Anti-Inflammatory Effect of Mangostenone F in Lipopolysaccharide-Stimulated RAW264.7 Macrophages by Suppressing NF-κB and MAPK Activation

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Abstract

Mangostenone F (MF) is a natural xanthone isolated from Garcinia mangostana. However, little is known about the biological activities of MF. This study was designed to investigate the anti-inflammatory effect and underlying molecular mechanisms of MF in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. MF dose-dependently inhibited the production of NO, iNOS, and pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) in LPS-stimulated RAW264.7 macrophages. Moreover, MF decreased the NF-κB luciferase activity and NF-κB DNA binding capacity in LPS-stimulated RAW264.7 macrophages. Furthermore, MF suppressed the NF-κB activation by inhibiting the degradation of IκBα and nuclear translocation of p65 subunit of NF-κB. In addition, MF attenuated the AP-1 luciferase activity and phosphorylation of ERK, JNK, and p38 MAP kinases. Taken together, these results suggest that the anti-inflammatory effect of MF is associated with the suppression of NO production and iNOS expression through the down-regulation of NF-κB activation and MAPK signaling pathway in LPS-stimulated RAW264.7 macrophages.

Key Words: Mangostenone F, NO, iNOS, NF-κB, MAPK

INTRODUCTION

Inflammation is induced by physical or noxious chemical stimuli or microbiological toxins as the normal response of living tissue. It is well known that chronic inflammation can cause inflammatory diseases, such as arthritis, asthma, multiple sclerosis, inflammatory bowel disease, and atherosclerosis (Shin et al., 2010). Macrophages are activated by various factors such as pro-inflammatory cytokines, bacterial lipopolysaccharide (LPS), and phorbol esters. Activated macrophages produce many cytokines, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and other inflammatory mediators such as nitric oxide (NO) and prosta-glandin E2 (PGE2) (Reddy and Reddanna, 2009). NO is a free radical produced by nitric oxide synthase (NOS), which exists as three NOS isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). Macrophages after LPS stimulation produce NO by up-regulating iNOS expression through mitogen-activated protein kinases (MAPK) and NF-κB signaling pathways (Pansanit et al., 2013). In response to macrophage activation, LPS stimulates a Toll-like receptor 4 (TLR4)-mediated myeloid differentiation factor (MyD88)-dependent pathway, which in turn activates the transforming growth factor-β-activated protein kinase 1 (TAK1), which subsequently results in activation of nuclear factor-κB (NF-κB) and activating protein-1 (AP-1), and produces inflammatory cytokines including TNF-α, IL-6, and IL-1β (Kawai and Akira, 2006). Therefore, inhibition of these inflammatory mediators has been considered as an effective strategy for the development of anti-inflammatory drugs (Shin et al., 2010; Pansanit et al., 2013).

Mangosteen (Garcinia mangostana) is a tropical tree from Southeast Asia including Malaysia, India, Myanmar, Philippines, and Thailand. The seedcases of mangosteen-fruit have been traditionally used for treating skin infections and wounds.

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for centuries by Southeast Asians. It is also used to treat inflammation, diarrhea, cholera, and dysentery in Ayurvedic medicine (Pedraza-Chaverri et al., 2008). Phytochemical studies have shown that *G. mangostana* contains a variety of secondary metabolites such as oxygenated and prenylated xanthones (Suksamrarn et al., 2002). These xanthone compounds have been reported to indicate various biological activities such as antioxidant, antitumor, anti-inflammatory, antiallergy, antibacterial, antifungal, antiviral, and antimalarial properties (Suksamrarn et al., 2002; Pedraza-Chaverri et al., 2008). A recent study documented that 12 xanthones including new xanthone mangostenone F (MF) isolated from the seedcases of *G. mangostana* indicate neuraminidase inhibitory activity (Ryu et al., 2010). It has also been reported that mangosteen’s xanthones including MF indicate α-glucosidase inhibition and antihyperglycemic activity (Ryu et al., 2011). In addition, it has been demonstrated that MF from the seedcases of *G. mangostana* inhibits melanin formation in B16F10 cells by down-regulating the tyrosinase expression (Ryu et al., 2012). However, the anti-inflammatory activity of MF has not yet been elucidated. Therefore, in the present study, we evaluated the anti-inflammatory effects of MF isolated from the seedcases of *G. mangostana* in LPS-stimulated RAW264.7 macrophages.

**MATERIALS AND METHODS**

**Materials**

Mangostenone F (MF, Fig. 1A) used in the present study was obtained from Dr. Hyung Won Ryu of Korea Research Institute of Bioscience and Biotechnology and dissolved in dimethyl sulfoxide. LPS, a Griess reagent, an NP40 cell lysis buffer, a protease inhibitor cocktail, and a NuCLEAR Extraction Kit were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies for β-Tubulin, IκBα, NF-κB p65, and lamin B were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibody for iNOS was purchased from BD Pharmingen (San Diego, CA, USA). Antibodies for β-actin, ERK, phosphor-ERK (T202/Y204), JNK, phosphor-JNK (T183/Y185), p38, and phosphor-p38 (Thr180/Tyr182) were purchased from Cell Signaling Technology (Danvers, MA, USA). Goat anti-mouse IgG HRP-conjugated antibody was from SouthernBiotech (Birmingham, AL, USA). Goat anti-rabbit IgG HRP-conjugated antibody, Opti-MEM I medium, and Lipofectamine 2000 were purchased from Invitrogen (Carlsbad, CA, USA). The pNF-κB-Luc and pAP-1-Luc reporter vectors were purchased from Stratagene (La Jolla, CA, USA) and Panomics (Fremont, CA, USA), respectively. The pRL-TK internal control vector was purchased from Promega (Madison, WI, USA). Enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-6, and IL-1β were purchased from R&D Systems (Minneapolis, MN, USA).

**Cell culture**

RAW264.7 macrophage cells were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were grown in DMEM supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA), 100 units/ml of penicillin, and 100 μg/ml of streptomycin (Invitrogen, Carlsbad, CA, USA) in a humidified atmosphere at 37°C with 5% CO₂.