Short Communication

Curcumin Suppresses Lipopolysaccharide-Induced Cyclooxygenase-2 Expression by Inhibiting Activator Protein 1 and Nuclear Factor κB Bindings in BV2 Microglial Cells

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Received May 19, 2003; Accepted January 5, 2004

Abstract Inflammation is a significant component of chronic neurodegenerative diseases. Cyclooxygenase-2 (COX-2) is expressed in activated microglial cells and appears to be an important source of prostaglandins during inflammatory conditions. To investigate the effect of curcumin on COX-2 gene expression in microglial cells, we treated lipopolysaccharide (LPS)-challenged BV2 microglial cells with various concentrations of curcumin. Curcumin significantly inhibited LPS-mediated induction of COX-2 expression in both mRNA and protein levels in a concentration-dependent manner. COX-2 enzyme activity was also inhibited in accordance with mRNA and protein levels. Furthermore, curcumin markedly inhibited LPS-induced nuclear factor κB (NF-κB) and activator protein 1 (AP-1) DNA bindings. These data suggest that curcumin suppresses LPS-induced COX-2 gene expression by inhibiting NF-κB and AP-1 DNA bindings in BV2 microglial cells.

Keywords: curcumin, cyclooxygenase-2, microglia

Microglia are the resident macrophages and immune surveillance cells of the central nervous system and play an active role in brain inflammatory, immune, and degenerative processes (1). Especially, activated microglia have been described in several human chronic neurodegenerative diseases, including Alzheimer’s disease (AD), AIDS dementia, and Parkinson’s disease (PD) (2, 3). Many of the effects of activated microglial cells are mediated by their numerous secretory products such as prostaglandins (PGs), a family of compounds derived from arachidonic acid through the cyclooxygenase (COX) pathway (4). COX-2 is the key enzyme in the formation of PGs (5) and is expressed in activated microglial cells and astrocytes which appear to be an important source of PGs during inflammatory conditions (6). PGs are potent mediators, and while low levels of these molecules may participate in protective responses leading to enhanced disease resistance, their excessive production may be involved in autotoxicity (7). The temporal correlation between increased levels of PGs and various neuropathological processes has led to the hypothesis that PGs contribute to neurodegeneration (8).

Curcumin is a phenolic antioxidant responsible for the major yellow pigment and active component of turmeric that has anti-inflammatory and anti-cancer properties (9). The anti-inflammatory properties of curcumin have been attributed, at least in part, to suppression of PG synthesis (9). Thus, the present study was undertaken to investigate the effects of curcumin on microglial COX-2 expression stimulated with bacterial endotoxin, LPS.

Curcumin and LPS were purchased from Sigma Chemical (St. Louis, MO, USA) and the stock solution was prepared by dissolving it in dimethylsulfoxide (Sigma) and stored in a brown bottle at −20°C until use. All the materials for cell culture were obtained from Gibco (Grand Island, NY, USA). Chemicals that are not specified here were obtained from Sigma.

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The immortalized murine BV2 cell line that exhibits phenotypic and functional properties of reactive microglial cells (10) was obtained from M. McKinney (Mayo Clinic, Jacksonville, FL, USA). The cells were grown and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal calf serum and penicillin/streptomycin at 37°C in a humidified incubator with 5% CO₂. When the cells became 80% – 90% confluent, they were subcultured.

BV2 microglial cells were preincubated for 1 h in the absence or presence of curcumin before the cells were stimulated with lipopolysaccharide (LPS) (0.2 ng/mL). Six hours after LPS treatment, a sample for analysis was collected; at this time, COX-2 mRNA level and nuclear proteins (activator protein (AP)-1 and nuclear factor (NF)-κB) binding activity achieve their maximum levels (data not shown). The RNA was extracted using an RNeasy kit (Qiagen, Valencia, CA, USA). COX-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antisense probes were made by using an in vitro transcription kit (Ambion, Austin, TX, USA), and gel-purified. RNase Protection Assay (RPA) was performed using a RPA II kit (Ambion). Protected fragments were resolved on 6% SDS-PAGE and quantitated with a PhosphorImager (BAS-2500; Fuji, Tokyo). Data are expressed as a ratio of the level of COX-2 mRNA normalized to that of GAPDH housekeeping genes. To evaluate the expression level of COX-2, the mRNA normalized to that of GAPDH housekeeping gene expression by preventing AP-1 and NF-κB DNA bindings, we examined the bindings of nuclear proteins to oligonucleotides containing sequences of various cytokines. The inappropriate regulation of NF-κB and AP-1 in the cells of the immune system, where they control the expression of various cytokines. The inappropriate regulation of NF-κB and AP-1 in their dependent genes have been associated with various pathological conditions including septic shock, inflammatory conditions, acute-phase response, viral replication, radiation damage, and cancer (13, 14). To determine whether curcumin suppresses the COX-2 expression by preventing AP-1 and/or NF-κB DNA bindings, we examined the bindings of nuclear proteins to oligonucleotides containing sequences of the AP-1 and NF-κB sites. Nuclear extracts from LPS-stimulated BV2 microglial cells formed a prominent DNA-protein complex with the AP-1 site (Fig. 1: B and C), whereas curcumin itself did not affect the basal level of COX-2 protein and its activity (data not shown). Cell death was not observed at the tested concentrations (2 – 16 μM) of curcumin.

NF-κB and AP-1 play critical roles in the cells of the immune system, where they control the expression of various cytokines. The inappropriate regulation of NF-κB and AP-1 and their dependent genes have been associated with various pathological conditions including septic shock, inflammatory conditions, acute-phase response, viral replication, radiation damage, and cancer (13, 14). To determine whether curcumin suppresses the COX-2 expression by preventing AP-1 and/or NF-κB DNA bindings, we examined the bindings of nuclear proteins to oligonucleotides containing sequences of the AP-1 and NF-κB sites. Nuclear extracts from LPS-stimulated BV2 microglial cells formed a prominent DNA-protein complex with the AP-1 site (>30 fold compared to basal levels), and curcumin suppressed the AP-1 binding activity observed in LPS-stimulated microglial cells in a concentration-dependent manner (Fig. 2). High concentration of curcumin (16 μM) decreased more than 50% of the LPS-induced AP-1 DNA bindings. We also examined the effect of curcumin on the activation of NF-κB. LPS treatment resulted in marked NF-κB DNA bindings (>4 fold compared to basal levels), and curcumin significantly suppressed LPS-induced NF-κB DNA binding in a concentration-dependent manner (Fig. 3). High concentration of curcumin (16 μM) decreased approximately 50% of LPS-induced NF-κB DNA binding. Given that the decrease of both AP-1 and NF-κB bindings by curcumin was in parallel with curcumin-mediated decrease of COX-2 mRNA expression in LPS-challenged cells, it is highly possible that curcumin suppresses the COX-2 gene expression by preventing AP-1 and NF-κB DNA bindings.

There is growing evidence that inflammation is
Curcumin Inhibits COX-2 Induction

Fig. 1. Inhibitory effects of curcumin on LPS-induced COX-2 mRNA (A), protein (B) expressions, and COX-2 enzyme activity (C) in BV2 microglial cells. BV2 microglial cells were pretreated with various concentrations of curcumin as indicated for 1 h prior to LPS treatment. Six hours after LPS treatment, a sample for analysis was collected. A: Representative autoradiograph (top) and quantitative analysis (bottom) of COX-2 mRNA expression. B: Representative immunoblot (top) and quantitative analysis (bottom) of COX-2 protein expression. C: PGE\(_2\) production determined by enzyme immunoassay. Data represent three independent experiments and were expressed as the mean ± S.E.M. *P<0.05, **P<0.01: LPS alone vs LPS + curcumin.

Fig. 2. Suppression of LPS-induced AP-1 DNA binding activity by curcumin in a concentration-dependent manner. BV2 microglial cells were pretreated with various concentrations of curcumin as indicated for 1 h prior to LPS treatment. Six hours later, nuclear extracts were prepared and AP-1 DNA binding was determined by EMSA. Top panel shows a representative autoradiograph and bottom panel shows quantitative values of autoradiographs. Data represent three independent experiments and were expressed as the mean ± S.E.M. *P<0.05, **P<0.01: LPS alone vs LPS + curcumin.

Fig. 3. Suppression of LPS-induced NF-κB DNA binding activity by curcumin in a concentration-dependent manner. BV2 microglial cells were pretreated with various concentrations of curcumin as indicated for 1 h prior to LPS treatment. Six hours later, nuclear extracts were prepared and NF-κB DNA binding activity was determined by EMSA. Top panel shows a representative autoradiograph and bottom panel shows quantitative values of autoradiographs. Data represent three independent experiments and were expressed as the mean ± S.E.M. *P<0.05; LPS alone vs LPS + curcumin. **P<0.01: LPS alone vs no-treatment control. No significant difference between the no-treatment control and curcumin alone.
involved in the pathogenesis of many chronic neurodegenerative diseases such as AD and ALS and that inhibitors of COX-2 activity are useful for treating inflammation (15, 16). In an animal model of AD, ibuprofen and curcumin were effective on the removal of neuritic plaques and memory improvement (17). Therefore, the inhibitory effects of curcumin on COX-2 expression might be one of the mechanisms of neuroprotection in this animal model.

In conclusion, our data show that curcumin contributes to the suppression of microglia by limiting the expression of COX-2 by inhibiting AP-1 and NF-κB DNA bindings. Suppression of COX-2 expression in microglia may represent a neuroprotection that limits microglial activation in pathological conditions. As a consequence, the pharmacological manipulation of COX-2 expression may potentially be exploited to control the extent of microglial activation in inflammation and neurodegenerative diseases.

Acknowledgments

This work was supported by Kangwon National University (G.K.).

References