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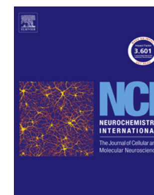

Dalesconols B inhibits lipopolysaccharide induced inflammation and suppresses NF-κB and p38/JNK activation in microglial cells

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ABSTRACT

Therapeutic strategies designed to inhibit the activation of microglia may lead to significant advancement in the treatment of most neurodegenerative diseases. Dalesconols B, also termed as TL2, is a newly found polyketide from a mantis-associated fungus and has been reported to exert potent immunosuppressive effects. In the present study, the anti-inflammatory effects of TL2 was investigated in lipopolysaccharide (LPS)-treated BV2 microglia and primary microglia cells. Our observations indicated that pretreatment with TL2 significantly inhibited the production of NO and PGE2 and suppressed the expression of pro-inflammatory mediators such as inducible nitric oxide synthase (iNOS), COX-2, TNF-α, IL-1β, IL-6, MCP-1 and MIP-1α in LPS-stimulated BV2 microglia. The nuclear translocation of NF-κB and the phosphorylation level of Akt, p38 and JNK MAP kinase pathways were also inhibited by TL2 in LPS-treated BV2 microglia. Moreover, TL2 also decreased Aβ-induced production of TNF-α, IL-1β and IL-6 in BV2 microglia. Additionally, TL2 protected primary cortical neurons against microglia-mediated neurotoxicity. Overall, our findings suggested that TL2 might be a promising therapeutic agent for alleviating the progress of neurodegenerative diseases associated with microglia activation.

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1. Introduction

Microglia, resident immune cells of central nervous system (CNS), are active sensors and versatile effectors in normal and pathologic brain (Hanisch and Kettenmann, 2007). Under normal conditions, microglia are essential for various cellular functions (Graeber, 2010; Nimmerjahn et al., 2005), including clearance of cellular debris, release of neurotrophic factors, synaptic plasticity, tissue repair through alternative activation, et.al. In the presence of diverse stimuli (Block et al., 2007), such as pathogens, neuron damage, β-amyloid (Aβ), microglia are activated and produce a variety of cytotoxic and pro-inflammatory factors, such as nitric oxide (NO), prostaglandin E2 (PGE2), cyclooxygenase (COX)-2, tumor necrosis factor-α (TNF-α), interleukin(IL)-1β, IL-6, monocyte chemotactic protein-1 (MCP-1/CCL2) and macrophage inhibitory factor-1α (MIP-1α/CCL3) (Block et al., 2007; McGeer and McGeer, 1995). While limited production of these factors are essential for host defense and tissue repair, uncontrolled accumulation of these factors released by hyperactivated microglia is detrimental to neighboring neurons (Block et al., 2007), and has been thought to be associated with the pathogenesis of a number of neurodegenerative diseases, including Parkinson disease (PD) and Alzheimer's disease (AD) (Block and Hong, 2005; Block et al., 2007; Perry et al., 2010). Therefore, attenuation of microglia hyperactivation has been considered as a promising therapeutic approach for a number of neurodegenerative diseases.

LPS, a major component of the outer cell wall of Gram-negative bacteria, is a well established activator of microglia (Nakamura, 2002). Recognized by Toll-like receptor (TLR)-4 expressed on microglia, LPS triggers the activation of a cascade of enzymes and transcription factors, including nuclear transcription factor kappa-

Abbreviations: Aβ, β-amyloid; AD, Alzheimer's disease; CNS, central nervous system; COX-2, cyclooxygenase-2; eNOS, endothelial NOS; ERK, extracellular signal-regulated kinases; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; iNOS, inducible NOS; JNK, c-Jun N-terminal kinases; LPS, lipopolysaccharide; MAP, mitogen-activated protein; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inhibitory factor-1α; nNOS, neuronal NOS; NO, nitric oxide; NOS, Nitric oxide synthase; PD, Parkinson's disease; PGE2, prostaglandin E2; TNF-α, tumor necrosis factor-α.

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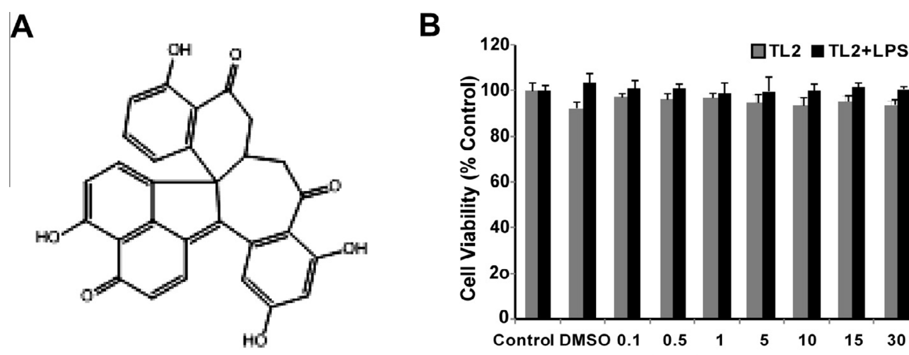

Fig. 1. Cell toxicity of TL2. (A) Structure of TL2. (B) BV-2 microglia were treated with various concentrations of TL2 in the absence or presence of 0.1 μg/ml LPS for 24 h. Cell viability was measured by MTT assay.

B (NF-κB) and mitogen-activated protein kinases (MAPKs), such as p38 MAP kinase, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK), resulting in the induction of numerous pro-inflammatory mediators such as NO, TNF-α, IL-1β and IL-6 (Block and Hong, 2005; Rankine et al., 2006). LPS-stimulated microglia has been a commonly used model to study microglia activation *in vitro*.

Inspired by the discovery of CsA, rapamycin and FK506, microorganisms are thought as a reliable source of immunosuppressing compounds (Zhang et al., 2008). Recently, a number of studies focused on symbionts, particularly some insect-associated fungi, as they can produce metabolites with potent immunoalleviating activity during longtime co-evolution with hosts (Thomas and Read, 2007; Zhang et al., 2008). Dalesconols B, also termed as TL2 (Fig. 1A), is a newly discovered polyketide isolated from the scaled-up fermentation of the mantis species *Tenodera aridifolia*-associated fungus *Daldinia eschscholzii* IFB-TL01 (Zhang et al., 2008). T-cell-proliferation assay show TL2 exerts potent immunosuppressive effect comparable to that of cyclosporine A (CsA), a well established immunocompromising compound, suggesting that TL2 may be a promising immunosuppressive agent (Zhang et al., 2008). Based on this, the anti-inflammatory and neuroprotective effects of TL2 on LPS-activated microglia and the underlying mechanisms were investigated in the present study.

2. Material and methods

2.1. Materials

TL2 was kindly provided by Prof. Renxiang Tan (Nanjing University, Nanjing, China). Stock solutions of TL2 were prepared in dimethy sulfoxide (DMSO) and diluted into culture medium before experiments. Dulbecco's Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin, and trypsin/EDTA were purchased from Hyclone (Logan, UT). LPS from *Escherichia coli* serotype O55:B5 was from Sigma–Aldrich (St. Louis, USA). Antibodies against Akt, p38, JNK, ERK1/2, phospho-Akt, phospho-p38, phospho-JNK, phospho-ERK1/2, and NF-κB were purchased from Cell Signaling Biotechnology (Hertfordshire, England). Antibodies against iNOS and COX-2 were from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody against β-actin and the secondary antibodies were obtained from Bioworld technology (Nanjing, China). FITC labeled goat anti-rabbit IgG and goat anti-mouse IgG were purchased from Invitrogen (Frederick, MD, USA). Enhanced Chemiluminescence (ECL) kit was from Millipore (Amersham Pharmacia Biotech, Piscataway, NJ). PCR primers were synthesized at Invitrogen (Frederick, MD, USA). The total nitrate assay kit was obtained from Beyotime (Nanjing, China).

2.2. Cell culture

BV2 microglia cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (100 U/ml penicillin and 100 μg/ml streptomycin) at 37 °C under a humidified 5% CO_2 atmosphere. Primary cultures of cortical neurons were prepared from C57/B6 mice at embryonic day 18 (E18) as described previously (Zhu et al., 2012). Cells were grown on poly-D-lysine coated 96-well plates and maintained in neuron culture medium at 37 °C in 5% CO_2. After 7 days *in vitro*, cells were treated with the BV-2 conditioned medium.

Mice primary microglia cells were prepared from 1–2 day-old C57BL/6J mice as previously described (Kitayama et al., 2011) with only modest modification. Briefly, the cerebral cortex was gently dissociated and digested in 0.25% trypsin for 10 min at 37 °C. The resulting cells were passed through a 200 μm pore filter and the primary mixed glial cells were resuspended in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were seeded in 75 cm^2 flasks for 10–12 days. Microglia cells were separated from the mixed glial cells by shaking the flasks for 5 h at 180 r.p.m. in a rotary shaker at 37 °C. Detached cells were cultured in the complete medium and seeded into 24-well plates at a density of 5×10^5 cells/well for 2–3 days. The purity of the primary microglia cells were more than 95% as determined by Iba1 staining.

2.3. Assessment of TL2 cytotoxicity

Cell viability was assessed by the conventional 3-[4,5-diethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay as described previously (Zhang et al., 2010). Briefly, BV2 microglia seeded in 96-well plates at a density of 5×10^3 cells/well were treated with TL2 in the presence and absence of LPS for 24 h. Subsequently, MTT solution was added and incubated at 37 °C in 5% CO_2 for 4 h. The dark blue formazan crystals were dissolved in DMSO and the absorbance at 540 nm was determined with a microplate reader. Results were expressed as the percentages of live cells over control cells.

2.4. Measurement of nitrite and PGE2

Cells were seeded at 1.0×10^5 cells/well in 24-well culture plates, treated with or without TL2 for 1 h followed by LPS treatment (0.1 μg/ml for BV2, 1 μg/ml for primary microglia) for 24 h. Cultured supernatants were collected. Accumulated nitrite was assessed using the Griess reaction. The absorbance was obtained at 570 nm and the results were expressed as mean change fold change of the control. Potassium nitrite was performed as a standard curve. The concentration of PGE2 was measured by a competitive enzyme

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