therefore, improvement in $A\beta$ clearance is an attractive disease-modifying strategy for AD therapy, and lysosomal function is a potential target to delay or even halt AD.

A recent report published in *The Journal of Neuroscience* (Chandra et al., 2018) has investigated the effects of low doses of aspirin, a classic and widely used nonsteroidal anti-inflammatory drug, in astrocytic lysosomal biogenesis and $A\beta$ clearance. Chandra et al. (2018) demonstrated that low doses of aspirin increased LysoTracker-Red signals and other lysosomal markers, such as lysosome-associated membrane protein 2 (LAMP2), in mouse primary astrocytes, indicating an improvement in lysosome biogenesis. Importantly, the boost in lysosome number was accompanied by greater lysosome function, assessed by catalytic activity of cathepsins and TPP1 (Chandra et al., 2018), two lysosomal proteases that facilitate $A\beta$ degradation (Wang et al., 2012; Solé-Domènech et al., 2018). In addition, Chandra et al. (2018) observed an increased number of autophagic vesicles in different stages of maturation in aspirin-treated primary astrocytes. Together, these results shed light on the potential usefulness of aspirin in reversing brain lysosomal dysfunction and related diseases.

To clarify the role of aspirin in lysosomal biogenesis, Chandra et al. (2018) examined

Received Sept. 4, 2018; revised Nov. 2, 2018; accepted Nov. 14, 2018.

I thank the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro for the postdoctoral fellowship.

The authors declare no competing financial interests.

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<https://doi.org/10.1523/JNEUROSCI.2283-18.2018>

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whether this drug affected the major regulator of the autophagy-lysosomal system, the transcription factor EB (TFEB). Aspirin increased *Tfeb* mRNA and TFEB protein levels surrounding the nucleus in mouse primary astrocytes. In addition, LAMP2 was upregulated in astrocytes after aspirin exposure, and this was abolished by TFEB silencing (Chandra et al., 2018). Overall, these findings suggested upregulation of TFEB as a key mechanism in aspirin-induced lysosomal biogenesis.

Recently, the presence of the peroxisome proliferator responsive element (PPRE) in the *Tfeb* gene promoter has been identified as a potential site of TFEB regulation by PPAR α , a transcription factor that regulates numerous genes. This regulatory mechanism has been proposed as a potential therapeutic target to improve lysosome function in lysosomal storage disorders (Ghosh et al., 2015; Ghosh and Pahan, 2016). Accordingly, using a double labeling of PPAR α and GFAP Chandra et al. (2018) showed that aspirin induced nuclear translocation of PPAR α in primary astrocytes. Importantly, aspirin-induced localization of PPAR α in the nucleus and perinuclear space has been related to an increased DNA-binding activity of PPAR α to PPRE site on the *Tfeb* gene, observed through shifted DNA migration rate in an electrophoretic mobility shift assay (Chandra et al., 2018). These findings suggested that aspirin-induced TFEB upregulation and lysosomal biogenesis in astrocytes were modulated by increased PPAR α activation.

Chandra et al. (2018) investigated whether specifically activation of PPAR α would transcriptionally regulate *Tfeb* expression. Notably, astrocytes transfected with the pPPRE-luciferase construct showed a marked increase in the PPRE-driven luciferase activity after aspirin treatment, which is prevented by deletion of *Ppara*, but not the *Pparb* gene (Chandra et al., 2018). Similarly, using primary astrocytes transfected with a construct containing a mutated PPRE core sequence on the TFEB promoter, Chandra et al. (2018) observed a reduction in the TFEB-driven luciferase activity induced by aspirin. In line with these findings, PPAR α antagonist (GW6471), unlike PPAR β (GSK0660) or PPAR γ (GW9662) antagonists, abolished aspirin-induced *Tfeb* promoter-driven luciferase activity in primary astrocytes. Collectively, these results confirmed the specific role of PPAR α in the aspirin-mediated transcriptional induction of the TFEB promoter (Chandra et al., 2018) and delineated a new pharmacological action for aspirin.

Finally, Chandra et al. (2018) explored how positive effects of aspirin in astrocytic lysosome biogenesis affect amyloid pathology *in vivo*. Oral administration of low doses of aspirin (2 mg/kg body weight/d; 1 month) increased PPAR α recruitment in hippocampal astrocytes of 5XFAD mice, an AD model that rapidly develops severe amyloid pathology. Notably, aspirin also induced higher levels of TFEB and LAMP2 in hippocampal astrocytes, indicating an improvement in lysosomal biogenesis *in vivo*.

Importantly, previous findings demonstrated that the activation of TFEB and increased lysosome number improved astrocytic A β clearance and reduced amyloid plaque load in the hippocampus of APP/PS1 mice (Xiao et al., 2014). Notably, Chandra et al. (2018) observed a remarkable reduction in A β peptide and plaque load in the hippocampus of 5XFAD mice treated with aspirin. Furthermore, in 5XFAD-*Ppara*-null mice, aspirin treatment was unable to lower A β pathology, demonstrating the relevance of PPAR α activation in the anti-amyloidogenic effects of aspirin. Nevertheless, it is possible that aspirin also stimulates non-amyloidogenic processing of APP in a PPAR α -dependent manner in line with previous findings (Corbett et al., 2015).

A remaining question is how aspirin treatment and reduction in amyloid load might improve memory deficits, the main clinical characteristic of AD, in transgenic mice. Recently, Patel et al. (2018) demonstrated that aspirin acted as a PPAR α ligand in the hippocampal neurons. Interestingly, this activity stimulated hippocampal plasticity via transcriptional activation of cAMP response element-binding protein and improved hippocampal plasticity and memory deficits in FAD5X mice, but not in FAD5X-*Ppara*-null mice. These findings indicate a positive pleiotropic action of aspirin in different pathological aspects of AD (Patel et al., 2018).

In conclusion, Chandra et al. (2018) have established a new function of aspirin in PPAR α -mediated upregulation of TFEB, leading to enhanced astrocytic lysosome biogenesis and function. Moreover, aspirin-induced lysosome biogenesis was associated with improved A β pathology in a mouse model of AD. These data suggest a new use for aspirin, one of the most widely used medications in the world, for reducing amyloid pathology in AD. This work should encourage further studies to assess how this old drug can contribute to the current challenge of treating AD.

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