Molecular Immunology 99 (2018) 95–103

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Oleanolic acid protects against cognitive decline and neuroinflammation-mediated neurotoxicity by blocking secretory phospholipase A2 IIA-activated calcium signals

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ARTICLE INFO

Keywords:
Oleanolic acid
Alzheimer's disease
Cognitive decline
Neuroinflammation
Neurotoxicity
Astrocyte

ABSTRACT

Neuroinflammation causes neurotoxic injury and underlies the pathogenesis of neurodegenerative disorders including Alzheimer's disease (AD). Astrocytes are the predominant immunoregulatory cells in AD. Oleanolic acid (OA) is a promising anti-inflammatory therapeutic agent that can ameliorate cerebral damage in ischemic environments, but its role in AD remains poorly elucidated. Here, preconditioning with OA inhibited the transcription and secretion of inflammatory cytokines IL-6, TNF-α, and IL-1β in amyloid-beta peptide (Aβ)-activated astrocytes. Moreover, OA ameliorated primary neuron death triggered by incubation in conditioned medium from Aβ-treated astrocytes. Furthermore, OA also suppressed Aβ-induced expression and production of group IIA secretory phospholipase A2 (sPLA2-IIA) in astrocytes. Supernatants supplemented with exogenous sPLA2-IIA reversed the protective role of OA against astrocyte activation-mediated neurotoxicity by suppressing cell viability and increasing LDH release, apoptosis, the contents of neurotoxic mediator arachidonic acid, and prostaglandin D2. Simultaneously, treatment with sPLA2 inhibitor aristolochic acid also counteracted neurotoxicity induced by Aβ-activated astrocytes through increasing cell viability, inhibiting cell apoptosis, and reducing the releases of arachidonic acid and prostaglandin D2. Additionally, OA restrained Ca^{2+} influx in neurons after incubation with supernatants from Aβ-activated astrocytes, which was abrogated by adding sPLA2-IIA. Activating Ca^{2+} signaling with BayK, an L-type Ca2 + channel agonist, reversed the beneficial role of OA against neurotoxicity induced by astrocyte activation-mediated inflammatory response. OA also ameliorated cognitive deficits in an adolescent rat model of Aβ-evoked AD. These findings confirm that OA abrogates neuroinflammation and subsequent neurotoxicity induced by conditioned media from Aβ-activated astrocytes in sPLA2-IIA mediated-calcium signals. Therefore, OA may protect neurons from injury caused by neighboring astrocyte activation in AD, indicating a promising therapeutic strategy against AD.

1. Introduction

Neurodegenerative diseases (ND) constitute the greatest health risk in the elderly with a hallmark of neuron deterioration and loss. Alzheimer's disease (AD) is among the most common causes of progressive dementia form worldwide, accounting for approximately 60%–80% of dementia cases (Alzheimer's Association, 2016). The clinical symptoms of AD include progressive cognitive decline, memory deterioration and inability to perform daily activities(Finder, 2010). Therefore, AD often represent an enormous disease load, regarding the gloomy life-quality and heavy economic cost. With the continuously increasing incidence, the number of patients with AD is projected to increase to 115.4 million by 2050(Abdulrahman, 2014).

Neuroinflammatory response has emerged recently as a predominant factor in AD(Calsolaro and Edison, 2016; Sawikr et al., 2017). Astrocytes, the most abundant glial cells in the central nervous system (CNS), are endowed to support neuronal and synaptic functions. Intriguingly, astrocytes have been recently identified as critical contributors to neuroinflammatory processes in AD, based on their activation in various pathological conditions (such as infection, ischemia, and ND)(Steardo et al., 2015). AD is characterized by amyloid-beta peptide (Aβ) deposition in senile plaques and intracellular neurofibrillary tangles comprising hyperphosphorylated tau. In AD, aberrant formation and deposition of Aβ injures neuron(Rama Rao and Kielian et al., 2015; Urrutia et al., 2017). Furthermore, Aβ stimulation facilitates AD by inducing tau phosphorylation, aggregation and fibrillization(Ballatore et al., 2007). Notably, astrocytes are necessary for soluble oligomeric Aβ-induced tau phosphorylation and neurotoxicity

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https://doi.org/10.1016/j.molimm.2018.04.015
Received 6 November 2017; Received in revised form 27 April 2018; Accepted 28 April 2018

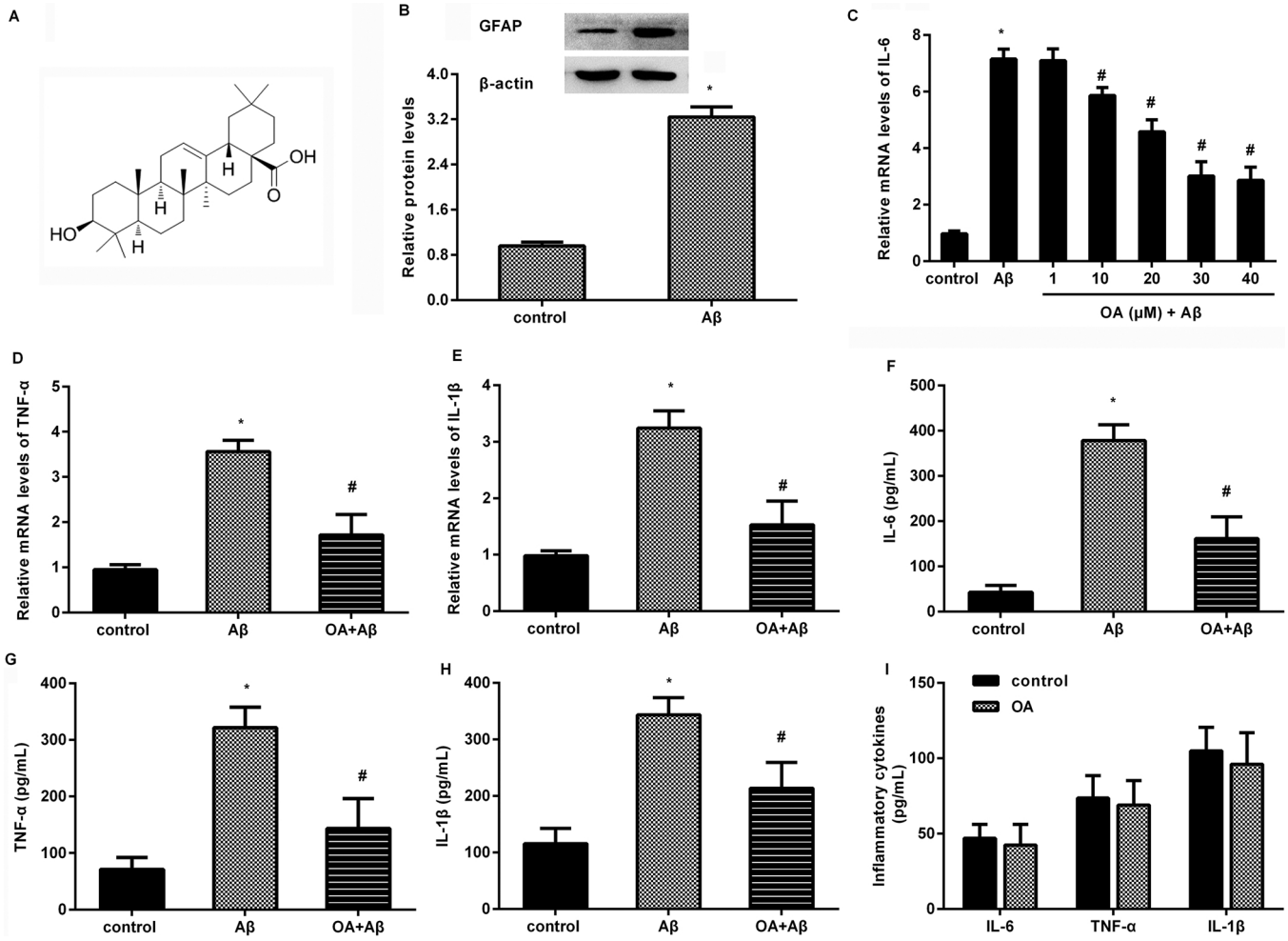

Fig. 1. OA inhibits inflammatory response in Aβ-activated astrocytes. (A) Chemical structure of OA. (B) Astrocytes were exposed to 10 μg/ml Aβ1-42 for 24 h. The protein levels of GFAP were determined by western blotting. (C) Before exposure to Aβ for 24 h, astrocytes were preconditioned with the indicated doses of OA for 5 h. The mRNA levels of IL-6 were evaluated by qRT-PCR. (D, E) The transcript levels of TNF-α (D) and IL-1β (E) were also measured by qRT-PCR. (F–H) The content of IL-6 (F), TNF-α (G) and IL-1β (H) in the medium of astrocytes were detected by ELISA. (I) Astrocytes were incubated with OA (30 μM) for 5 h, or not. ELISA assay was performed to determine the inflammatory cytokine levels of IL-6, TNF-αand IL-1β. $^{*}P < 0.05$ vs. control group. $^{\#}P < 0.05$vs. Aβ-treated group.

(Garwood et al., 2011). Oligomeric form of Aβ1–42 exhibits stronger neurotoxicity than fibrillar forms(Jang et al., 2017; Takahashi et al., 2004). More importantly, neurotoxic soluble oligomeric Aβ is a major contributor to cognitive dysfunction in AD(Jang et al., 2017; Takahashi et al., 2004). The amyloid-β_{42} proxy, amyloid-β (25–35), induces normal human cerebral astrocytes and cortical neurons to produce amyloid-β_{42}(Armato et al., 2013; Dal Pra et al., 2011). Stimulation with Aβ also activates astrocytes with a feature of intermediate filament glial fibrillary acidic protein (GFAP) overexpression, and subsequently perturb neuron homeostasis and induce neurotoxic injury by exerting inflammatory effector functions(Rama Rao and Kielian et al., 2015; Urrutia et al., 2017). Blocking astrocyte activation abrogates astrocytic inflammatory response, and decreases neuron death(Garwood et al., 2011).

Oleanolic acid (OA) is a bioactive triterpenoid compound with a pentacyclic structure (Fig. 1A) that is abundant in plants. It is traditionally used to treat hepatitis in China. However, recent study shows that OA plays important roles in numerous biological processes, such as anti-oxidative stress, anti-apoptosis and anti-inflammation(Kashyap et al., 2016; Tsao and Yin, 2015; Zhao et al., 2017). Increasing evidence has revealed OA as a promising therapeutic agent against inflammation-related diseases(Choi et al., 2016; Kashyap et al., 2016). For instance, treatment with OA rejuvenates testicular function by deactivating the NF-κB pathway, which suppresses inflammatory cytokine production and germ cell damage (Zhao et al., 2017). Moreover, OA protects bronchial epithelial cells against H2O2-induced apoptotic, oxidative, and inflammatory stress(Tsao and Yin, 2015). Emerging research substantiates the neuroprotective effects of OA on cerebral damage in ischemic environment(Caltana et al., 2015; Rong et al., 2011). Unfortunately, the function of OA in AD remains poorly elucidated. Thus, we aimed to explore the role and underlying mechanism of OA in astrocyte activation-induced neurotoxicity.

2. Materials and methods

2.1. Experiment animals and ethics statement

Male Sprague-Dawley (SD) rats (weighing 220–250 g, 7–8 weeks old) were purchased from the Center of Laboratory Animals at the Fourth Military Medical University. Before the experiment, rats were acclimatized for at least 1 week. All animals were housed in a room with 12 h light/dark cycles and maintained at 25 °C, during which they had free access to food and water. All experimental procedures were conducted according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Cangzhou Central Hospital.