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AMELIORATION OF AMYLOID β-INDUCED RETINAL INFLAMMATORY RESPONSES BY A LXR AGONIST TO901317 IS ASSOCIATED WITH INHIBITION OF THE NF-κB SIGNALING AND NLRP3 INFLAMMASOME

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Abstract—Amyloid β (Aβ) is a pathogenic peptide associated with many neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. The retinal inflammation in response to Aβ is implicated in the pathogenesis of several ocular diseases including age-related macular degeneration, Alzheimer's-related optic neuropathy and glaucoma. In the present study, we found that a single intravitreal injection of oligomeric Aβ1-40 in mouse activated the NLRP3 inflammasome and the NF-κB signaling, induced the production of inflammatory cytokines including TNF-α and IL-6. In addition, Aβ1-40 caused retinal function impairment while no noticeable morphological changes were observed under light microscope. Furthermore, immunohistochemical results showed that Aβ1-40 enhanced the number of Iba1-positive cells in the inner retina. The mRNA expressions of LXRα and LXRβ decreased in the neuroretina of the Aβ1-40-injected mice. No significant difference was found on the protein expressions of LXRs and ABCA1 in both neuroretina and RPE/choroid complex between the Aβ1-40-injected group and the control group. A synthetic LXR ligand, TO901317 (TO90), enhanced the expressions of LXRα and ABCA1 at both mRNA and protein levels in the Aβ1-40-injected mice, while the LXRβ expression was unchanged. TO90 preserved ERG a- and b-wave amplitudes and reduced the number of Iba1-positive cells in the Aβ1-40-treated retina. Furthermore, TO90 down-regulated the mRNA levels of TNF-α and IL-6, as well as the expressions of p-IκBα, NLRP3, caspase-1 and IL-1β in the Aβ1-40-injected animals. We suggest that activation of LXRα and its target gene ABCA1 exerts potent anti-inflammatory effect on the Aβ-treated retina.

Key words: Amyloid β, liver x receptors, NLRP3 inflammasome, NF-κB, retinal inflammatory response.

INTRODUCTION

Amyloid β (Aβ) is a pathogenic peptide associated with many neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's Disease (PD) (Criscuolo et al., 2017; Shao et al., 2017; Takata et al., 2012). Recently, it has been shown that Aβ-related amyloidosis also occurs during several ocular diseases including Alzheimer's-related optic neuropathy, glaucoma and age-related macular degeneration (AMD) (Criscuolo et al., 2017; Lynn et al., 2017; Masuzzo et al., 2016). It has been suggested that Aβ activates microglia (Santos et al., 2017) and promotes the generation of cytokines and oxygen species, including nitric oxide (NO) and tumor necrosis factor-α (TNF-α) in AD (Xie et al., 2002). Aβ exists in two forms: Aβ1-42 is mainly associated with AD plaques while Aβ1-40 is more prevalent in eye diseases (Ding et al., 2011; Isas et al., 2010; Kurji et al., 2010). Recent studies showed that a single intravitreal injection of Aβ1-40 induced retinal inflammatory responses which mimicked those observed in the early phases of AMD (Liu et al., 2013, 2014).

It was reported that activation of the nucleotide-binding oligomerization domain leucine-rich repeats containing pyrin domain 3 (NLRP3) inflammasome and NF-κB inflammatory pathway is involved in Aβ-induced neuroinflammation (Halle et al., 2008; Liu et al., 2013, 2014; Shi et al., 2013; Zhao et al., 2015). Emerging evidence in animal models showed that activation of NLRP3 inflammasome accelerated the progression of neuroinflammation in different neurodegenerative conditions, including dry AMD (Doyle et al., 2012; Liu et al., 2013;

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Abbreviations: ABCA1, ATP-binding cassette transporters A1; AD, Alzheimer's disease; AMD, age-related macular degeneration; Aβ, Amyloid β; CNV, choroidal neovascularization; DMSO, dimethyl sulfoxide; EAU, experimental autoimmune uveitis; ERG, electroretinogram; Iba1, ionized calcium-binding adaptor molecule 1; IL-6, interleukin-6; IκB, inhibition factor-κB; LXRs, liver X receptors; NF-κB, nuclear factor kappa B; NLRP3, nucleotide-binding oligomerization domain leucine-rich repeats containing pyrin domain 3; NMDA, N-methyl-D-aspartate; PD, Parkinson's disease; PVDF, polyvinylidenedifluoride; RIPA, Radio Immuno Precipitation Assay; RPE, retinal pigment epithelium; TNF-α, tumor necrosis factor-α; TO90, TO901317; VEGF, vascular endothelial growth factor.

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Marneros, 2013; Tarallo et al., 2012), diabetic retinopathy (Devi et al., 2012), and glaucoma (Chi et al., 2015). NLRP3 inflammasome is an innate immune complex. Once activated, it cleaves caspase-1 precursor (pro-caspase-1) into an active caspase-1. Caspase-1 results in the cleavage of IL-1β and IL-18 from its proform such as pro-IL-1β and pro-IL-18 to its enzymatically active mature cytokines IL-1β and IL-18 (Liu et al., 2013; Tarallo et al., 2012). Nuclear factor kappa B (NF-κB), the important priming signaling in the activation of NLRP3 inflammasome, mediates the synthesis of the inactive pro-IL-1β which converts into an active mature IL-1β by caspase-1-dependent cleavage. NLRP3 inflammasome couples with NF-κB inflammatory pathway to mediate IL-1β transcription (Bauernfeind et al., 2009).

Liver x receptors (LXRs) are members of the nuclear receptor superfamily of ligand-activated transcription factors and are involved in the regulation of cholesterol homeostasis and lipid metabolism (Calkin and Tontonoz, 2010; Chen and Smith, 2013; Sene et al., 2013). Both LXR isoforms, LXRα and LXRβ, are expressed in numerous tissues including the retina and different receptor subtypes are activated by LXR agonists at different conditions: LXRα is activated in experimental autoimmune uveitis (EAU) while LXRβ is activated in N-methyl-D-aspartate (NMDA)-induced retinal neuron damage (Yang et al., 2014; Zheng et al., 2015). As one important target gene of LXRs, ATP-binding cassette transporters A1 (ABCA1) seems to play a key role in the regulation of inflammation, retinal neuron damage, as well as cholesterol homeostasis.

The anti-inflammatory activity of TO901317 (TO90), an LXR agonist, has been primarily ascribed to the inhibition of NF-κB and the activation of ABCA1. However, it remains unknown whether LXR agonist could suppress NLRP3 inflammasome in the process of retinal inflammation. In this study, we tested the hypothesis that by activating ABCA1, TO90 presented dual inhibition on the activation of NLRP3 inflammasome and NF-κB signaling in a mouse model of retinal inflammation induced by Aβ1-40.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed in 6- to 8-week-old C57BL/6J mice purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and housed in the Laboratory Animal Center of Chongqing Medical University (Chongqing, China). Mice were maintained on a 12-hour light/dark cycle and provided with food and water ad libitum. All experimental procedures involving animals were performed in accordance with the ARVO statement for the use of Animals in Ophthalmic and Vision Research. The protocol was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. Every effort was made to minimize animal discomfort and stress.

Preparation of Aβ1-40

Previous reports illustrated that the deposit of fibrillar material stayed at the vitreal interface after intravitreal injection (Jen et al., 1998). However, diffuse staining of soluble amyloid crossed the retina and extended as far as the outer plexiform layer (Howlett et al., 2011). Besides, it is likely that Aβ-induced toxicity in the retina, as in the brain, is due to the formation of toxic amyloid structures, inasmuch as Aβ oligomers exert cellular toxicity, whereas soluble Aβ monomers do not (Glabe, 2004, 2008). Therefore, we choose the oligomeric form of Aβ in our study.

Human Aβ1-40 (Catalog Number: 03-136) and Aβ40-1 (Catalog Number: 03-245) were purchased from (Invitrogen, Carlsbad, CA, USA). Oligomeric Aβ1-40 was prepared as reported (Liu et al., 2013). Briefly, soluble Aβ1-40 peptide (1.0 mg) was dissolved in 400 μl of hexafluoroisopropanol for 10–20 min at room temperature. 100 μl of the resulting seedless solution was added to 100 μl sterile phosphate-buffered saline (PBS, without Ca^{+}) in a siliconized Eppendorf tube. After 10–20 min incubation at room temperature, the samples were centrifuged for 15 min at 14,000×g, and the supernatant fraction was transferred to a siliconized tube and subjected to a gentle stream of nitrogen for 5–10 min to evaporate the hexafluoroisopropanol. The final concentration was 2.5 μg/μl. The samples were stirred at 500 rpm for 24–48 h at 22 °C. Aliquots of this solution were kept at −80 °C until use. The reverse peptide Aβ40-1 was prepared in an identical manner.

To determine the optimal concentration and time for inducing and measuring the inflammatory responses, different concentrations of oligomeric Aβ1-40 (2 μl, 0.5, 1.0, 1.5, 2.0 μg/μl) and PBS (2 μl, as the control) were injected intravitreally. The expressions of inflammatory cytokines IL-6, TNF-α and NLRP3 were measured with real-time PCR at different time points (day 1, 4, and 14) post injection, the results are shown in Fig. 1. In accordance with other studies (Liu et al., 2013, 2014), oligomeric Aβ1-40 enhanced the expressions of the pro-inflammatory cytokines at day 1 and day 4. Based on these preliminary results, an Aβ1-40 concentration of 1.0 μg/μl and a time at day 4 were selected in following experiments.

Treatment with TO90

TO90 (Cayman, Ann Arbor, MI, USA) was dissolved in 100% dimethyl sulfoxide (DMSO) and then diluted with PBS to a final DMSO concentration of 2%, as described previously (Yang et al., 2014; Zheng et al., 2015). The 2% DMSO with PBS as the vehicle. TO90 (50 mg/kg/d) or vehicle was intragastrically administered daily from 3 days before to 4 days after Aβ injection.

Induction of retinal inflammatory response

To detect whether Aβ could activate microglial cells in the retina, a small part of C57BL/6J mice were randomly divided into three groups: (1) the PBS-treated group as a control; (2) the Aβ40-1-treated group; (3) the Aβ1-40-treated group.

In the following experiments, C57BL/6J mice were randomly divided into three groups: (1) Aβ1-40 plus TO90 group, in which mice were treated with TO90 then injected