Curcumin Suppresses the Production of Pro-inflammatory Cytokine Interleukin-18 in Lipopolysaccharide Stimulated Murine Macrophage-Like Cells

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Abstract Curcumin is a major bioactive compound of turmeric that exerts its anti-inflammatory effects by suppressing the many pro-inflammatory cytokines and chemokines in a number of cell types and pathologic conditions. Interleukin-18 (IL-18) is a novel pro-inflammatory cytokine which plays an important role not only in generating Th1 responses but also in inducing severe inflammatory reactions. As curcumin induced inhibition of IL-18 production in keratinocytes and mice is well known, effect of curcumin on IL-18 release in macrophages remains unknown. Hence, this present study has been designed to evaluate the effect of curcumin on IL-18 production and necrotic cell death in murine macrophages-like cells treated with or without lipopolysaccharide (LPS). The IL-18 secretion in cell culture supernatants was assayed by enzyme-linked immunosorbent assay and cytotoxicity was determined by lactate dehydrogenase release assay. Our results demonstrate that curcumin significantly inhibited the production of pro-inflammatory cytokine IL-18 in *E.coli* LPS stimulated murine macrophage-like cells RAW264.7 in a concentration-dependent manner. Interestingly, curcumin had no cytotoxic effect on murine macrophage-like cells. Our findings suggest that curcumin may be used as a potential therapeutic agent for the treatment of inflammatory diseases.

Keywords Curcumin · Murine macrophage-like cells · IL-18 · Necrosis

Introduction

Curcuma longa Linn. is a perennial flora of family Zingiberaceae, cultivated throughout the tropical and sub tropical regions of the world especially in Asian countries like India and China. In India, it is grown as spice popularly known as *Haldi* (turmeric), a yellow color producing rhizome. In Ayurveda, curcuma has been documented as one of the potential plants having strong medicinal properties used in treatment of various ailments like dyspepsia, liver dysfunction, urinary tract infections, flatulence and sprains. It also acts as ‘blood purifier’ and causes rapid healing of skin wounds and a wide spectrum of skin diseases [1, 2].

The yellow color of curcuma is thought to be due to the presence of principal bioactive compound ‘curcumin’. Curcumin was first isolated from turmeric in 1815 by Vogel and Pelletier and its chemical characterization was made in 1910 which confirmed that curcumin is chemically a diferuloylmethane [3]. It constitutes yellow pigment along with others curcuminoids. Curcumin is a highly pleotropic compound regulates a complex cascade of immune system and influences many of biological functions. It is not only a potent anti-cancer, anti-inflammatory, anti-oxidant, anti-viral, anti-bacterial, anti-angiogenic and anti-proliferative agent but also one of the major pro-apoptotic compounds helping the host in eliminating the infection as well as functionally abnormal cellular bodies. In addition, it has been observed that it exerts hypocholesterolemic effects in patient suffering with diabetes and reduces the level of oxidative stress [4].

Interleukin-18 (IL-18) is a novel cytokine which regulates both innate and acquired immunity. It facilitates the

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clearance of a wide variety of intracellular bacteria, fungi and protozoa from the host body by inducing the interferon gamma mediated killing of microbes [5]. IL-18 was originally named as interferon gamma inducing factor [6] and first identified in the livers of mice infected with *Propionibacterium acnes* [7] but later on its production was observed in a multiple cell types including macrophages, dendritic cells (DCs), osteoblasts, intestinal epithelial cells, Kupffer cells, microglial cells, synovial fibroblasts, keratinocytes and adrenal cortex cells [5].

Like other cytokines, the cellular profile of IL-18 is also significantly modulated by the presence of external agents like microbes and chemicals [5]. There are a very few studies in which it was shown that curcumin decreases the production of IL-18 in UVB-irradiated human keratinocytes [8], mice [9, 10] and rat [11] but it is not known whether or not curcumin also modulates the production of IL-18 in macrophages. In the present study, we have investigated the effect of curcumin on the IL-18 production in LPS activated murine macrophage-like cells RAW264.7. In addition, role of curcumin in necrotic cell death of RAW264.7 cells was also examined.

Materials and Methods

Preparation of Curcumin Stock Solution

A 100 μ M stock solution of commercially available curcumin (Sigma-Aldrich, St. Louis, MO) was prepared in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO) and stored as small aliquots at -20°C . Just before adding to the cultured macrophage-like cells RAW264.7, curcumin was diluted in cell culture medium as needed. The final concentration of dimethyl sulfoxide was kept at 1 %.

Cell Culture and Curcumin Treatment

Murine macrophage-like cell line RAW264.7 (American Type Culture Collection, Rockville, MD) was cultured and routinely maintained in DMEM (Gibco, Invitrogen Corporation, USA) media supplemented with 10 % (v/v) heat inactivated fetal bovine serum (Hyclone, Thermo Scientific, USA), 1.46 g/l L-glutamine (Sigma-Aldrich, St. Louis, MO), 2.3 g/l HEPES (Gibco, Invitrogen Corporation, USA) and 3.7 g/l NaHCO_3 (Sigma-Aldrich, St. Louis, MO), 10 ml/l of Pen Strep (Gibco, Invitrogen Corporation, USA) containing 100 U/ml penicillin-100 μ g/ml streptomycin at 37°C in a 5 % CO_2 humidified incubator. For an experiment, 0.5×10^6 cells were seeded onto 100 mm tissue culture dish (Falcon, BD Bioscience, MA) and incubated for 4 h to allow them to adhere to the bottom of the dishes. After washing with plain DMEM media, cells were pre-treated with or without various

concentrations (1, 25, 50 μ M) of curcumin for 4 h followed by stimulation with or without 1 μ g/ml *E.coli* LPS (Sigma-Aldrich, St. Louis, MO) for 12 h. Supernatants of treated cells were collected after 12 h for cytokine assay.

Cytokine Assay

The amount of IL-18 secreted into the culture supernatants was determined by enzyme-linked immunosorbent assay (ELISA) using commercially available Mouse IL-18 Platinum ELISA Kit (Bender MedSystems, San Bruno, CA) according to the manufacturer's protocols.

Cytotoxicity Assay

Curcumin mediated cell death was determined by measuring the release of intracellular enzyme lactate dehydrogenase (LDH) in the culture supernatants of RAW264.7 macrophages treated with or without varying amounts (1, 25, 50 μ M) of curcumin for 24 h following the protocols provided with CytoTox96[®] Non-Radioactive Cytotoxicity Assay Kit (Promega, Madison, WI).

Statistical Analysis

Analysis of variance (ANOVA) and Newman-Keuls multiple comparison test were performed to compare the statistical significance between various groups. *p* values were considered significant if $p \leq 0.05$. GraphPad Prism[®] 5.0 software was used for statistical analyses.

Results and Discussion

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) seems to exhibit its anti-inflammatory effects via suppression of production of major pro-inflammatory cytokines such as IFN- γ , TNF- α , IL-2, IL-6, IL-8, IL-12, IL-13, IL-15 and some chemokines in a variety of cell types and diseases [12, 13] but its role in modulation of IL-18 release in macrophages is not known. Therefore, in the present study we have investigated the effect of curcumin on the production of IL-18 in *E.coli* LPS stimulated murine macrophages-like cells RAW264.7. Our data demonstrate that pre-treatment of RAW264.7 cells with different concentrations (1, 25, 50 μ M) of curcumin significantly inhibited the IL-18 production in concentration-dependent manner when compared with control samples ($p < 0.0001$) (Fig. 1). Previously, similar results were observed in UVB-irradiated human keratinocytes [8], mice [9, 10] and rat [11].

The mechanism(s) by which curcumin suppresses the IL-18 production in LPS stimulated macrophage-like cells

Fig. 1 Effect of curcumin on IL-18 production. Murine macrophage-like cells RAW264.7 were incubated with different concentrations of curcumin (1, 25 and 50 μM) in the presence of *E.coli* LPS (1 μg/ml). Cell supernatants were collected after 12 h and assayed for IL-18. The data were expressed in mean ± SD and derived from three independent experiments. $p < 0.0001$ versus control (only LPS treated cells)

Fig. 2 Effect of curcumin on the rate of necrosis. Murine macrophage-like cells RAW264.7 were incubated with different concentrations of curcumin (1, 25 and 50 μM) for 24 h. Cell supernatants were collected after 24 h and assayed for release of LDH. The data were expressed in mean ± SD and derived from three independent experiments. $p > 0.05$ versus control

remains unclear. However, it is suggested that the inhibition of transcription factor NF-κB and signaling pathway MAPK may be one of the possible reasons behind such event. In previous studies, many researchers have reported that curcumin abolishes the activity of NF-κB proteins [14] which is thought to play a central role not only in regulating the expression of many pro-inflammatory cytokines and chemokines but also in governing the expression of a wide range of genes critically required for cell differentiation, cell proliferation, cell survival, invasion, apoptosis, angiogenesis, tumorigenesis, metastasis, radioresistance and chemoresistance and other member proteins of NF-κB signaling cascade namely IκB, IκB kinase β [13]. In addition, MAP kinase signaling pathway is also concerned with the regulation of cytokine production [15]. A number of studies illustrated that curcumin suppressed the expression of inflammatory cytokines via inhibition of

components of MAPK signaling channel such as p38, JNK and ERK [16–18].

Subsequently, we evaluated the effect of curcumin on macrophage cell death by performing the LDH release based cytotoxicity assay. Our results showed that curcumin did not exert any statistically significant effect on necrotic cell death of treated RAW264.7 cells as compared with untreated control samples ($p > 0.05$) (Fig. 2). Earlier report also demonstrated that curcumin did not cause cellular toxicity (in the term of necrosis or apoptosis mediated cell death) to non-tumor/normal cells [19].

Conclusion

Our study showed that curcumin suppresses the production of IL-18 in macrophage-like cells RAW264.7 when stimulated with LPS. Moreover, curcumin had no cytotoxic effect on RAW264.7 cells. It is suggested that curcumin induced inhibition of IL-18 may be useful in treatment of inflammatory diseases.

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