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Tolfenamic acid downregulates BACE1 and protects against lead-induced upregulation of Alzheimer's disease related biomarkers

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ABSTRACT

Environmental exposure to lead (Pb) early in life results in a latent upregulation of genes and products associated with Alzheimer's disease (AD), particularly the plaque forming protein amyloid beta (Aβ). Furthermore, animals exposed to Pb as infants develop cognitive decline and memory impairments in old age. Studies from our lab demonstrated that tolfenamic acid lowers the levels of the amyloid β precursor protein (APP) and its aggregative cleavage product Aβ by inducing the degradation of the transcription factor specificity protein 1 (Sp1). These changes were accompanied by cognitive improvement in transgenic APP knock-in mice. In this study, we examined the effects of tolfenamic acid on beta site APP cleaving enzyme 1 (BACE1) which is responsible for Aβ production and tested its ability to reverse Pb-induced upregulation in the amyloidogenic pathway. Mice were administered tolfenamic acid for one month and BACE1 gene expression as well as its enzymatic activity were analyzed in the cerebral cortex. Tolfenamic acid was also tested for its ability to reverse changes in Sp1, APP and Aβ that were upregulated by Pb *in vitro*. Differentiated SH-SY5Y neuroblastoma cells were either left unexposed, or sequentially exposed to Pb followed by tolfenamic acid. Our results show that tolfenamic acid reduced BACE1 gene expression and enzyme activity in mice. In neuroblastoma cells, Pb upregulated Sp1, APP and Aβ, while tolfenamic acid lowered their expression. These results along with previous data from our lab provide evidence that tolfenamic acid, a drug that has been used for decades for migraine, represents a candidate which can reduce the pathology of AD and may mitigate the damage of environmental risk factors associated with this disease.

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1. Introduction

A century has passed since the disease was first described by Alois Alzheimer and about 35 million patients around the world suffer today from Alzheimer's disease (AD) without any potential cure (Anstey et al., 2013; Selkoe, 2012). The majority of AD cases are sporadic and the exact causes of the disease are unknown. As no means for prevention are available, the number of AD cases and the enormous economic costs of this devastating disease will continue to grow at an alarming rate. Knowledge on the pathophysiology of the disease continues to be gathered and reveal more possible drug targets and disease biomarkers. Two types of deposits are found in the AD brain, the amyloid plaques and the tau neurofibrillary tangles (Terry et al., 1964; Tomlinson, 1982). A lot of attention has been directed to the plaques and their main constituent amyloid beta (Aβ) as well as intermediates in Aβ production or degradation, especially after the development of the amyloid cascade hypothesis which views Aβ as a major trigger in AD pathology (Hardy and Selkoe, 2002; Hardy and Higgins, 1992). However, so far no disease-modifying drug for AD is available.

Aβ is generated following the sequential enzymatic processing of the amyloid β precursor protein (APP) by β-secretase and γ-secretase (Shoji et al., 1992). The produced Aβ is normally secreted, but also can accumulate and form insoluble aggregates (Shoji et al., 1992; Urbanc et al., 1999). The levels and activity of β-secretase are elevated in AD brains compared to control (Holsinger et al., 2002; Li et al., 2004). β-APP cleaving enzyme 1 (BACE1) is the main form of β-secretase that cleaves APP to generate Aβ (Cai et al., 2001). In an alternative pathway for processing APP, it can be cleaved by the enzyme α-secretase within the Aβ fragment resulting in non-amyloidogenic products (Selkoe, 1994). Aβ is found as 36–43

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; ANOVA, analysis of variance; APP, amyloid β precursor protein; BACE, beta-site APP cleaving enzyme; FBS, fetal bovine serum; Pb, lead; SEM, standard error of the mean; Sp1, specificity protein 1; SP1, Sp1 protein; YAC, yeast artificial chromosome.

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amino-acid-long peptides of which $A\beta_{40}$ is the most abundant and $A\beta_{42}$ is the most aggregative and is proposed to trigger plaque formation in AD (Iwatsubo et al., 1994; Nakano et al., 1999; Naslund et al., 2000).

Specificity protein 1 (Sp1) is a transcription factor that has been associated with the pathology of AD (Basha et al., 2005; Santpere et al., 2006; Zawia and Basha, 2005). Sp1 acts as a co-activator of APP transcription and regulates the expression of BACE1 (Christensen et al., 2004; Docagne et al., 2004). Sp1 regulates gene transcription by binding to GC rich promoter regions in genes like APP and BACE1 whose binding to Sp1 increases their transcription (Christensen et al., 2004; Docagne et al., 2004; Hoffman and Chernak, 1995; Pollwein et al., 1992). Overexpression of Sp1 increases BACE1 promoter activity, while the decline in Sp1 reduces BACE1 gene transcription (Christensen et al., 2004). Immunohistochemical studies from our laboratory demonstrated that Sp1 protein (SP1), APP, and Aβ co-localize in brain neurons, and that cortical and hippocampal areas with higher SP1 levels express more Aβ (Brock et al., 2008). Therefore, changes in Sp1 expression can influence APP and BACE1 transcription and consequently alter the levels of their downstream product Aβ. Sp1 represents a potential AD target, where its abnormal and elevated expression has been associated with the disease decline (Brock et al., 2008; Christensen et al., 2004; Citron et al., 2008; Hoffman and Chernak, 1995; Santpere et al., 2006; Zawia and Basha, 2005).

Exposure to the environmental toxicant lead (Pb) is considered a risk factor with detrimental effects on various organs especially the brain (White et al., 2007; Zawia and Basha, 2005; Zawia et al., 2009). Experiments conducted at our lab demonstrated that Pb exposure early in life results in AD like pathology *in vitro* and *in vivo*, in rodents and primates (Basha et al., 2005; Wu et al., 2008). Pb administration caused the upregulation of Sp1, APP, Aβ, BACE1 and other intermediates implicated in AD later in life (Basha et al., 2005; Bihaqi et al., 2013, 2011; Bihaqi and Zawia, 2012; Huang et al., 2011; Wu et al., 2008; Zawia et al., 2009). Our most recent studies revealed that these molecular changes were accompanied by cognitive deterioration in mice administered Pb compared to controls (Bihaqi et al., 2013). On the other hand, studies in our lab also showed that promoting SP1 degradation by oral administration of the anti-migraine drug tolfenamic acid to mice reduces APP and Aβ levels as well as improves cognition (Adwan et al., 2011; Subaiea et al., 2013).

Since the transcription factor Sp1 is vital for the regulation of several genes involved in AD including BACE1, this research study was conducted to assess the effect of tolfenamic acid administration to APP yeast artificial chromosome (YAC) transgenic mice on BACE1, as a major enzyme in the production of Aβ, that is under Sp1 regulation. We also utilized an *in vitro* model of Pb exposure established in our lab to test the ability of tolfenamic acid to rescue proteins upregulated following Pb exposure, which induces molecular consequences that resemble pathological events observed in late onset AD (Bihaqi and Zawia, 2012; Huang et al., 2011). Following cell viability studies, differentiated SH-SY5Y cells were exposed to Pb, tolfenamic acid or both agents in chronological order and the changes on SP1, APP and Aβ were examined in comparison to control. An illustration of the mechanism of action of tolfenamic acid induced APP and BACE1 reduction can be found in Fig. 1.

2. Materials and methods

2.1. Animals

Female hemizygous APP YAC transgenic mice (line R1.40) were used in this study. The B6.129-Tg(APPSw)40Btla/Mmjax strain was obtained from the Jackson Laboratory, Bar Harbor, ME. Animals were bred in-house and the age of the mice used in this study was between 14 and 20 months. This AD animal model contains the entire human APP gene including the regulatory fragments and expresses elevated levels of Aβ especially the longer more aggregative forms $A\beta_{42}$ and $A\beta_{43}$ (Lamb et al., 1999, 1997; Lehman et al., 2003). Animals were housed in designated

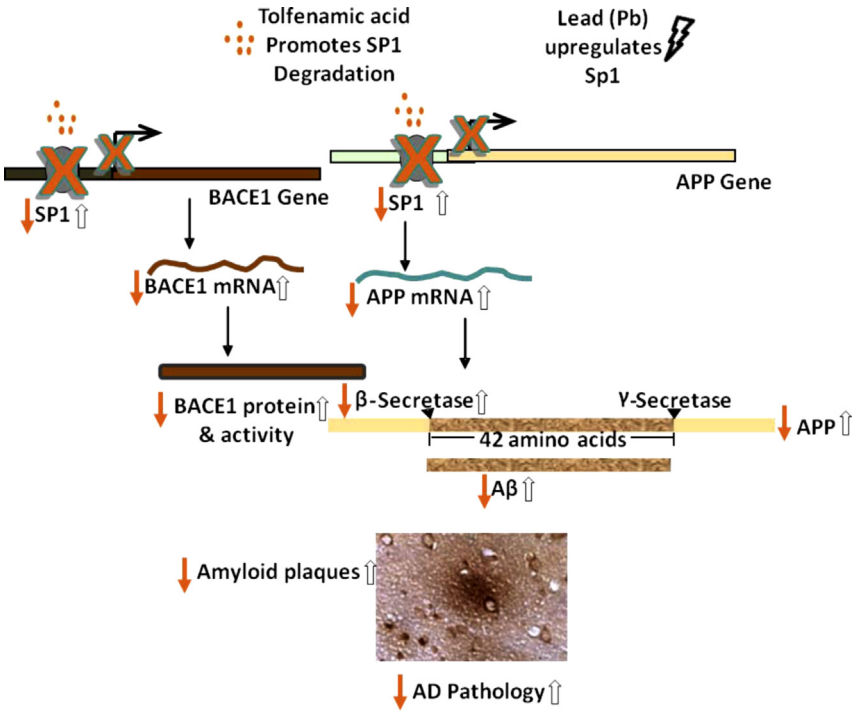

Fig. 1. Downregulation of BACE1 and APP by tolfenamic acid and their upregulation by early Pb exposure. Tolfenamic acid stimulates the degradation of the transcription factor Sp1, which reduces the transcription of APP and BACE1, consequently reducing the expression of BACE1 and APP as well as the aggregative product Aβ and the associated AD pathology. On the other hand, studies from our lab have revealed that Pb exposure early in life upregulates the expression of Sp1, APP, BACE1, Aβ and induces AD like pathology later in life. The solid arrows represent the hypothetical consequences following tolfenamic acid exposure, whereas the hollow arrows represent the latent effects after Pb exposure.

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