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Inhibitory effects of bisdemethoxycurcumin on mast cell-mediated allergic diseases

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ABSTRACT

Most allergic reactions are induced by mast cell activation. Mast cells play vital roles in the pathogenesis of allergic diseases. Bisdemethoxycurcumin (BDMC), a natural curcuminoid, has potential anti-allergic effects. Hence, we explored the effect of BDMC on mast cell-mediated allergic diseases. The study proved that BDMC suppresses β-hexosaminidase release, granule release, and membrane ruffling in monoclonal anti-2,4,6-dinitrophenyl-immunoglobulin (Ig) E/human serum albumin (DNP-IgE/HSA)-stimulated rat basophilic leukaemia cells (RBL-2H3 cells), and BDMC suppressed ovalbumin (OVA)-induced allergic rhinitis (AR) symptoms and OVA-specific IgE levels in AR mice. Furthermore, BDMC increased the survival of compound 48/80 anaphylaxis shock mice and elevated the decreased rectal temperature in OVA-induced active systemic anaphylaxis mice. These findings indicate that BDMC regulates the degranulation of mast cells, demonstrating its potential in the treatment of mast cell-induced allergic reactions.

1. Introduction

Allergic diseases, as the body's immune response to antigens, are characterised by physiological disorders and tissue or cell damage, which include eczema, allergic rhinitis, asthma, allergic dermatitis, allergic conjunctivitis, anaphylaxis, and food or drug allergic reactions. Robust epidemiological data have shown a real and alarming increase in IgE-mediated allergic rhinitis in particular. The increase in allergic disease prevalence was first observed in the developed world but is now becoming a major problem in emerging economies and the developing world [1]. Climate change, urbanisation, emissions, and air pollutants are among the most important challenges to the health and quality of life of the still-increasing number of allergic patients today and in the coming decades [2].

Mast cells play essential roles in the pathogenesis of allergic diseases [3]. When an antigen enters the body through the skin or mucous membranes, it induces B lymphocyte proliferation and production of allergen-specific immunoglobulin (Ig) E. IgE, as a pro-cellular antibody, binds to the high-affinity Fc epsilon receptor I (FcεRI) expressed on the surface of mast cells and then sensitises the mast cells. When the same antigen re-enters the body, it crosslinks with the IgE-FcεRI complexes on the surface of mast cells and then promotes mast cell degranulation and release of inflammatory mediators, such as histamine, tryptase, leukotriene C_4 (LTC_4), prostaglandin D_2 (PGD_2), and cytokines. These mediators cause telangiectasia, permeability increase, smooth muscle contraction, inflammation, injuries and other symptoms [4–9].

Currently, various treatments are used to treat allergic diseases. Histamine H_1-receptor antagonists, which block H_1-receptors in addition to suppressing Th2-type cytokine production, are used as a therapeutic strategy for the treatment of allergic diseases [10]. However, adverse reactions of H_1-receptor antagonists, including cardiotoxicity, neurotoxicity, and cholinergic inhibition, limit its clinical application [11,12]. The use of steroids in the treatment of allergic diseases has decreased in clinical practice due to its adverse effects, such as skin toxicity, intestinal toxicity and liver toxicity [2,13,14]. Mast cell stabilisers suppress the release of inflammatory mediators by inhibiting degranulation of mast cells and then exert anti-allergic effects. Nevertheless, the side effects of mast cell stabilisers include drowsiness, upset stomach, chest congestion and dry mouth [12,15,16]. Therefore, natural products have attracted widespread attention due to their low adverse reactions and high anti-allergic activity.

Curcuminoids, such as the diketone compound extracted from the roots and stems of Zingiberaceae and Araceae plants, include curcumin (~77%), demethoxycurcumin (DMC, ~18%), and bisdemethoxycurcumin (BDMC, ~5%), which have been reported to have neuroprotective, liver protective, antioxidative, and anti-tumour effects. In addition, numerous studies have proven that curcumin exerts anti-allergic colitis, anti-food allergy, and anti-allergic rhinitis effects [17–19].

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Similarly, bisdemethoxycurcumin has been demonstrated to inhibit the release of cytokines and NF-κB activation and IκB degradation [20]. Taken together, these data suggest that curcuminoids may have beneficial effects in mast cell degranulation and may prevent the development of mast cell-mediated allergic diseases.

Rat basophilic leukaemia cells (RBL-2H3 cells) are widely used to study IgE-mediated cell activation due to their strong surface expression of the IgE receptor FcεRI [21]. We investigated the effect of curcuminoids on degranulation of mast cells in a monoclonal anti-2,4,6-dinitrophenyl-immunoglobulin (Ig) E/human serum albumin (DNP-IgE/HSA)-stimulated mast cell model, and the results showed the half maximal inhibitory concentration (IC50) values of inhibition of β-hexosaminidase release by curcumin (99.47 μM), demethoxycurcumin (31.15 μM), and bisdemethoxycurcumin (17.25 μM). Hence, we explored the anti-allergic effects of bisdemethoxycurcumin on RBL-2H3 cells, allergic rhinitis, anaphylaxis shock, and systemic anaphylaxis in mice.

2. Materials and methods

2.1. Cell culture

The RBL-2H3 cells were obtained from the National Infrastructure of Cell Line Resource (Shanghai, China) and maintained in minimum essential medium (MEM) with 15% foetal bovine serum (FBS), 100 μg/mL streptomycin, 100 U/mL penicillin, 1.5 mg/mL sodium bicarbonate, and 110 μg/mL sodium pyruvate at 37 °C in a humidified incubator with 5% CO_2.

2.2. Animals

Male BALB/c mice weighing 18–22 g (8 weeks old), and male ICR mice weighing 18–22 g (5 weeks old) were obtained from the Laboratory Animal Center of Jilin University. The mice had free access to food and water, and these animals were housed under 12-hour light/12-hour dark conditions in an air-conditioned room (ambient temperature 23 ± 2 °C, relative humidity 55 ± 5%). The procedures in this study were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Jilin University.

2.3. Cell viability assay

Cell viability was determined using the MTT [3-(3, 5)-dimethylthiazol-2,5-diphenyltetrazolium bromide] assay according to the method of Kim, Y et al. [22]. In brief, RBL-2H3 cells were seeded on a 48-well plate (6×10^5 cells/well) in MEM medium containing 15% FBS at 37 °C overnight. The cells were incubated with 100 ng/mL monoclonal anti-2, 4,6-dinitrophenyl-IgE (DNP-IgE, Sigma-Aldrich, LA, USA) for 12 h. The sensitised cells were washed with modified Tyrode's buffer [126 mM NaCl, 5.6 mM glucose, 4.0 mM KCl, 0.6 mM KH_2PO_4, 10.0 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.6 mM $MgCl_2/6H_2O$, 1.0 mM $CaCl_2$ and 0.1% bovine serum albumin (BSA)] and then pretreated for 1 h. The pretreated cells were stimulated with 100 ng/mL DNP-HSA (Biorearch, Petaluma, USA) for 12 h at 37 °C to activate the cells and evoke the allergic reaction (degranulation). MTT (5 mg/mL) was added to each well and then further incubated for 4 h. The formazan crystals produced in the cells were dissolved with 200 μL DMSO per well. The absorbance was measured at 490 nm using a spectrophotometer. Throughout this study, the positive drug control used was ketotifen fumarate, which is a clinically used anti-histamine drug for the treatment of general allergy by inhibiting mast cell degranulation.

2.4. β-Hexosaminidase release assay

The release of β-hexosaminidase was used as a marker of mast cell degranulation. The β-hexosaminidase release assay was implemented as described previously with slight modifications [23]. After DNP-IgE/HSA sensitisation, the cells were incubated in an ice bath for 10 min to stop the reaction and centrifuged (300 × *g* for 10 min) at 4 °C. The 50 μL supernatant was mixed with 50 μL substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 0.1 M sodium citrate buffer, pH 1.5), and the fixative solution was incubated for 1.5 h at 37 °C. The reaction was terminated by adding 200 μL of buffer (0.1 M $Na_2CO_3/NaHCO_3$, pH 10.0). The absorbance was measured at 405 nm using a spectrophotometer.

2.5. Toluidine blue staining

To visualise the release of granules by RBL-2H3 cells during degranulation, the RBL-2H3 cells were stained by toluidine blue as previously described [24]. The RBL-2H3 cells sensitised by DNP-IgE/HSA were washed with phosphate buffered saline (PBS) and then incubated with 250 μL 4% paraformaldehyde/PBS for 30 min at room temperature (RT). The mixture was discarded, and fixed cells were stained with 300 μL of toluidine blue dye (1% *w/v* in 0.9% NaCl solution, pH 2.5) for 30 min. Images of the stained cells were then examined and captured using an inverted microscope.

2.6. F-actin microfilament staining

Phalloidin, a useful tool to investigate the distribution of filamentous actins (F-actin), specifically binds to F-actin in cells. The cells were stained with Alexa Fluor-488-phalloidin as previously described with slight modifications [25]. The RBL-2H3 cells were seeded in 4-well chamber slides and then sensitised with 100 ng/mL DNP-IgE for 12 h. The sensitised cells were pretreated with BDMC or ketotifen fumarate for 1 h, followed by stimulation with DNP-HSA for 1 h. The cells were then washed with PBS and fixed using 4% paraformaldehyde/PBS for 1 h and 30 min. The fixed cells were washed with PBS and then permeated with 0.1% Triton X-100/PBS for 3 min. The permeated cells were washed with PBS and then stained using Alexa Fluor 488-phalloidin diluted in 1% BSA (1:1000) for 30 min. Finally, F-actin fibres were examined using a Leica DM2500 Microscope equipped with excitation (490 nm) and emission (520 nm) filters.

2.7. OVA-induced allergic rhinitis

The experimental protocol for the OVA-induced allergic rhinitis model was implemented as previously described with modulation [26,27]. BALB/c mice were intraperitoneally injected with OVA (50 μg) (Sigma, St. Louis, MO, USA) in aluminium hydroxide (2 mg) (Sigma, St. Louis, MO, USA) once every two days from day 0 to day 14. Then, local immunization was performed once a day from day 15 to day 24 by dripping the OVA into the nasal vestibule bilaterally. Before the experiment, mice were placed into an observation cage (320 × 180 × 150 mm) for approximately 15 min for acclimatization. Then, 10 μL of 10% OVA solution was dropped into the bilateral nasal cavities of the mice. 1 h after administration BDMC, demethoxycurcumin, curcumin (100 mg/kg), or BDMC (50, 100 and 200 mg/kg), ketotifen fumarate (50 mg/kg), the frequencies of nose rubbing was then counted for 10 min to evaluate early allergic responses.

In the comparative efficacy experiment of BDMC, demethoxycurcumin, curcumin, pretreatment of BDMC (≥98% purity, Chengdu Must Bio-Technology Co., Ltd., Sichuang China), demethoxycurcumin (≥98% purity, Chengdu Must Bio-Technology Co., Ltd.), curcumin (≥98% purity, Chengdu Must Bio-Technology Co., Ltd.) at 100 mg/kg were administered orally before the local immunization once daily for 10 days. Normal and OVA-induced AR mice received distilled water