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Research Report

R-flurbiprofen improves tau, but not Aß pathology in a triple transgenic model of Alzheimer's disease

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ARTICLE INFO

Article history:
Accepted 15 October 2013
Available online 22 October 2013

Keywords:
R-flurbiprofen
Hyperphosphorylated tau
Aß
3xTg-AD mice
Magnetic resonance spectroscopy

ABSTRACT

We have previously reported that chronic ibuprofen treatment improves cognition and decreases intracellular Aß and phosphorylated-tau levels in 3xTg-AD mice. Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) that independently of its anti-inflammatory effects has anti-amyloidogenic activity as a gamma-secretase modulator (GSM) and both activities have the potential to decrease Aß pathology. To further understand the effects of NSAIDs in 3xTg-AD mice, we treated 3xTg-AD mice with R-flurbiprofen, an enantiomer of the NSAID flurbiprofen that maintains the GSM activity but has greatly reduced anti-inflammatory activity, and analyzed its effect on cognition, Aß, tau, and the neurochemical profile of the hippocampus. Treatment with R-flurbiprofen from 5 to 7 months of age resulted in improved cognition on the radial arm water maze (RAWM) test and decreased the level of hyperphosphorylated tau immunostained with AT8 and PHF-1 antibodies. No significant changes in the level of Aß (using 6E10 and NU-1 antibodies) were detected. Using magnetic resonance spectroscopy (MRS) we found that R-flurbiprofen treatment decreased the elevated level of glutamine in 3xTg-AD mice down to the level detected in non-transgenic mice. Glutamine levels correlated with PHF-1 immunostained hyperphosphorylated tau. We also found an inverse correlation between the concentration of glutamate and learning across all the mice in the study. Glutamine and glutamate, neurochemicals that shuttles between neurons and astrocytes to maintain glutamate homeostasis in the synapses, deserve further attention as MR markers of cognitive function.

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0006-8993/$ - see front matter Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.brainres.2013.10.025

1. Introduction

Alzheimer's disease (AD) is an age dependent neurodegenerative disorder clinically characterized by progressive memory loss and cognitive decline. AD is the most common cause of dementia. It affects 5% of the population over the age of 65 and more than 30% of individuals over 80 years of age (Brookmeyer et al., 2007). There is no effective treatment for AD and as the society ages, AD is prone to become a major health problem. AD pathology develops over the course of decades (Braak and Braak, 1997; Jack et al., 2010; Price and Morris, 1999) but early diagnostic is not available and the definitive diagnosis only comes from the post-mortem examination of the brain.

The neuropathological hallmarks of AD are extracellular plaques, intraneuronal neurofibrillary tangles (NFT) and the degeneration and loss of neurons and synapses (Blennow et al., 2006; Selkoe, 2001). Plaques are mainly composed of fibrils of beta-amyloid (Aß) peptides surrounded by dystrophic neurites, reactive astrocytes and activated microglia. Aß peptides are produced by the sequential proteolytic cleavage of the transmembrane amyloid precursor protein (APP) by the action ß-secretase at the amino-termini and by γ-secretase at the carboxy-termini (Bayer et al., 2001). γ-Secretase is a multi-unit enzyme that cleaves APP (and other type I transmembrane proteins such as Notch) within its transmembrane domain (Lichtenthaler et al., 2002). γ-Secretase does not provide strict sequence specificity and generates Aß peptides of different lengths, mainly peptides of 40 (Aβ40) and 42 (Aβ42) residues. The longer Aß42 peptide is more prone to aggregation and despite of being much less abundant than Aβ40 is the initial and the most predominant Aβ species in plaques. Soluble Aβ42 peptides and oligomers are neurotoxic and thought to be central to AD pathogenesis (Lacor et al., 2007; Shankar et al., 2008). The amyloid hypothesis, the most popular working hypothesis for AD pathogenesis, supports that the modest increase of Aß42 peptide in the brain is sufficient to cause AD with complete penetrance (Hardy and Selkoe, 2002). NFT are intracellular fibrillar aggregates primarily composed of abnormally hyperphosphorylated forms of the microtubule-associated protein tau. Phosphorylated tau sequesters normal tau and other microtubule-associated proteins causing disassembly of microtubules and impaired axonal transport, compromising neuronal/synaptic function (Iqbal et al., 2005). Tau pathology is initially evidenced by the somatodendritic accumulation of conformationally altered, non-fibrillar tau, followed by the accumulation of progressively hyperphosphorylated tau detected by CP-13 antibody first and later by AT8 and PHF-1 antibodies. PHF-1 labels the fibrillar NFT in neuronal perikarya and neuritic processes (Greenberg et al., 1992; Lewis et al., 2001). It is widely considered that in AD tau phosphorylation is a consequence of Aß accumulation despite the fact that hyperphosphorylation and accumulation of pathological forms of tau is found in other neurodegenerative diseases in the absence of Aß and has been extensively linked to neurodegeneration (Lee et al., 2001).

Another important feature of AD pathology is the local inflammatory response, particularly involving microglia. Aß plaques are consistently surrounded by microglia that display an activated phenotype characterized by enhanced expression of immune cell surface markers and the production of proinflammatory cytokines and chemokines (Akiyama et al., 2000). The finding in multiple epidemiological studies that treatment with non-steroidal anti-inflammatory drugs (NSAIDs) was associated with a reduced risk of developing AD was initially taken as proof that inflammation is a pathogenic factor in AD (Aisen, 2002; McGeer and Rogers, 1992; McGeer et al., 1996; McGeer and McGeer, 2007; Townsend and Pratico, 2005). However, the results of pharmacological studies with anti-inflammatory agents have been inconsistent in both mice and humans (Imbimbo, 2009a).

Interest in the effects of NSAIDs on AD grew faster after a subset of NSAIDs was found to decrease the production of the more amyloidogenic $Aß_{42}$ peptide at the expense of shorter and less toxic Aß forms (Eriksen et al., 2003; Weggen et al., 2001). The $Aß_{42}$-lowering action of some NSAIDs is independent of their anti-inflammatory action on cyclooxygenase (COX) and appears to involve the allosteric modulation of γ-secretase (Lleo et al., 2004). The effect of $Aß_{42}$-lowering NSAIDs, unlike the action of γ-secretase inhibitors that uncovered the essential role of γ-secretase activity in the proteolytic processing of the Notch receptor (Beher et al., 2004), is specific to the production of Aß without significantly perturbing the action of γ-secretase on other substrates (Eriksen et al., 2003; Weggen et al., 2001; Weggen et al., 2003). The increasing number of small molecules able to decrease production of $Aß_{42}$ is collectively termed γ-secretase modulators (GSMs) (Bulic et al., 2011). Among NSAIDs, ibuprofen and flurbiprofen are among the most prominent GSM. Treatment with ibuprofen, that has dual anti-inflammatory and GSM activity, has shown striking preventive effects in several mouse models of AD reducing $Aß_{42}$ deposition and/or Aß load (Choi et al., 2010; Dedeoglu et al., 2004; Heneka et al., 2005; Jantzen et al., 2002; Lim et al., 2000; Lim et al., 2001; Yan et al., 2003), and significantly improving performance on behavioral tasks (Lim et al., 2000; Lim et al., 2001; Yan et al., 2003). In the triple transgenic mouse model of AD (3xTg-AD) that develops Aß and tau pathology (Oddo et al., 2003), ibuprofen reduced both the level of Aß accumulation and tau phosphorylation together with improved spatial learning and memory (McKee et al., 2008). Treatment with R-flurbiprofen, an enantiomer of flurbiprofen that maintains the GSM activity in vitro and in vivo (Eriksen et al., 2003; Morihara et al., 2002), but lacks anti-inflammatory activity (Geisslinger et al., 1994; Wechter et al., 1994), attenuated learning impairments in the APP Tg2576 mouse model of AD (Kukar et al., 2007) and lowered $Aß_{42}$ in vivo (Eriksen et al., 2003; Morihara et al., 2002). A phase 2 trial of R-flurbiprofen suggested that patients with mild AD, but not moderate AD, had a dose-related slower rate of decline than those treated with placebo, and that the drug was well tolerated with few adverse effects (Wilcock et al., 2008). On the basis of these promising results, a large phase 3, randomized, placebo controlled trial of tarenflurbil was conducted in patients with mild AD. Disappointing results of the phase 3 trial showed no effect of R-flurbiprofen slowing cognitive decline or loss of activities of daily living in patients with mild AD nor did