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Effect of berberrubine on interleukin-8 and monocyte chemotactic protein-1 expression in human retinal pigment epithelial cell line

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Abstract

We examined the effects of berberrubine, a protoberberine alkaloid, on interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) expression in a human retinal pigment epithelial cell line (ARPE-19) stimulated with interleukin-1β (IL-1β) or tumor necrosis factor α (TNF-α). ARPE-19 cells were cultured to confluence. Berberrubine and IL-1β or TNF-α were added to the medium. IL-8 and MCP-1 protein concentrations were measured using enzyme-linked immunosorbent assay. IL-8 and MCP-1 mRNA were measured by real time polymerase chain reaction. Nuclear factor κB (NF-κB) translocation was examined by immunofluorescent staining/microscopy. Berberrubine dose-dependently inhibited IL-8 and MCP-1 protein levels in the media and mRNA expression of the cells stimulated with IL-1β or TNF-α. Immunofluorescent staining/microscopy of NF-κB in the nucleus of unstimulated cells was faint (51±14 arbitrary units). Fluorescein was dense (215±42 or 170±24 arbitrary units, respectively) 30 min after stimulation with IL-1β or TNF-α and was decreased to 62±18 or 47±16 arbitrary units, respectively, by berberrubine. Berberrubine dose-dependently inhibited IL-8 and MCP-1 expression and protein secretion induced by IL-1β or TNF-α. Possibly, the effect on chemotactic factors may be via suppression of NF-κB translocation.

Keywords: ARPE-19 cells; Berberrubine; Interleukin 1β; Tumor necrosis factor α; Interleukin 8; Monocyte chemotactic protein 1

Introduction

The retinal pigment epithelial (RPE) cells play important roles on physiology and pathology of the retina. Human RPE cells isolated from donor eyes express interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) by stimulation with interleukin 1β (IL-1β) or tumor necrosis factor α (TNF-α) (Elner et al., 1990; 1991). Crane et al. (2000) reported that MCP-1 and IL-8 are produced at much higher levels than other chemokines tested in RPE cells. MCP-1 and IL-8 are called CCL2 and CXCL8, respectively, according to a new classification system recommended by Zlotnik and Yoshie (2000). Dunn et al. (1996) reported that ARPE-19, a human retinal pigment epithelial cell line, has structural and functional properties characteristic of RPE cells.

The roots and barks of Berberis species are used in traditional Chinese–Korean–Japanese medicine for treatment of various inflammatory diseases. Berberine is a major alkaloid isolated from some herbs such as Berberis species, Coptidis species rhizoma (Huanglian, in Chinese; Ohren, in Japanese), and Phellodendri species cortex (Huangbai, in Chinese; Ohbaku, in Japanese). The alkaloid has multiple pharmacological actions including anti-inflammatory effects (Fukuda et al., 1999; Zhou and Mineshita, 2000; Kupeli et al., 2002; Lee et al., 2003; Kuo et al., 2004), antipyretic action (Kupeli et al., 2002), diarrhea-treating action (Luo, 1955), glucose-lowering potential in vitro and in vivo (Yin et al., 2002; Leng et al., 2004), insulin sensitizing and insulinotropic action (Ko et al., 2005) and cholesterol-lowering effect (Kong et al., 2004). We previously reported that berberine dose-dependently inhibited the expression of IL-8 and MCP-1 in ARPE-19 cells induced by IL-1β or TNF-α (Cui et al., 2006). Berberrubine, 9-demethylberberine, is a protoberberine alkaloid, which has antimicrobial effects

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(Hong et al., 2000; Kim et al., 2002) and antitumor activities (Kobayashi et al., 1995; Kim et al., 1998; Kang and Chung, 2002). In the present study, we investigated the effects of berberrubine on IL-8 and MCP-1 expression and NF-κB translocation in ARPE-19 cells stimulated with IL-1β or TNF-α.

Materials and methods

Cell culture

ARPE-19, a human RPE cell line, was purchased from American Type Culture Collection (No. CRL-2302, Rockville, MD, USA). The cells were maintained in Dulbecco's modified essential medium and Ham's F12 (DMEM/F12; 1:1; Gibco-BRL, NY, USA) supplemented with 10% fetal bovine serum (FBS); penicillin, 100 U/ml; and streptomycin, 100 μg/ml, to obtain confluent cells. All cells were cultured at 37 °C under 10% CO_2 and 90% moist air. Media were changed twice a week. The viability of ARPE-19 cells after incubation was assessed using trypan blue dye.

Synthesis of berberrubine compound

Berberrubine was a gift from Dr. Zuo Feng (Institute of Natural Medicine, University of Toyama), who made it from berberine (Sigma Chemicals, St Louis, MO, USA) by the conversion method using microwave irradiation (Das and Srinivas, 2002), purified it by silica gel column chromatography (yield: 76%), (Hong et al., 2000) and identified by liquid chromatography-tandem mass spectrometry (LC/MS-MS) (Esquire 3000 plus mass spectrometer, Bruker Daltonics Inc, USA). Chemical structures of berberine and berberrubine are shown in Fig. 1.

IL-8 and MCP-1 protein concentration by enzyme-linked immunosorbent assay (ELISA)

Berberrubine was dissolved in 50 mM dimethyl sulfoxide (DMSO) just before use. The final concentration of DMSO was kept at ≤0.05% in the culture media. ARPE-19 cells were seeded in 24-well culture plates (averaging 2.0×10^5 cells/well) and incubated for 14 days in DMEM/F12 medium containing 10% FBS. The cells were washed twice with serum-free medium and incubated in serum-free medium for 2 h. ARPE-19 cells were preincubated in serum-free medium with 0.2, 1, 5, and 25 μM of berberrubine for 30 min. Then, recombinant human IL-1β (Wako Pure Chemicals, Osaka, Japan) or TNF-α (Wako Pure Chemicals, Osaka, Japan) was added to the medium, and it was incubated for 24 h. Thereafter, the supernatant was collected and stored at −70 °C until assay. IL-8 and MCP-1 protein concentration were determined using ELISA (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) and were calculated based on standard curves using concentrations of recombinant IL-8 and MCP-1 in the ranges of 25–1000 pg/ml and 51–2000 pg/ml, respectively. All assays were performed in duplicate.

Total RNA extraction and IL-8 and MCP-1 mRNA expression by real time polymerase chain reaction

ARPE-19 cells were planted and cultured in 6-well culture plates (averaging 2.0×10^6 cells/well) for 14 days in DMEM/F12 medium containing 10% FBS. After the cells were washed twice with serum-free medium, ARPE-19 cells were preincubated for 2 h in serum-free medium with 0.05% DMSO or 0.2, 1, 5, and 25 μM of berberrubine for 30 min. Then, recombinant human IL-1β (Wako Pure Chemicals, Osaka, Japan) or TNF-α (Wako Pure Chemicals, Osaka, Japan) was added to the medium, and incubated. Total RNA was extracted from the cells using RNeasy Protect Mini Kit (Qiagen, Hilden, Germany), and treated with RNase-free DNase Set (Qiagen, Hilden, Germany) to remove any residual genomic DNA. One microgram of each total RNA was reverse transcribed using Random Hexamers[b], MultiScribe Reverse Transcriptase (Applied Biosystems, Branchburg, NJ, USA), and thermal cycler (Gene Amp PCR System 2400; Applied Biosystems, Norwalk, CT, USA). Condition of the reverse transcription included incubation at 25 °C for 10 min, reverse transcription at 48 °C for 30 min, and reverse transcriptase inactivation at 95 °C for 5 min.

cDNA was used to detect real time PCR products for IL-8 and MCP-1 using TaqMan Universal Master Mix (Applied Biosystems, Branchburg, NJ, USA) and ABI PRISM™ 7700 Sequence Detector (Applied Biosystems, Foster, CA, USA) with TaqMan® Pre-Developed Assay Reagents (Applied Biosystems, Foster, CA, USA) for human IL-8 and MCP-1. The thermal profile for each primer consisted of 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and

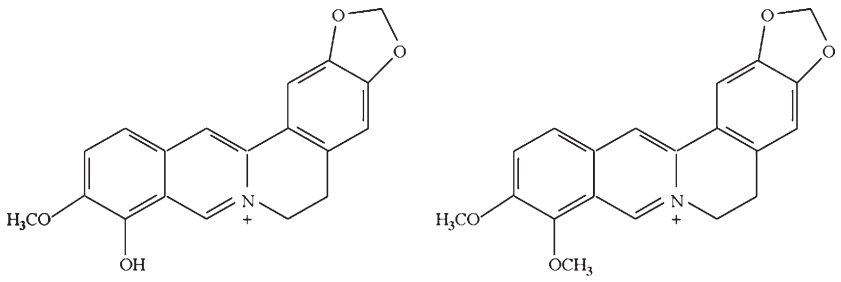

Fig. 1. Chemical structures of berberrubine and berberine.