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Anti-allergic effects of scoparone on mast cell-mediated allergy model

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ABSTRACT

Scoparone is known to have a wide range of pharmacological properties *in vitro*. However, the roles of scoparone in immediate-type allergic reactions have not yet been investigated. In this study, we demonstrated that scoparone attenuated IgE-mediated allergic response in mast cells. Oral administration of scoparone inhibited passive cutaneous anaphylaxis in rats. Presence of scoparone dose-dependently decreased histamine release from rat peritoneal mast cells (RPMC) stimulated by anti-dinitrophenyl IgE. Moreover, scoparone reduced the expression and secretion of pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6 in RPMC. Pretreatment with scoparone inhibited the calcium uptake and p38 mitogen-activated protein kinase (MAPK) activity. Furthermore, scoparone blocked translocation of nuclear factor-kappa B (NF-κB) p65 subunit by suppressing IκBα phosphorylation in RPMC. Reduced calcium uptake as well as the suppressed activity of p38 MAPK and NF-κB might be involved in the inhibitory effect of scoparone on the secretory response. Our findings suggest that scoparone may serve as an effective therapeutic agent for allergic diseases.

Introduction

Allergy, especially immediate hypersensitivity such as asthma, allergic rhinoconjunctivitis, atopic dermatitis and eczema, is a principal health problem, and the prevalence of allergy has increased during the past two decades. Mast cells play a key role in immediate-type allergic response through the release of a number of mediators and cytokines. The secretory response of mast cells can be induced by aggregation of their cell surface-specific receptors (FcεRI) for IgE by the corresponding antigen (Kim et al. 2004). Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as leukotrienes, prostaglandins, proteases and several pro-inflammatory and chemotactic cytokines such as tumor necrosis factor (TNF)-α, and interleukins (IL)-6, IL-4, IL-13, IL-8 (Kalesnikoff and Galli 2008). Among them, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Petersen et al. 1996).

The signaling pathway leading to degranulation of mast cells has been extensively characterized. Activation of mast cells leads to phosphorylation of tyrosine kinase, mobilization of internal Ca^{2+} and external Ca^{2+} influx. This is followed by activation of protein kinase C, mitogen-activated protein kinases (MAPKs) and transcription factor nuclear factor-kappa B (NF-κB) and releasing of inflammatory cytokines (Kalesnikoff and Galli 2008). MAPKs and NF-κB have important activities as mediators of cellular responses to extracellular signals. Some of the MAPKs important to mammalian cells include extracellular signal regulated kinase (ERK), c-jun N-terminal kinase (JNK) and p38. p38 MAPK and NF-κB are thought to play an important role in the regulation of pro-inflammatory cytokines on cellular responses, especially TNF-α, IL-1b and IL-6 (Azzolina et al. 2003; Lorentz et al. 2003).

Coumarins comprises of a group of phenolic compounds widely distributed in natural plants. They have recently attracted much attention because of their broad pharmacological activities. Scoparone (6,7-dimethoxycoumarin) (Fig. 1), a derivative of coumarin, has been shown to have biological properties including immunosuppressive effects, vasodilation, lipid lowering activity, antioxidant and anti-inflammatory effects (Huang et al. 1991, 1993; Jang et al. 2006). It has also been documented to exert strong anti-asthmatic action in the guinea pig model (Fang et al. 2003). However, the direct effect of scoparone on IgE-mediated mast cell activation has not yet been reported. Thus, we investigated whether scoparone can inhibit IgE-mediated allergic response in mast cells and its mechanism of action. Our results will give an insight into the prevention or treatment of mast cell-dependent allergic diseases.

Materials and methods

Materials

Scoparone (purity 98%, HPLC), azelastine, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA) and HEPES were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

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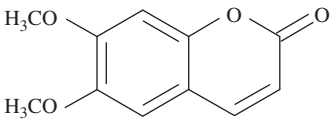

Fig. 1. Chemical structure of scoparone.

Percoll solution was purchased from Pharmacia (Uppsala, Sweden). Scoparone was dissolved in dimethyl sulfoxide (DMSO) and freshly diluted in HEPES-Tyrode buffer for all *in vitro* experiments.

Experimental animals

Male Sprague-Dawley rats (8 weeks old, 230-280 g each) were purchased from Damool Science (Daejeon, Korea). Animals were housed 3-5 per cage in laminar air-flow cabinet maintained at 22 ± 1 °C and relative humidity of 55 ± 10% throughout the study. All animal experiments were performed in compliance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23; revised 1985) and were approved by the Institutional Animal Care and Use Committee of Yanbian University School of Medical Sciences.

Anti-DNP IgE-mediated passive cutaneous anaphylaxis (PCA)

Anti-DNP IgE-mediated PCA was examined as reported previously (Choi et al. 2006). Rats were sensitized in the right dorsal skin by the intradermal injection of 500 ng anti-DNP IgE in 50 μl PBS and were given a sham PBS injection in the left dorsal skin. Twenty-four hours later, the rats received into the penile vein an injection of 200 μl of PBS containing 100 μg DNP-HSA with 1% Evans blue. Scoparone [10, 25, 50 mg/kg body weight (BW)] was administered orally at 1 h before the antigen challenge. As a control, a mast cell-stabilizing agent, azelastine, was orally given at 1 h prior to the challenge with antigen. Thirty minutes after the challenge, the rats were killed by terminal anaesthesia, tissue sections around the intradermal injection site were excised and weighed, followed by extraction of extravasated Evans blue dye by incubation of biopsies in 1 ml formamide at 55 °C for 24 h and measurement of absorbance at 620 nm using a spectrophotometer (Spectra MAX PLUS, Molecular Devices, CA, USA). Tissue Evans blue concentrations were quantified by interpolation on a standard curve of dye concentrations in the range of 0.01 to 30 μg/ml.

Preparation of rat peritoneal mast cells (RPMC) suspension

RPMC were isolated as described previously (Choi et al. 2006). Isolated RPMC were purified by using a Percoll density gradient as described in detail elsewhere (Hachisuka et al. 1988). RPMC preparations were at least 95% pure as assessed by toluidine blue staining and at least 98% of these cells were viable as judged by trypan blue exclusion. Purified mast cells (1×10^6 cells/ml) were resuspended in HEPES-Tyrode buffer.

Assay of histamine release

Mast cell suspensions (2×10^5 cells in 200 μl) were sensitized with 10 μg/ml anti-DNP IgE for 6 h and preincubated with scoparone (25, 50 and 100 μM), the vehicle (0.1% DMSO, used as a control) or azelastine at 37 °C for 30 min. The cells were stimulated with DNP-HSA (100 ng/ml) for 30 min. Following centrifugation at $150 \times g$ for 10 min, the amount of histamine in the supernatant was determined by the radioenzymatic method (Harvima et al. 1988). The inhibition percentage of histamine release was calculated using the following formula: Inhibition $(\%) = (A–B) \times 100/A$

(A): histamine release without scoparone; (B): histamine release with scoparone

Assay of TNF-α and IL-6 secretion

RPMC were sensitized with 10 μg/ml anti-DNP IgE for 6 h and then stimulated with 100 ng/ml DNP-HSA for 30 min with or without scoparone. TNF-α and IL-6 concentrations in the supernatant were determined by using commercial ELISA kits, according to the manufacturer's instructions (Invitrogen-Biosource Cytokine & Signaling, Camarillo, CA, USA).

Western blot analysis

RPMC extracts were prepared by lysis procedure described previously (Nunomura et al. 2006). Samples (30 μg of protein per lane) were loaded on a 12% SDS-PAGE gel. After electrophoresis at 120 V for 90 min, separated proteins were transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA) by the wet transfer method (250 mA, 90 min). The amount of TNF-α and IL-6 was determined using anti-TNF-α and -IL-6 antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). The p38 MAPK, ERK and JNK activation was evaluated using anti-phopspho-p38, -ERK and -JNK antibodies (Cell Signaling, Beverly, MA, USA). Immunodetection was done using enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Measurement of ^{45}Ca uptake

The calcium uptake of mast cells was measured according to the method described by Choi et al. (2006). Purified mast cells were resuspended in HEPES-Tyrode buffer containing ^{45}Ca (1.5 mCi/ml; $1\ Ci = 3.7 \times 10^{10}$ becquerels; Perkin-Elmer Life Sciences, MA, USA) and incubated at 4 °C for 10 min. Mast cell suspensions were sensitized with 10 μg/ml anti-DNP IgE for 6 h and preincubated with scoparone at 37 °C for 30 min prior to the challenge with DNP-HSA (100 ng/ml). The reaction was stopped by the addition of 1 mM lanthanum chloride. The samples were centrifuged 3 times at $150 \times g$ for 10 min at 4 °C, and then the mast cells were lysed with 10% Triton X-100 and vigorous shaking. Radioactivity of the solution was measured in a scintillation ß-counter (Liquid Scintillation Analyzer, A Canberra Company, Australia).

Cytosolic and nuclear protein extractions for analysis of NF-κB

RPMC were sensitized with 10 μg/ml anti-DNP IgE for 6 h and preincubated with scoparone or the vehicle at 37 °C for 30 min prior to the challenge with DNP-HSA (100 ng/ml). The cells were harvested and washed twice with ice-cold PBS. The washed cell pellets were resuspended in 2 volumes of buffer A (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% glycerol, 0.5 mM DTT, 5 mM $MgCl_2$, 1 mM PMSF and protease inhibitor cocktails). This suspension was centrifuged at $1{,}000 \times g$ for 15 min at 4 °C. The supernatant fraction was incubated on ice for 10 min and centrifuged at $100{,}000 \times g$ for 1 h at 4 °C to obtain cytosolic protein extracts. The pellets were washed twice in buffer A and resuspended in buffer B (1.3 M sucrose, 1.0 mM $MgCl_2$ and 10 mM potassium phosphate buffer, pH 6.8) and pelleted at $1{,}000 \times g$ for 15 min. The pellets were suspended in buffer B with a final sucrose concentration of 2.2 M and centrifuged at $100{,}000 \times g$ for 1 h. The resulting nuclear pellets were washed once with a solution containing 0.25 M sucrose, 0.5 mM $MgCl_2$ and 20 mM Tris-HCl, pH 7.2, and centrifuged at $1{,}000 \times g$ for 10 min. The pellets were solubilized