OK205 Regulates Production of Inflammatory Cytokines in HMC-1 Cells

In-Young Choi, a,b Hyuk-Sang Jung, a Hyung-Ryong Kim, c Eon-Jeong Lee, d Eun-Hee Lee, e Tae-Yong Shin, f Hyung-Min Kim, g and Seung-Heon Hong*. b

a College of Oriental Medicine, Kyung Hee University; 1 Hoegi-dong, Dongdaemun-gu, Seoul 130–701, Republic of Korea; b Department of Oriental Pharmacy, College of Pharmacy; c Department of Dental Pharmacology and Nano Science & Technology Research Institute, School of Dentistry; d College of Oriental Medicine, Wonskwang University; Iksan, Jeonbuk 570–749, Republic of Korea; e Division of Beauty Arts, Jeonbuk Science College; Jeonegeup, Jeonbuk 580–712, Republic of Korea; f College of Pharmacy, Woosuk University; Jeonju, Jeonbuk 565–701, Republic of Korea.

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OK205 is a traditional Korean prescription containing water-soluble chitosan, glucosamine HCl, chondroitin sulfate, and extract of herbal medicine, and has been used commercially to treat rheumatoid arthritis (RA). Because infiltrated mast cells and their mediators may contribute to the initiation and progression of the inflammatory process and matrix degradation of RA, we tested the inhibitory effects of OK205 on cytokine production in a human mast cell line (HMC-1 cells). Production of tumor necrosis factor-α was significantly decreased to 0.091±0.010 ng/ml after treatment of HMC-1 cells with OK205 100 μg/ml. The inhibition rate was about 43.57%. In addition, production of interleukin-6 in OK205 1 pg/ml-treated cells was 2.77±0.071 ng/ml, and the inhibition rate was about 50.22%. However, OK205 did not significantly inhibit the production of interleukin-8. These findings may help in understanding the mechanism of action of OK205, leading to control of mast cells in inflammatory conditions like RA.

Key words OK205; rheumatoid arthritis; human mast cell line; tumor necrosis factor-α; interleukin-6; interleukin-8

OK205 is composed of several materials and the components are interesting from the viewpoint of relieving rheumatoid arthritis (RA). However, it is still unclear how it regulates the immune or inflammatory responses.

RA is a typical rheumatic disease characterized by chronic inflammatory disease that leads to cartilage destruction, joint deformity, and disability.1,2) In the rheumatoid lesion, profound hypertrophic changes of the synovium with infiltration of immune cells, increased vascularity, and hyperplasia result in the formation of a synovial pannus that invades cartilage and bone.3) The cellular composition at sites of cartilage erosion varies greatly, such as macrophages, fibroblasts, mast cells, polymorphonuclear lymphocytes, and dendrocytes.4) Although mast cells have been viewed primarily in the central role of immediate-type hypersensitivity reactions,5,6) the significant contribution of the mast cells in the pathogenesis of rheumatic diseases has recently become more evident. Accumulation of mast cells and their activation/degranulation have been demonstrated in rheumatoid synovial tissues as well as in the synovial fluids.7–9) Activated mast cells synthesize prostaglandins and leukotrienes and produce both preformed and newly synthesized cytokines such as tumor necrosis factor (TNF-α) and interleukins.10) Thus infiltrated mast cells and their mediators may contribute to the initiation and progression of the distributive cytokine gene expression such as that of TNF-α, IL-5, IL-6, and IL-8.27,28) Moreover, treatment with CsA plus another RA agent like methotrexate was effective in treating RA.29)

In the present study, we showed that OK205 inhibited the production of phorbol-12-myristate-13-acetate (PMA) plus A23187-induced cytokines from a human mast cell line (HMC-1 cells).

MATERIALS AND METHODS

Reagents and Antibodies Cell culture medium, Iscove’s modified Dulbecco’s medium (IMDM), was purchased from Gibco BRL (Grand Island, NY, U.S.A.). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT), PMA, A23187, 2,2-aziobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), CsA, and other reagents were obtained from Sigma (St. Louis, MO, U.S.A.). Anti-human TNF-α and IL-1β Ab, biotinylated anti-human TNF-α and IL-1β Ab, and recombinant human TNF-α and IL-1β were purchased from R&D Systems (Minneapolis, MN, U.S.A.). Anti-human IL-6, and IL-8 Ab, biotinylated anti-human IL-6 and IL-8 Ab and recombinant human IL-6 and IL-8 were obtained from Pharmingen (Cambridge, U.K.).

Preparation of OK205 OK205 was composed of water-
RESULTS

statistically significant. Between the groups. Results of 

soluble chitosan, glucosamine HCl, chondroitin sulfate, and extract of herbal medicine in a ratio of 3:3:3:1. First, the extract of herbal medicine was prepared by decocting the dried prescription of seven herbs with 11 of distilled water. The extraction decocted for approximately 2 h was filtered, lyophilized, and kept at 4 °C. It includes Codonopsis pilosula NANNF, Angelica koreana MAXIM, Acanthopanax sessiliflorus SEEM, Chaenomeles sinensis KOEINE, Eucommia ulmoides OLIV, Poria cocos WOLF, and Glycyrrhiza uralensis FISCH (20 : 15 : 15 : 15 : 12 : 8). These were dissolved in PBS and filtered with a 0.2-μm syringe filter. Water-soluble chitosan, glucosamine HCl, and chondroitin sulfate were obtained from Chito153 (Seoul, South Korea). All plants were obtained from the Daehak Oriental Pharmacy (Iksan, South Korea).

Cell Culture and Stimulation HMC-1 cells were grown in IMDM supplemented with penicillin 100 units/ml, streptomycin 100 μg/ml, monothioglycerol 10^{-5} M, and 10% heat-inactivated FBS at 37 °C in 5% CO2 and 95% humidity. HMC-1 cells (1×10^6 cells/ml) were treated with OK205 or CsA (5 μg/ml) for 30 min prior to stimulation with PMA 50 nm plus 1 μM A23187 and incubated at 37 °C for an additional time.

MTT Assay To test the viability of cells, the MTT colorimetric assay was performed as described previously. Briefly, HMC-1 cells (1×10^6 cells/ml) were incubated for 8 h after stimulation in the presence or absence of OK205 (10 ng/ml, 10 μg/ml). After the addition of MTT solution, the cells were incubated at 37 °C for 4 h. The crystalized MTT was dissolved and the absorbance was measured at 540 nm.

Assay of Cytokine Production Secreted cytokine levels in culture supernatants of HMC-1 were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) according to manufacturer’s protocol (for TNF-α and IL-1β assay, R&D Systems; for IL-6 and IL-8 assay, PharMingen). Absorption of the avidin-horseradish peroxidase color reaction was measured at 405 nm and compared with serial dilutions of human recombinants as a standard.

Statistical Analysis Each value represents the mean±S.E.M. of different experiments under the same conditions. Student’s t-test was used to make a statistical comparison between the groups. Results of p<0.05 were considered statistically significant.

RESULTS

Effects of OK205 on HMC-1 Viability To test the cytotoxic effects of OK205, we performed the MTT assay in HMC-1 cells. OK205 (10 ng/ml, 10 μg/ml) did not significantly affect cell viability in each condition and had no toxicity on HMC-1 cells (data not shown).

Effects of OK205 on Cytokine Production HMC-1 cells can induce the activation of nonphysiologic agents such as PMA or A23187 and release various cytokines. The concentration of TNF-α was 0.021±0.006 ng/ml in nonstimulated cells and 0.145±0.007 ng/ml in PMA plus A23187-stimulated cells (p<0.001). However, the production was significantly decreased to 0.091±0.010 ng/ml after treatment with OK205 100 μg/ml (p<0.001). CsA (5 μg/ml) also inhibited TNF-α production by 0.053±0.005 ng/ml (Fig. 1). However, 10 ng/ml, 0.1 ng/ml, and 1 μg/ml of OK205 did not inhibit TNF-α production by activated HMC-1 cells (data not shown).

The concentration of IL-6 was 0.308±0.087 ng/ml in nonstimulated cells and 5.272±0.190 ng/ml in PMA plus A23187-stimulated cells. We also measured the IL-6 concentration in supernatants incubated with OK205 or CsA. The concentration of IL-6 in OK205 1 pg/ml-stimulated cells was 2.779±0.071 ng/ml, and the inhibition rate was about 50.22%. CsA (5 μg/ml) decreased IL-6 production (4.082±0.124 ng/ml), and the inhibition rate was about 23.97% (Fig. 2).

IL-8 production was 0.089±0.013 ng/ml in nonstimulated cells and 2.599±0.116 ng/ml in PMA plus A23187-stimu-
success.31,32) Higher levels of IL-6 have been found in the sera of RA patients compared with controls, and in synovial fluid from RA patients.33,34) It was reported that chitosan can regulate inflammatory cytokine production such as TNF-α, IL-1β, IL-6, and IL-8.21,38) OK205 also inhibited the inflammatory cytokines such as TNF-α, IL-1β, and IL-6, but not IL-8. The human chemokine IL-8 released from mast cells might act on surrounding cells such as neutrophils, T cells, B cells, and eosinophils and induce migration and activation of inflammatory effector cells.41–44) The OK205 doses used may not affect the migration of these cells or the release response may be different from that of Daeganghwal-tang and Cool-cool. The main effect of OK205 in RA may be associated with inflammatory cytokine blockage.

This study investigated the effects of OK205 in an in vitro experimental model. The effective concentration is too high, which means that a clinical application is likely not possible. The results of this study may provide clues to the pharmacologic function of the formula for future in vivo study. In addition, because TNF-α, IL-6, and IL-1β are dominantly produced in other types of cells, such as macrophages, synoviocytes, T cells, and so on, we need to confirm the present results in these cells.

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REFERENCES


Fig. 3. Effect of OK205 on PMA Plus A23187-Induced IL-8 Production from HMC-1 Cells

Cells (1×10⁶ cells/ml) were incubated in the presence or absence of OK205 (1 pg/ml—100 μg/ml) for 30 min prior to stimulation with PMA plus A23187, and IL-8 in the supernatant was measured after 8-h incubation. CsA (5 μg/ml) was used as a positive control compared with OK205. Asterisks correspond to the levels of significance assessed using the Student’s t-test. *p<0.01 compared with nonstimulated cells, and **p<0.001 compared with the PMA plus A23187-stimulated value. All data represent the mean±S.E.M. of four independent experiments. 1, Nonstimulated cells; 2, PMA plus A23187; 3, OK205 (1 pg/ml)+PMA plus A23187; 4, OK205 (10 pg/ml)+PMA plus A23187; 5, OK205 (100 pg/ml)+PMA plus A23187; 6, OK205 (10 μg/ml)+PMA plus A23187; 7, OK205 (1 μg/ml)+PMA plus A23187; 8, OK205 (10 μg/ml)+PMA plus A23187; 9, OK205 (100 μg/ml)+PMA plus A23187; 10, CsA (5 μg/ml)+PMA plus A23187.


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