Synthetic 3',4’-Dihydroxyflavone Exerts Anti-Neuroinflammatory Effects in BV2 Microglia and a Mouse Model

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Abstract

Neuroinflammation is an immune response within the central nervous system against various proinflammatory stimuli. Abnormal activation of this response contributes to neurodegenerative diseases such as Parkinson disease, Alzheimer’s disease, and Huntington disease. Therefore, pharmacologic modulation of abnormal neuroinflammation is thought to be a promising approach to amelioration of neurodegenerative diseases. In this study, we evaluated the synthetic flavone derivative 3',4'-dihydroxyflavone, investigating its anti-neuroinflammatory activity in BV2 microglial cells and in a mouse model. In BV2 microglial cells, 3’,4’-dihydroxyflavone successfully inhibited production of chemokines such as nitric oxide and prostaglandin E₂ in proinflammatory cytokines such as tumor necrosis factor alpha, interleukin 1 beta, and interleukin 6 in BV2 microglia. It also inhibited phosphorylation of mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB activation. This indicates that the anti-inflammatory activities of 3’,4’-dihydroxyflavone might be related to suppression of the proinflammatory MAPK and NF-κB signaling pathways. Similar anti-neuroinflammatory activities of the compound were observed in the mouse model. These findings suggest that 3’,4’-dihydroxyflavone is a potential drug candidate for the treatment of microglia-related neuroinflammatory diseases.

Key Words: Microglia, Neuroinflammation, Lipopolysaccharide, BV2 microglia, Flavone

INTRODUCTION

Neuroinflammation is an immune response to proinflammatory stimuli within the central nervous system (CNS). It is a major contributing factor to neurodegenerative diseases, including multiple sclerosis, Parkinson disease, Alzheimer disease, and Huntington disease (Kim and Choi, 2015). Microglia are the resident macrophages of the CNS, with important roles in immune regulation and brain homeostasis (Biber et al., 2016). In the normal immune response to various factors such as xenobiotics, microglia can be activated to release neurotrophic factors and support neurogenesis, helping maintain CNS homeostasis. However, abnormal activation of microglia may lead to neuronal injury and production of neurotoxic molecules, such as prostaglandin E₂ (PGE₂) and nitric oxide (NO), and proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α (Block and Hong, 2005). Activated microglia also migrate toward damaged areas and further promote neuroinflammation (de Haas et al., 2008), and the microglia themselves or microglia-derived inflammatory products eventually accelerate neurodegeneration (Cherry et al., 2014; Kim and Choi, 2015). Novel therapeutic agents are needed to inhibit the production of neurotoxic chemokines and cytokines by microglia, blocking their contribution to neuroinflammatory and neurodegenerative conditions.

The anti-inflammatory effects of flavonoids have been reported from in vitro and in vivo studies, as well as clinical trials (Perez-Cano and Castell, 2016; Zeinali et al., 2017). Flavones, a type of flavonoid, are natural products isolated from a number of medicinal plants. Synthetic derivatives of these compounds are based on the 2-phenylchromen-4-one backbone (An et al., 2017). The anti-inflammatory activities of flavones have been extensively studied.
vones have been investigated in many studies, but most have been focused on natural products such as luteolin. In an effort to identify more potent anti-inflammatory flavones, we synthesized a number of flavone derivatives and evaluated their abilities to inhibit NO and PGE₂ production in a cell-based assay using RAW 264.7 cells (An et al., 2017). A novel and potent anti-inflammatory flavone derivative identified in that study was 3’,4’-dihydroxyflavone. In the present study, we evaluated the antineuroinflammatory effects of 3’,4’-dihydroxyflavone and investigated the mechanisms by which it might counteract the effects of lipopolysaccharide (LPS) stimulation in BV2 microglial cells. To confirm the in vitro results, we also examined the antineuroinflammatory effects of 3’,4’-dihydroxyflavone in the brains of mice injected with LPS.

MATERIALS AND METHODS

Chemicals and reagents

We synthesized 3’,4’-dihydroxyflavone and flavone (Fig. 1A) using our previously reported procedure (An et al., 2017). LPS from Escherichia coli serotype O55:B5 (L6529) and luteolin (L9283) were purchased from Sigma-Aldrich (MO, USA). HyClone (GE Healthcare) culture media and other materials were used for cell culture. Rabbit antibodies against p38 (#9212S), phosphor-p38 (#9215S), JNK (#9252S), phosphor-JNK (#9251S), ERK (#9101L), phosphor-ERK (#9102), iNOS (#2982S) and COX-2 (#4842S) were purchased from Cell Signaling Technology (MA, USA). Antibodies against β-actin (SC-47778 HRP) and NF-κB (rabbit, SC-372) were purchased from SantaCruz Biotechnology (CA, USA). A rabbit antibody against anti-ionized calcium-binding adapter molecule 1 (Iba-1, #019-19741) was purchased from Wako (Tokyo, Japan). Secondary antibodies were purchased from Bio-Rad Laboratories (CA, USA), SantaCruz Biotechnology and Invitrogen (CA, USA). Mouse TNF-α and IL-6 ELISA kits were purchased from BD. A mouse IL-1β kit was purchased from Invitrogen.

Cell culture and assessment of cytotoxicity

Murine BV2 microglial cells were cultured in DMEM with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C in a humidified 5% CO₂ atmosphere. Cell viability and toxicity were determined by the WST-1 assay (Roche, Mannheim, Germany). Briefly, BV2 cells in sterile plates were incubated with the indicated concentrations of 3’,4’-dihydroxyflavone for 24 h. After replacing the culture media with fresh medium, WST-1 solution was added to each well and the cells were incubated for 2 h. Absorbance at 450 nm was then measured with a plate reader. In some experiments, the cells were incubated with different concentrations of 3’,4’-dihydroxyflavone and then incubated with LPS (500 ng/ml) for 24 h.

Measurement of NO and PGE₂

BV2 cells were incubated with various concentrations of 3’,4’-dihydroxyflavone for 2 h prior to incubation with or without LPS (500 ng/ml) for a further 22 h. The NO and PGE₂ concentrations in culture supernatants were assessed using the Griess reaction kit (#KA1342; Abnova, Taipei City, Taiwan) and the enzyme immune assay kit (#ADI-900-001; Enzo Life Sciences, NY, USA), respectively, according to the manufacturer’s instructions.