Epigallocatechin-3-gallate Inhibits LPS-Induced NF-κB and MAPK Signaling Pathways in Bone Marrow-Derived Macrophages

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Background/Aims: Epigallocatechin-3-gallate (EGCG), the primary catechin in green tea, has anti-inflammatory and anti-oxidative properties. The aim of the current study was to characterize the impact of EGCG on lipopolysaccharide (LPS)-induced innate signaling in bone marrow-derived macrophages (BMMs) isolated from ICR mice. Methods: The effect of EGCG on LPS-induced pro-inflammatory gene expression and nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signaling was examined using reverse transcription-polymerase chain reaction, Western blotting, immunofluorescence, and the electrophoretic mobility shift assay. Results: EGCG inhibited accumulation of LPS-induced IL-12p40, IL-6, MCP-1, ICAM-1, and VCAM-1 mRNA in BMMs. EGCG blocked LPS-induced IκBα degradation and RelA nuclear translocation. EGCG blocked the DNA-binding activity of NF-κB. LPS-induced phosphorylation of ERK1/2, JNK, and p38 was inhibited by EGCG. U0126 (an inhibitor of MEK-1/2) suppressed the LPS-induced IL-12p40, IL-6, MCP-1, ICAM-1, and VCAM-1 mRNA accumulation in BMMs. Conclusions: These results indicate that EGCG may prevent LPS-induced pro-inflammatory gene expression through blocking NF-κB and MAPK signaling pathways in BMMs. (Gut Liver 2012;6:188-196)

Key Words: Epigallocatechin-3-gallate; Nuclear factor-κB; Mitogen-activated protein kinase; Macrophage

INTRODUCTION

Complementary and alternative medicine such as herbal/dietary therapy is becoming an increasingly attractive approach to the treatment and prevention of various inflammatory disorders, including inflammatory bowel disease (IBD). However, despite their clear popularity, absence of empirical data showing efficacy and mechanisms of action in vivo prevents their incorporation into mainstream medicine.

Green tea is one of the widely consumed beverages in the world. Many epidemiologic studies showed that green tea consumption has beneficial effects in preventing the development of atherosclerosis and prostatic cancer. It is generally agreed that these beneficial effects of green tea are mediated by its polyphenols. Green tea contains four polyphenolic compounds known as catechin; (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate and (-)-epigallocatechin-3-gallate (EGCG). EGCG is the most abundant polyphenol in green tea, and has a variety of modulatory actions on physiological functions, such as anti-inflammatory, anti-oxidative, anti-mutagenic, and anti-carcinogenic effects.

IBD, such as Crohn’s disease and ulcerative colitis, is a chronic and relapsing intestinal inflammation of unknown etiology. It has been proposed that IBD is caused by aberrant mucosal immune responses to nonpathogenic bacteria and bacterial products in the intestine. In IBD, the intestinal epithelial cell damage results in an increased uptake of luminal antigens, including bacteria and bacterial products, and thus leads to the activation of immune cells in lamina propria and the mounting of inflammatory responses. Among the immune cells, macrophages and monocytes play an important role in the initiation, development and outcome of immune response and are also found in the inflamed gut mucosa. However, until now, the direct effects of EGCG on bone marrow-derived macrophages (BMMs) in intestinal inflammation have not been fully investigated.

Lipopolysaccharide (LPS), a gram negative bacteria-derived cell wall product, stimulates over-production of nitric oxide, release of inflammatory cytokines and recruitment of immune
cells. Inflammatory cytokines are important pro-inflammatory mediators and may be responsible for the induction of chemokines, enzymes and adhesion molecules in intestinal inflammation. Most inflammatory cytokines are induced by the activation of transcription factors and protein kinases such as the transcriptional nuclear factor-kB (NF-kB) and mitogen-activated protein kinase (MAPK). Mucosal inflammation in patients with IBD and in experimental models of intestinal inflammation is accompanied by elevated levels of activated NF-kB. It has been shown previously that MAPK plays a critical role in the transduction of inflammatory response in variable cell types. MAPK differentially regulates the production of pro- and anti-inflammatory cytokines in immune cells including dendritic cells.

In the present study, we investigated the impact of EGCG on LPS-induced innate signaling and proinflammatory gene expression in BMMs.

MATERIALS AND METHODS

1. Isolation and culture of BMMs

Bone marrow cells were isolated from 5- to 8-week-old ICR mice (Samtako Science, Daejeon, Korea) as previously described. Mice were sacrificed by cervical dislocation. Femora and tibiae were aseptically removed and dissected free of adherent soft tissue. The bone ends were cut, and the marrow cavity was flushed out into a petri dish by slowly injecting MEM-α medium (Hyclone, Logan, UT, USA) at one end of the bone using a sterile 21-gauge needle. The bone marrow suspension was carefully agitated with a plastic Pasteur pipette to obtain a single cell suspension. Bone marrow cells were washed and depleted of red blood cell (RBC) by hypotonic lysis using RBC lysing buffer (Sigma-Aldrich, St. Louis, MO, USA). After washing twice with phosphate-buffered saline (PBS), the cells were suspended in MEM-α medium supplemented with 10% FBS and 50 μg/mL streptomycin (Gibco, Grand Island, NY, USA). The number of viable cells was determined with trypan blue (Gibco) and bone marrow cells were cultured on 1 cm² tissue culture dishes in total amount of 2×10⁵ cells/dish. 10 ng/mL of mouse macrophage colony stimulating factor (M-CSF; BioSource, Camarillo, CA, USA) was added to every 10 cm² dish to differentiate BMMs. On day 3, non-adherent cells were discarded and adherent cells (immature BMMs) were suspended in fresh MEM-α with M-CSF and used in subsequent experiment. All of the cells were cultured at 37°C under a humidified atmosphere containing 5% CO₂. EGCG was obtained from Sigma-Aldrich and stored as 50 mM stock solutions in 4°C. LPS from Escherichia coli (serotype 0111:B4) was also purchased from Sigma-Aldrich and dissolved in sterile, pyrogen free PBS. U0126 (a specific inhibitor of MEK-1/2, an upstream effector of ERK1/2) was obtained from Calbiochem (San Diego, CA, USA). Cells were pretreated with various concentrations of EGCG (0-100 μM) or U0126 (20 μM) after which they were stimulated with LPS (0.5-1 μg/mL) for times indicated (0 to 1 hour). All methods used in this study was approved by the Animal Care and Use Committee at the Chonnam National University Medical School Research Institution and conformed to US National Institutes of Health (NIH publication No. 86-23 revised 1985) guidelines.

2. Cell viability

BMMs were plated to a 96-well plate at a density of 1×10⁵ cells/well and incubated in medium with various concentrations of EGCG and LPS. After incubation for 24 hours, cell viability was determined by EZ-CyTox (tetrrazolium salts, WST-1) cell viability Assay kit (Daeil Lab Inc., Seoul, Korea). After WST-1 reagent was added for 1 to 2 hours at 37°C, the absorbance was determined using a microplate reader (Infinite M200; Tecan Austria GmbH, Grödig, Austria) with Magellan V6 data analysis software (Tecan, Austria GmbH, Austria). Triplicate wells were used for each experimental condition and all experiments were repeated at least three times.

3. Western blotting

The cells were exposed to LPS (1 μg/mL) in the absence or presence of 100 μM EGCG-pretreatment. Following 10 or 30 minutes of incubation at 37°C, cells were washed twice with cold PBS and lysed with RIPA buffer (1 M Tris-HCl, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA) with 1 mM PMSF, Halt Protease inhibitor and Halt Phosphatase inhibitor and Halt Protease inhibitor cocktail (Thermo) for 15 minutes at 4°C. Lysates were cleared by centrifugation at 14,000 g for 20 minutes at 4°C. The protein concentrations of cell lysates were determined using BCA protein assay (Thermo, Rockford, IL, USA). Equivalent amounts of proteins were separated by 12% SDS-PAGE and electrophoretically transferred to PVDF membrane (Millipore, Billerica, MA, USA). The analysis was used primary antibodies as described by the manufacturer: polyclonal anti-IκBα, phospho-IκBα, ERK1/2, phospho-ERK1/2, JNK, phospho-JNK, p38, phospho-p38 (Cell Signaling, Danvers, MA, USA), polyclonal anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washing three times with TBST, membranes were incubated with secondary HRP-conjugated anti-mouse IgG for 1 hour. After washing, the blots were detected with chemiluminescence (ECL) HRP substrate (Millipore) by image reader (Ras-4000; Fujifilm, Tokyo, Japan).

4. Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the instructions provided by the manufacturer. The quantity and purity of total RNA were determined by measuring the optical density using Nanodrop (Nanodrop Technologies, Wilmington, DE, USA). RT was carried out...