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Anti-inflammatory activity of fisetin in human mast cells (HMC-1)

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Abstract

Mast cells play an important role in the pathogenesis of allergic diseases through the release of inflammatory mediators such as histamine, cysteinyl leukotrienes, cytokines, and chemokines. Flavonoids, like fisetin are naturally occurring molecules with antioxidant, cytoprotective, and anti-inflammatory actions. The aim of our study was to examine whether fisetin modulates inflammatory reaction in stimulated human mast cells (HMC-1). Fisetin decreased phorbol-12-myristate 13-acetate plus calcium ionophore A23187 (PMACI)-stimulated gene expression and production of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-4, IL-6, and IL-8 in HMC-1 cells. Fisetin inhibited PMACI-induced phosphorylation of p38 mitogen-activated protein kinase, extracellular-regulated kinase, and c-Jun N-terminal kinase. In addition, fisetin suppressed nuclear factor (NF)-κB activation induced by PMACI, leading to expression of IκB-α phosphorylation and degradation. Fisetin suppressed powerful induction of NF-κB promoter-mediated luciferase activity. These pharmacological actions of fisetin produce new suggestion that fisetin is a potential medicine for treatment of inflammatory diseases through the down-regulation of mast cell activation.

Keywords: Fisetin; Inflammatory reaction; Mast cells; Inflammatory cytokine

1. Introduction

Mast cells are broadly distributed throughout mammalian tissues and play a critical role in a wide variety of biological responses. Typically, mast cells have been considered not only in the association of immediate-type hypersensitivity, but also in late reactions like inflammatory responses, which are mast cell dependent [1,2]. In inflammatory process, several pro-inflammatory cytokines such as, tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-4, IL-6, and IL-8, recruit activated immune and inflammatory cells to the site of lesions, thereby amplifying and perpetuating the inflammatory condition [3,4]. Mast cell-derived pro-inflammatory cytokines play an important role in the development of acute- and late-phase allergic inflammatory reactions. Therefore, inhibition of the production and secretion of these mediators is likely to provide a major mechanism to enhance efficacy in the treatment of inflammatory diseases [5–7].

Mitogen-activated protein kinases (MAPKs) activated by various different stimuli regulate the transcriptional activity of mammalian cellular physiology. Those extracellular stimuli commence specific biological responses involving differentiation, proliferation and apoptosis through the activation of MAPK signalling cascades, which constitute three major subfamilies, such as extracellular signal-regulated kinase (ERK), p38 MAPK and *c-Jun* N-terminal kinase (JNK). These three types of MAPKs play an significant role in the signalling of apoptosis and cytokine expression [8–10]. Nuclear factor (NF)-κB is a substantial transcription factor required for the

Abbreviations: HMC-1, human mast cell; TNF, tumor necrosis factor; IL, interleukin; NF, nuclear factor; MAPK, mitogen-activated protein kinase; ERK, extracellular-regulated kinase; JNK, c-Jun N-terminal kinase; PMA, phorbol 12-myristate 13-acetate; PMACI, PMA plus calcium ionophore A23187; EMSA, electrophoretic mobility shift assay; RT-PCR, reverse transcription-polymerase chain reaction

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expression of many inflammatory genes [11]. NF-κB plays a serious role in the expression of cytokines such as TNF-α, IL-6, which mediate response to inflammatory stimuli in the allergic inflammatory responses [12]. For this reasons, NF-κB is an obvious target of anti-inflammatory treatment [6,13,14].

Flavonoids are low molecular weight compounds rich in seeds, citrus fruits, red wine, tea, and olive oil. Some flavonoids, such as quercetin, myricetin and rutin have diverse biological effects including antioxidant, anti-platelet, anti-thrombotic action, cytoprotective, anti-allergic, anti-viral, anti-carcinogenic activities, and anti-inflammatory activities [12,15–18]. Fisetin (3,7,3′,4′-tetrahydroxy flavone) is a member of flavonoids and it has been reported to attract significant consideration from biological as well as other important perspective. Lately, it has been reported that fisetin has strong antioxidant properties in membrane environments and is suggested as a potentially useful therapeutic agent against various free radical-mediated diseases [19,20]. However, the anti-inflammatory activities and the possible mechanism of action focusing on the expression of pro-inflammatory cytokines are still unknown. In the present study, to clarify the mechanism of fisetin that accounts for its anti-inflammatory effect, we examined the potential role of fisetin on gene expression of inflammatory cytokines, and the possible mechanisms of action in PMACI-stimulated human mast cells.

2. Materials and methods

2.1. Reagents

Fisetin, phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187 (Calcymycin; C29H37N3O6) were purchased from Sigma Chemical Co. (St. Louis, MO), and liquefied in DMSO. Iscove's modified Dulbecco's medium (IMDM) was purchased from Life Technologies (Grand Island, NY). Anti-human NF-κB (p65) and -IκBα antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-p38, -JNK, and -ERK antibodies and anti-p38, -JNK, and -ERK antibodies were purchased from Cell Signalling (Beverly, MA). Specific inhibitors for NF-κB (pyrrolidine dithiocarbamate, PDTC), p38 MAPK (SB203580), ERK (PD98059), and JNK (SP600125) were purchased from Biomol (Plymouth Meeting, PA).

2.2. Cell culture

HMC-1 was grown in IMDM supplemented with 100 μg ml^{-1} streptomycin, 100 U ml^{-1} penicillin, and 10% heat-inactivated fetal bovine serum (FBS) at 37 °C in 5% CO_2. HMC-1 was treated with fisetin (3–30 μM) for 30 min (except luciferase assay) prior to stimulation with 40 nM of PMA and 1 μM of A23187.

2.3. RT-PCR for inflammatory cytokines secretion

Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNF-α, IL-1β, IL-4, IL-6, IL-8 and β-actin (internal control). The respective primer decides were chosen by Primer3 program (Whitehead Institute, Cambridge, MA). Total RNA was separated from HMC-1 according to the manufacturer's instruction [7]. Each sample was reverse-transcribed to cDNA for 60 min at 45 °C using a cDNA synthesis kit (Amersham Biosciences Inc., Piscataway, NJ). PCR was carried out with following primers for TNF-α (s 5′-cct acc aga cca agg tca ac-3′; as 5′-agg ggg taa taa agg gat tg-3′), IL-1β (s 5′-aaa cag atg aag tgc tcc tt-3′; as 5′-tgg aga aca cca ctt gtt gc-3′), IL-4 (s 5′-atg ggt ctc acc tcc caa ctg ct-3′; as 5′-cag ctc gaa cac ttt gaa tat ttc tct ctc-3′), IL-6 (s 5′-aaa gag gca ctg gca gaa aa-3′; as5′-atc tga ggt gcc cat gct ac-3′), and IL-8 (s 5′-aca gca gag cac aca agc tt-3′; as 5′ctg gca acc cta caa cag ac-3′). The β-actin (s 5′-gga ctt cga gca aga gat gg-3′; as 5′-agc act gtg ttg gcg tac ag-3′) was used to verify that equal amounts of RNA were used for reverse transcription and PCR amplification from different experimental conditions. The annealing temperature and cycles were 57 °C, 26 cycle for TNF-α, 57 °C, 28 cycle for IL-1β, 57 °C, 26 cycle for IL-4, 59 °C, 26 cycle for IL-6, 59 °C, 26 cycle for IL-8 and 54 °C, 23 cycle for β-actin. Amplified fragment sizes for TNF-α, IL-1β, IL-4, IL-6, IL-8 and β-actin were 279, 391, 461, 412, 247 and 234 bp, respectively. Products were separated by electrophoresis on a 1.2% agarose gel and visualized by staining with ethidium bromide. The gels were certificated using a Kodak DC 290 digital camera (Eastman Kodak, Rochester, NY) and digitized using UN-SCAN-IT software (Silk Scientific, Orem, UT).

2.4. Enzyme-linked immunosorbent assay (ELISA)

TNF-α, IL-1β, IL-4, IL-6, and IL-8 secretion were measured by ELISA. HMC-1 cells were sensitized with PMA (40 nM) and A23187 (1 μM) for 12 h with or without fisetin. The ELISA was performed by coating 96-well plates with 6.25 ng well^{-1} of monoclonal antibody with specificity for TNF-α, IL-1β, IL-4, IL-6, and IL-8, respectively. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. For the standard curve, rTNF-α, rIL-1β, rIL-4, rIL-6, and rIL-8 were added to the serum which was previously determined to be negative to endogenous TNF-α, IL-1β, IL-4, IL-6, and IL-8. After exposure to the medium, the assay plates were exposed sequentially to biotinylated 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) tablet substrates. Optical density was read within 10 min of the addition of the substrate with a 405 nm filter.

2.5. Preparation of cytoplasmic and nuclear extract

Nuclear and cytoplasmic extracts were prepared as described somewhere else [21]. In brief, after stimulation for the indicated times, 3×10^6 cells were washed with ice-cold PBS and centrifuged at $400 \times g$ for 5 min at 4 °C, resuspended in 100 μg of hypotonic buffer A (10 mM HEPES/KOH, 2 mM $MgCl_2$, 0.1 mM EDTA, 10 mM KCl, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride, pH 7.9). The cells were allowed to swell on ice for 15 min, lysed gently with 2.5 μl of 10% Nonidet P-40. After centrifugation at $400 \times g$ for 10 min at 4 °C, the supernatant was acquired in the form of a cytoplas-