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Anti-neuroinflammatory effects of citreohybridonol involving TLR4-MyD88-mediated inhibition of NF-κB and MAPK signaling pathways in lipopolysaccharide-stimulated BV2 cells

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ABSTRACT

In the course of searching for anti-neuroinflammatory metabolites from marine fungi, citreohybridonol was isolated from marine-derived fungal strain *Toxicocladosporium* sp. SF-5699. Citreohybridonol inhibited production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) in BV2 cells stimulated by lipopolysaccharide (LPS). Citreohybridonol also suppressed the expression of inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), and other pro-inflammatory cytokines including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the LPS-stimulated cells. In the further study, citreohybridonol disturbed nuclear translocation of nuclear factor-kappa B (NF-κB) in LPS-stimulated BV2 cells by inhibiting the phosphorylation of the inhibitor kappa B-α (IκB-α). Citreohybridonol also had inhibitory effect on the LPS-stimulated phosphorylation of p38 mitogen-activated protein kinase (MAPK). Finally, citreohybridonol suppressed the protein expression of Toll-like receptor 4 (TLR4) and myeloid differentiation factor 88 (MyD88) in LPS-induced BV2 cells. These results suggest that citreohybridonol has anti-neuroinflammatory effect in LPS-stimulated BV2 cells by modulating TLR4-mediated several inflammatory pathways such as NF-κB and p38 MAPK pathways.

1. Introduction

Inflammation is an important body defense mechanism against pathogens and diverse external stimuli. Numerous endogenous inflammatory mediators, including cytokines, chemokines, prostaglandins and nitric oxide (NO), are present in the body. Among these mediators, interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) are representative pro-inflammatory cytokines, and their overproduction exacerbates various inflammatory states, including sepsis and rheumatoid arthritis (Hohki et al., 2010; Hack et al., 1989). The occurrence of inflammation is a complicate process, regulated by nitric oxide (NO), prostaglandins (PGs), and cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β) (Guzik et al., 2003).

Neuroinflammation is an important mechanism within the brain that leads to neuronal damage and generates neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), HIV-associated dementia (HAD), stroke, and multiple sclerosis (MS) (Mrak and Griffin, 2001; Nguyenet et al., 2002). Microglia are resident macrophages in the brain and play a critical role in the immune defense in the central nervous system (CNS) (Rivest, 2009; Moss and Bates, 2001). Once microglia stimulated by stimuli such as lipopolysaccharide (LPS) and interferon-gamma (IFN-γ), microglia produce inflammatory mediators including NO, TNF-α, and PGE_2 (Agostinho

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Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
CD14	Cluster of differentiation 14
cDNA	Complementary DNA
CNS	Central nervous system
COX-2	Cyclooxygenase-2
DEPC	Diethyl pyrocarbonate
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ECL	Enhanced chemilumescent
ELISA	Enzyme-linked immunosorbent assay
ERK1/2	Extracellular signal-regulated kinase
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High-performance liquid chromatography
HSQC	Heteronuclear Single Quantum Coherence
IL-1β	Interleukin-1β
IL-6	Interleukin-6
iNOS	Inducible NO synthase
Iκ-B	Inhibitor kappa B-α
JNK	c-Jun N-terminal kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCP-1	monocyte chemotactic protein 1
MS	mass spectrometry
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
MyD88	Myeloid differentiation primary response 88
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
PCNA	Proliferating cell nuclear antigen
PD	Parkinson's disease
PGE_2	Prostaglandin E_2
PMSF	Phenylmethylsulfonlyfluoride
RNA	Ribonucleic acid
TAK1	Transforming growth factor beta-activated kinase 1
TLR4	Toll-like receptors 4
TRAF6	TNF receptor associated factors 6
TRIF	TIR-domain-containing adapter-inducing interferon-β

et al., 2010; Nakamura et al., 1999). Sustained and uncontrolled activation of microglia can lead to an excessive production of inflammatory mediators, which promote neuronal injury. Thus, intervention in a microglial activation process could be a promising approach for the treatment of many neurodegenerative diseases (Block et al., 2007).

NF-κB, a major transcription factor, modulates inflammatory system through expressing proinflammatory genes including inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) (Ghosh et al., 1998; Siebenlist et al., 1994). Under normal conditions, NF-κB is resident as an inactivation form complex with inhibitors of κB (IκB) in the cytoplasm. Once stimulated by inflammatory signals such as LPS and TNF-α, IκB is phosphorylated and degraded resulting in free NF-κB (Lappas et al., 2002; Karin and Ben-Neriah, 2000). Afterward, NF-κB p50/p65 heterodimers translocate into the nucleus and binds to the DNA binding site related to regulating the transcriptions of its target genes, triggering expression of pro-inflammatory enzymes and cytokines such as iNOS, COX-2, TNF-α, and IL-β.

Mitogen-activated protein kinases (MAPKs) are one of the major kinase families associated with cellular processes such as differentiation, stress responses, apoptosis, and immune defense. There are three major mitogen-activated protein kinase (MAPK) signaling pathways, which are mediated by extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2), c-Jun N-terminal kinases (JNKs), and p38 MAPK (Bennett and Tonks, 1997). NF-κB and MAPK signaling pathways are involved in the regulating the inflammatory process, and it has been reported that natural products can exhibit anti-neuroinflammatory effects by inhibiting the NF-κB and MAPK signaling pathways (Ha et al., 2012; Zeng et al., 2010; Park et al., 2012; Himaya et al., 2012).

Once LPS binds to Toll-like receptor 4 (TLR4) on the surface of microglia, it leads to the activation of several signal transduction pathways of inflammatory processes (Medzhitov, 2001). Activated TLR4 transfers the signal through the two main downstream pathways: First, the TLR4-mediated myeloid differentiation factor 88 (MyD88)-dependent pathway and second, the Toll/IL-1 receptor domain-containing adapter induction of the interferon-β (TRIF)-dependent pathway (Noman et al., 2009). MyD88 is an adapter protein which mediates signaling pathway for most TLRs, which leads to activation of NF-κB and MAPKs (Park et al., 2011).

Marine microorganisms have been recognized as promising sources of metabolites with bioactivity, and have inspired the development of new classes of drugs (Fenical and Jensen, 2006; Bugni and Ireland, 2004; Gerwick and Moore, 2012). In the course of searching for secondary metabolites from marine-derived fungi with biological activity, citreohybridonol, a meroterpenoid derivative, was isolated from the marine-derived fungal strain *Toxicocladosporium* sp. SF5699, exhibiting anti-neuroinflammatory activity. Although citreohybridonol has been previously isolated (Kosemura et al., 1994; Gao et al., 2012; Kosemura, 2003), its biological effect has not been known. This study describes isolation, structure elucidation, and anti-neuroinflammatory effect of citreohybridonol. Furthermore, molecular mechanism involved in the anti-neruoinflammatory effect of citreohybridonol was elucidated.

2. Material and methods

2.1. Instruments, fungal materials and isolation of citreohybridonol

ESIMS data were obtained using an ESI Q-TOF MS/MS system (AB SCRIEX Triple, USA). NMR spectra (1D and 2D) were recorded in DMSO-d_6 with a JEOL JNM ECP-400 spectrometer, and the chemical shifts were referenced relative to the residual solvent peaks ($\delta_H/\delta_C = 2.49/39.5$). HSQC and HMBC experiments were optimized for $^1J_{CH} = 140$ Hz and $^nJ_{CH} = 8$ Hz, respectively. HPLC (YOUNGLIN-YL9100, Younglin, Anyang, Korea) separation was performed using a PhenomenexSynergi 4u Polar-RP 80A, AXIA packed column (21.2 × 150 mm, 5-μm particle size) with a flow rate of 5 mL/min and the solvents used for HPLC were all analytical grade.

Toxicocladosporium sp. SF-5699 (deposited at the College of Medical and Life Sciences fungal strain repository, Silla University) was isolated from the unidentified sponge that was collected at Cheju Island, Korea in February, 2011. This fungus was identified based on the analysis of its ribosomal RNA (rRNA) sequence. A GenBank search with the 28S rRNA gene of SF-5699 (GenBank