Inhibitory Effect of Persimmon on Mast Cell-Mediated Allergic Inflammation

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Abstract— Diospyros kaki ‘Fuyu’ (Persimmon) has been used in traditional medicine for the treatment of inflammation and microbial infection. In this study, we investigated the effect of cortex of Diospyros kaki ‘Fuyu’ (CDK) on mast cell-mediated allergic inflammation and study its possible mechanisms of action. Levels of histamine, expression and secretion of proinflammatory cytokines, and activation of nuclear factor (NF)-κB from mast cells were assayed. CDK reduced histamine release from human mast cells (HMC-1) activated by phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 (PMACI). CDK decreased PMACI-stimulated gene expression and secretion of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β. The inhibitory effect of CDK on proinflammatory cytokines was NF-κB dependent. Our results suggest a possible therapeutic application of CDK in allergic inflammatory disorders.

Keywords— Persimmon, Allergic inflammation, Histamine, Nuclear factor-κB, proinflammatory cytokines

I. INTRODUCTION

Diospyros kaki ‘Fuyu’, commonly called persimmon, is widely distributed in northeast Asian countries, and has been used in traditional medicine. Pharmacological effects of D. kaki such as inhibition of inflammation, microbial infection, blood pressure, and thrombosis have been reported [1]-[3]. D. kaki contains biological active compounds such as carotenoid, tannins, flavonoids, and catechin [4]. Recently, a report demonstrated that the concentrations of these compounds are higher in the cortex than in the pulp or leaf [5]. However the anti-allergic effect cortex of D. kaki has not been elucidated yet.

Mast cells are broadly distributed throughout mammalian tissue and play various functions as a regulator of allergic inflammation such as asthma, atopic dermatitis, eczema, and sinusitis. Mast cells have been considered not only in the association of immediate type hypersensitivity but also in late reaction like inflammatory responses [6], [7]. In mast cell-mediated inflammatory responses, histamine is one of the most characterized and important mediators implicated in the acute phase of immediate hypersensitivity [8], [9]. Mast cell activation is initiated by phosphorylation of tyrosine kinase which leads to activation of protein kinase C, mitogen-activated protein kinases (MAPKs), nuclear factor (NF)-κB, and expression of proinflammatory cytokines [10], [11].

In the present study, we investigated the effect of cortex of Diospyros kaki (CDK) on on phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 (PMACI)-induced histamine release from mast cells. The effect of CDK on PMACI-induced gene expression of proinflammatory cytokines and the role of NF-κB in this effect were investigated using human mast cells (HMC-1).

II. MATERIALS AND METHODS

A. Reagents and cell culture

Phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 were purchased from Sigma Chemical Co (St Louis, MO, USA). The human mast cell line (HMC-1) was grown in Iscove’s media (Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO2. The passage ranging 4-8 of HMC-1 was used throughout the study.

B. Extraction and isolation of active compound from CDK

D. kaki was supplied from Korea National Arboretum. The shade dried and powdered cortex of D. kaki (600 g) was extracted three times with hot water, and then filtered through Whatman No.1 filter paper. The extracts were combined and evaporated. The resultant water extract (70 g) was successively partitioned as chloroform, ethyl acetate, n-butanol and water soluble fractions. The ethyl acetate soluble fraction exhibited the most anti-allergic activity. Ethyl acetate soluble fraction was chromatographed on Sephadex LH-20 column (MeOH) to give five fractions (E1-E5). E2 was subjected to chromatography on Sephadex LH-20 gel column (MeOH) to give compound 1 (25 mg). In this study, we use whole water extract of CDK. The CDK was dissolved in saline or Tyrode buffer A (HEPES 10 mM, NaCl 130 mM, KCl 5 mM, CaCl2 1.4 mM, MgCl2 1 mM, glucose 1.4 mM, 0.1 % bovine serum albumin) and was filtered using a 0.45 μm syringe filter before use.

C. Determination of histamine level

Determination of histamine contents was examined as previously described [12]. The histamine contents were measured by the o-phthalaldehyde spectrofluorometric procedure. The fluorescent intensity was measured at emission 438 nm and excitation 353 nm using spectrofluorometer.
D. RNA extraction and mRNA detection

The total cellular RNA was isolated from the cells (1×10^6/well in a 24-well plate) after stimulation with PMA (20 nM) and A23187 (1 μM) with or without CDK for 2 h using TRI reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer’s protocol. The first strand complementary DNA (cDNA) was synthesized using the Superscript II reverse-transcriptase (Invitrogen, Carlsbad, CA, USA). A reverse-transcriptase polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNF-α, IL-1β, and β-actin (internal control). The conditions for the reverse transcription and PCR steps were similar to those described previously [13]. The amplified products were separated by electrophoresis on 2% agarose gel containing ethidium bromide, documented using a Kodak DC 290 digital camera and digitized with UN-SCAN-IT software (Silk Scientific, Orem, UT, USA). The band intensity was normalized to that of β-actin in the same sample.

E. Western blot analysis

HMC-1 were washed 3 times with PBS and resuspended in lysis buffer. Samples were electrophoresed using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described elsewhere [14], and then transferred to a nitrocellulose membrane. The nucleus and cytosolic p65 NF-κB and IκBα was assayed using anti-NF-κB (p65) and anti-IκBα antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunodetection was done by using Supersignal (Thermo scientific, Waltham, MA, USA).

F. Transient transfection and luciferase activity assay

For transient transfection, HMC-1 cells were seeded at 2×10^6 in a 6 well plate 1 day before transient transfection. The expression vectors containing the NF-κB luciferase reporter construct (pNF-κB-LUC, plasmid containing NF-κB binding site; Stratagene, Grand Island, NY, USA) were transfected with serum- and antibiotics-free Iscove’s medium containing 8 μl Lipofectamine 2000 reagent (Invitrogen). After 5 h of incubation, medium was replaced with Iscove’s medium containing 10% FBS and antibiotics. Cells were allowed to recover at 37°C for 30 h and subsequently were stimulated as indicated. Cell lysates were prepared and assay for luciferase activity using Luciferase Assay System (Promega, Madison, WI, USA), according to the manufacturer’s instructions.

G. Statistical analysis

Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC, USA). Treatment effects were analyzed using analysis of variance, followed by Duncan’s multiple range tests. P < 0.05 was to indicate significance.

III. RESULTS

A. Effect of CDK on histamine release

We estimated the reducing effects of CDK on the histamine release from PMACI-stimulated HMC-1 cells. Mast cells released a high level of histamine when stimulated with PMACI (Fig. 1).

When CDK was pretreated for 30 min, histamine level was dose-dependently inhibited in PMACI-induced HMC-1. Up to 1 mg/ml of CDK did not show cytotoxicity (data not shown).

Fig. 1 Effects of CDK on histamine release in HMC-1 cells.

B. Effect of CDK on the expression and secretion of pro-inflammatory cytokines

We investigated the inhibitory effect of CDK on the expression of proinflammatory cytokines such as TNF-α and IL-1β. Previously, we reported that gene expression of TNF-α and IL-1β was peaked at 4 h after treatment of PMACI [15]. Consequently, HMC-1 cells were stimulated by PMACI during 4 h, and the cells were preincubated with CDK for 30 min. Figure 2 shows the expression of proinflammatory cytokines was inhibited by CDK.

Fig. 2 Effects of CDK on gene expression of pro-inflammatory cytokines in HMC-1 cells.
C. Effect of CDK on the activation of NF-κB

To investigate the intracellular mechanism responsible for the inhibitory effect of CDK on the expression of proinflammatory cytokines, we examined the effect of CDK on the activation of transcription factors, NF-κB. NF-κB is an important transcriptional regulator of inflammatory cytokines and plays a crucial role in immune and inflammatory responses [10]. Stimulation of HMC-1 with PMACI induced the nuclear translocation of p65 NF-κB and degradation of IκBα after 2 h of incubation. CDK inhibited the PMACI-induced nuclear translocation of NF-κB and degradation of IκBα (Fig. 3A). To confirm the inhibitory effect of CDK on the NF-κB activation, we examined the effect of CDK on NF-κB-dependent gene reporter assay. HMC-1 was transiently transfected with a NF-κB-luciferase reporter construct or an empty vector. Exposure of cells to PMACI increased the luciferase activity in the cells transfected with NF-κB-luciferase reporter construct (Fig. 3B). CDK (100 μg/ml) significantly reduced PMACI-induced luciferase activity.

Fig. 3 Effects of CDK on the activation of NF-κB in mast cells.

IV. DISCUSSIONS

Anaphylaxis is a life-threatening syndrome induced by a sudden systemic release of inflammatory mediators such as histamine, various cytokines and lipid-derived mediators [16]. Histamine was originally considered as a mediator of acute inflammatory and immediate hypersensitivity responses. It has been reported that histamine affects chronic inflammation and regulates several essential events of immune response such as immune cell maturation, polarization, and lymphocyte responsiveness [17]. Using in vitro models, we showed that CDK reduces mast cell-mediated allergic inflammatory reactions. This result indicates that mast cell-mediated immediate-type allergic reactions are inhibited by CDK. This finding suggests that CDK might be useful in the treatment of allergic disease particularly skin reactions.

The HMC-1 cell line is one of the useful cells for studying cytokine activation pathways [10]. The various types of cytokines produced by HMC-1 with PMACI stimulation supports the well-recognized role of mast cells in immediate-type hypersensitivity. TNF-α and IL-1β, the known proinflammatory cytokines, play an important role in triggering and sustaining the allergic inflammatory response in mast cells [18], [19]. Mast cells are a principal source of TNF-α in human dermis. TNF-α has an major amplifying effect in asthmatic inflammation and potently stimulates airway epithelial cells to produce cytokines [20]. It promote inflammation, leukocyte infiltration, chemotaxis of neutrophils and T cells [21]. TNF-α is also involve in eosinophils survival, thereby contributes to chronic inflammation [22]. IL-1β induces expression of adhesion molecules on endothelium to enhance PMN transmigration [23]. These reports indicate that the reduction of proinflammatory cytokines from mast cells is one of the key indicators of reduced allergic symptoms. In the present study, CDK inhibited the expression of TNF-α and IL-1β in PMACI-stimulated mast cells. This result suggests that the anti-allergic effect of CDK results from its inhibition of TNF-α and IL-1β generation from mast cells.

Expression of TNF-α and IL-1β is regulated by the activation of transcription factor NF-κB [24]. NF-κB regulates the expression of multiple inflammatory and immune genes and plays a critical role in chronic inflammatory diseases. Activation of NF-κB required phosphorylation and proteolytic degradation of the inhibitory protein IκBα, an endogenous inhibitor that binds to NF-κB in the cytoplasm. In PMACI-stimulated mast cells, CDK decreased the degradation of IκBα and nuclear translocation of NF-κB. This data demonstrated that CDK attenuates activation of NF-κB and downstream cytokine expression such as TNF-α and IL-1β.

In summary, the present study demonstrates that CDK significantly reduced mast cell-mediated allergic inflammation in in vitro models. We suggest that CDK reduces histamine release. CDK inhibits expression and secretion of inflammatory cytokines by the suppression of NF-κB. We provide evidence that CDK could contribute to prevention or treatment of mast cell-mediated allergic inflammatory diseases.

REFERENCES


