



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 C₄ (LTC₄), prostaglandin D₂ (PGD₂), 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 H₁-receptor antagonists, which block H₁-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 H₁-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 neuro-protective, 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- κ B activation and I κ B 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 Fc ϵ RI [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 μ M), demethoxycurcumin (31.15 μ M), and bisdemethoxycurcumin (17.25 μ M). 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 μ g/mL streptomycin, 100 U/mL penicillin, 1.5 mg/mL sodium bicarbonate, and 110 μ g/mL sodium pyruvate at 37 °C in a humidified incubator with 5% CO₂.

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 $\pm$ 2 °C, relative humidity 55 $\pm$ 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 KH₂PO₄, 10.0 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.6 mM MgCl₂/6H₂O, 1.0 mM CaCl₂ and 0.1% bovine serum albumin (BSA)] and then pretreated for 1 h. The pretreated cells were stimulated with 100 ng/mL DNP-HSA (Bioresearch, 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 μ L 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 $\times$ g for 10 min) at 4 °C. The 50 μ L supernatant was mixed with 50 μ L 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 μ L of buffer (0.1 M Na₂CO₃/NaHCO₃, 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 μ L 4% paraformaldehyde/PBS for 30 min at room temperature (RT). The mixture was discarded, and fixed cells were stained with 300 μ L 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 μ g) (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 $\times$ 180 $\times$ 150 mm) for approximately 15 min for acclimatization. Then, 10 μ L 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 ($\geq$ 98% purity, Chengdu Must Bio-Technology Co., Ltd., Sichuan China), demethoxycurcumin ($\geq$ 98% purity, Chengdu Must Bio-Technology Co., Ltd.), curcumin ($\geq$ 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

alone on the same schedule. After each treatment, the incidences of nasal rubbing were counted. And in dose-dependent experiment of BDMC, BDMC (50, 100 and 200 mg/kg) and ketotifen fumarate (50 mg/kg) were administered orally before the local immunization once daily for 10 days. Normal and OVA-induced AR mice received distilled water alone on the same schedule. After each treatment, the incidences of nasal rubbing were counted. The serum was collected 24 h after the last OVA treatment. The serum was used to determine OVA-specific IgE and histamine levels using ELISAs.

2.8. Compound 48/80-induced anaphylactic shock

The compound 48/80-induced anaphylactic shock reaction was carried out according to P. Venkatesh et al. [28]. Briefly, ICR mice were randomly assigned to six groups, BDMC (50, 100, and 200 mg/kg) or ketotifen fumarate (50 mg/kg) was orally administered for 5 days, and injection of 8 mg/kg compound 48/80 (Sigma, LA, USA) was administered 1 h after the last oral administration. The control group was treated with saline. The mortality was measured 1 h after injection of compound 48/80.

2.9. OVA-induced active systemic anaphylaxis (ASA)

The study of OVA-induced ASA was performed based on the method of Yeon-Yong et al. with little modification [23]. Briefly, BALB/c mice were sensitised by intraperitoneal injection of an OVA mixture (100 µg of OVA and 2 mg of alum adjuvant in 200 µL of PBS) on day 0 and day 7. In the comparative efficacy experiment of BDMC, demethoxycurcumin, curcumin, pretreatment of BDMC, demethoxycurcumin, curcumin (100 mg/kg) were orally administered from day 8 to day 14. And in Dose-dependent experiment BDMC, BDMC (50, 100 and 200 mg/kg) and ketotifen fumarate (50 mg/kg) were orally administered from day 8 to day 14. Normal and OVA-induced AR mice received distilled water alone on the same schedule. The sensitised mice were challenged by OVA (200 µg in 200 µL of PBS) 1 h after the oral administration on day 14, and then, the rectal temperature was measured every 10 min for 75 min.

2.10. Statistical analysis

Data are presented as the mean ± Standard Error (SE). Statistical analyses were performed using SPSS. A one-way ANOVA was used for statistical comparisons between the groups. Bonferroni's multiple comparison test was carried out to compare the toxicant and test groups. A value of $p < 0.05$ indicated a significant difference.

3. Results

3.1. Effect of BDMC on cell viability

In this study, we first investigated the effect of DNP-IgE/HSA stimulation and BDMC on RBL-2H3 cell viability. As shown in Fig. 1, stimulation with DNP-IgE/HSA did not reduce the RBL-2H3 cell viability. Similarly, pretreatment with BDMC at concentrations up to 73.2 µM did not reduce the DNP-IgE/HSA-stimulated RBL-2H3 cell viability.

3.2. Effect of BDMC on β -hexosaminidase release from mast cells

β -Hexosaminidase is a marker of degranulation. To assess the effect of BDMC on β -hexosaminidase release from mast cells, we measured β -hexosaminidase activity (OD 405 nm) in the supernatant. The results indicated that BDMC and ketotifen fumarate both inhibited the β -hexosaminidase release of DNP-IgE/HSA-stimulated mast cells (Fig. 2).

3.3. Effect of BDMC on release of granules by DNP-IgE/HSA-stimulated RBL-2H3 cells

Toluidine blue is a synthetic basic dye, it shows blue when combines with the acidic material in mast cells, and appears purple while it combines with heparin, histamine and other heterochromatic substances in the granule. Therefore, we observed the effect of BDMC on release of granules in DNP-IgE/HSA-stimulated RBL-2H3 cells by toluidine blue staining, and the results are shown in Fig. 3. The normal RBL-2H3 cells were spindle shaped and had purple granules stored in the cells. However, the shape of the DNP-IgE/HSA-stimulated RBL-2H3 cells was irregular, and purple granules were released outside the cell. Pretreatment with BDMC or ketotifen fumarate markedly inhibited the morphological changes and degranulation.

3.4. Effect of BDMC on membrane ruffling in DNP-IgE/HSA-stimulated RBL-2H3 cells

Rearrangements of the F-actin cytoskeleton, a key step of the degranulation process, facilitate granule fusion with the plasma membrane. Phalloidin specifically combines with F-actin. Therefore, we observed the changes in F-actin of the DNP-IgE/HSA-sensitised RBL-2H3 cells after BDMC administration through Alexa Fluor 488-phalloidin staining. As shown in Fig. 4, the normal RBL-2H3 cells were spindle shaped with uniform distribution of F-actin at the cell periphery (Fig. 4A). The shapes of DNP-IgE/HSA-sensitised RBL-2H3 cells become round or elliptical because of disassembly of the F-actin cytoskeleton (Fig. 4B). Pretreatment with BDMC or ketotifen fumarate inhibited the shape change and the disassembly of the F-actin cytoskeleton (Fig. 4C–F).

3.5. Effect of BDMC, demethoxycurcumin, curcumin on OVA-induced allergic rhinitis mice

To evaluate whether BDMC, demethoxycurcumin, curcumin at the dose of 100 mg/kg inhibit OVA-induced allergic rhinitis, the number of nasal rubbing was counted. The number of nasal rubbing in BDMC group was significantly lower than demethoxycurcumin group and curcumin group (Fig. 5). Therefore, we chose BDMC for the follow-up experiment.

3.6. Effect of BDMC on OVA-induced allergic rhinitis mice

OVA-induced AR, a type I hypersensitivity reaction, is induced by mast cell activation. The incidences of nasal rubbing of the mice were counted after OVA intranasal challenge. As shown in Fig. 6 A, OVA challenge significantly increased the nasal rubbing. By contrast, this effect was suppressed by administering BDMC or ketotifen fumarate.

We next explored the influence of BDMC on OVA-specific IgE and histamine serum levels. The levels of OVA-specific IgE and histamine in AR mice were significantly increased. In contrast, BDMC exerted strong inhibitory effects on the increased levels of OVA-specific IgE and histamine (Fig. 6B, C).

3.7. Effect of BDMC on compound 48/80-induced anaphylaxis shock mice

Anaphylaxis shock was induced in mice by intraperitoneal injection of 8 mg/kg of compound 48/80. The effect of BDMC on anaphylaxis shock mice was determined by assessing survival. The results indicated that the survival of model mice was 100% after intraperitoneal injection with compound 48/80 for 75 min. Administration with BDMC or ketotifen fumarate significantly attenuated anaphylactic death (Fig. 7).

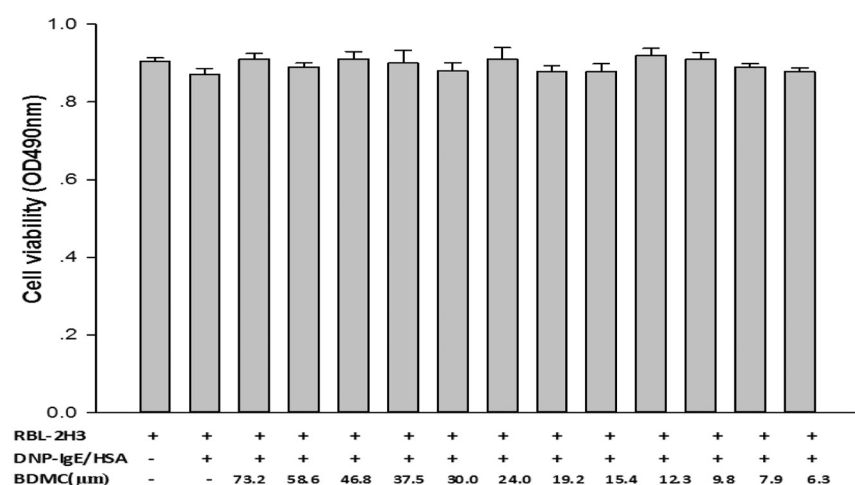


Fig. 1. Effect of BDMC on DNP-IgE/HSA-sensitised cell viability. RBL-2H3 cells (6×10^5) were seeded into 48-well microplates. Then, the cells were stimulated with DNP-IgE (100 ng/mL) for 12 h and pretreated with BDMC or solvent for 1 h. The pretreated cells were sensitised with DNP-HSA for 1 h to activate the cells. MTT was added to each well and then further incubated for 4 h. The formazan crystals produced in the cells were dissolved with DMSO. The absorbance was measured at 490 nm. The values are expressed as the mean $\pm$ SE.

3.8. Effect of BDMC, demethoxycurcumin, curcumin on ovalbumin-induced ASA

To evaluate whether BDMC, demethoxycurcumin, curcumin at the dose of 100 mg/kg inhibit OVA-induced ASA, the rectal temperature of the mice was determined. The effect of increasing rectal temperature of BDMC was more obvious than demethoxycurcumin and curcumin as compared with OVA group (Fig. 8). Therefore, we chose BDMC for the follow-up experiment.

3.9. Effect of BDMC on ovalbumin-induced ASA

After an intraperitoneal injection of OVA, the rectal temperature of the mice obviously decreased. When BDMC or ketotifen fumarate (Keto.) was orally administered to those mice, the decreasing rectal temperature was increased (Fig. 9).

4. Discussion

Cell-based techniques, such as in vitro approaches, are vital strategies in drug discovery, which will save further development costs in early drug screening and discovery. In our study, we first used the DNP-IgE/HSA-sensitised RBL-2H3 cell model to investigate the effect of

BDMC on mast cell activation. Degranulation is a fundamental step in mast cell activation to release inflammatory mediators [29]. Therefore, we explored the degree of degranulation by measuring β -hexosaminidase release, a degranulation marker. The results indicated that BDMC attenuated β -hexosaminidase release induced by DNP-IgE/HSA stimulation. During anaphylactic degranulation, cytoplasmic granules rapidly fuse with each other and with the plasma membrane, resulting in a massive release of mediators [5]. Hence, activation of mast cells is highly associated with typical morphological changes of the cells. We used toluidine blue staining to confirm that pretreatment with BDMC preserved the cell morphology in a spindle shape with few prominent granules in the cytoplasm. Meanwhile, disassembly of the cortical F-actin network is an essential process for mast cell exocytosis in the case of antigen stimulation [30]. Cortical actin filaments, made predominantly of actin and myosin, act as a barrier, which separates secretory granules from their fusion sites at the plasma membrane. Antigen stimulates mast cell-induced actin-filament remodelling, which promotes granule fusion with the plasma membrane to release pro-inflammatory mediators [31–33]. Thus, we observed F-actin cytoskeleton change by Alexa Fluor 488-phalloidin staining. The results suggest that BDMC suppresses rearrangement of the F-actin cytoskeleton. Overall, we deduced that BDMC exerts inhibitory effects on Ag-induced mast cell activation in vitro. In agreement with the previous report, the study

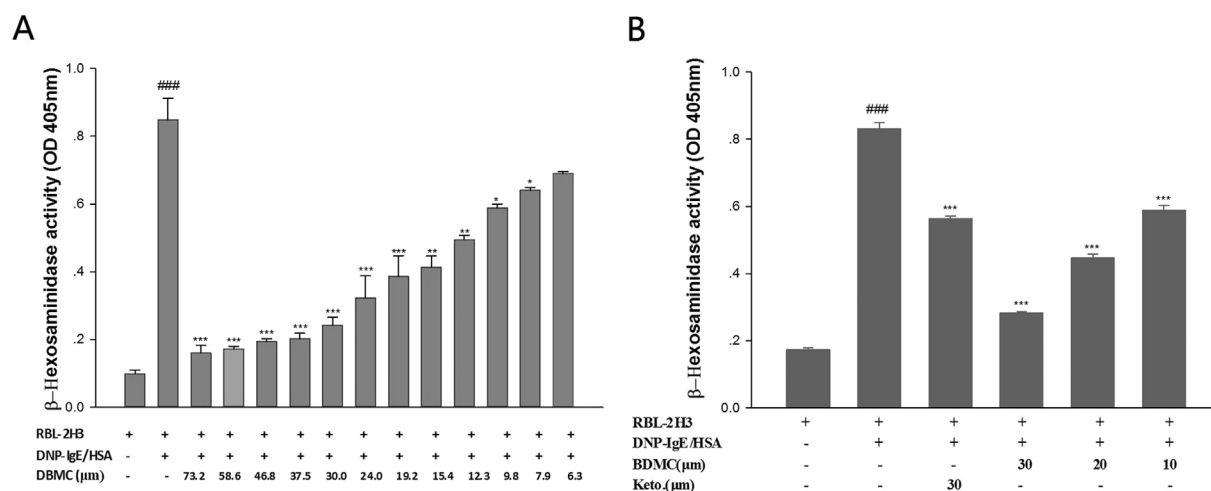


Fig. 2. Effect of curcuminoids on β -hexosaminidase release of DNP-IgE/HSA-sensitised cells. After DNP-IgE/HSA sensitisation, the cells were incubated in an ice bath for 10 min to stop the reaction and centrifuged at 4 °C. The 50 μ L supernatant was mixed with the 50 μ L substrate, and the fixative solution was incubated for 1.5 h at 37 °C. The reaction was terminated by adding stop buffer. The absorbance was measured at 405 nm using a spectrophotometer. The values are expressed as the mean $\pm$ SE. ### $p < 0.001$ was compared to the normal group; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ were compared to the DNP-IgE/HSA-sensitised group, $n = 10$ /group.

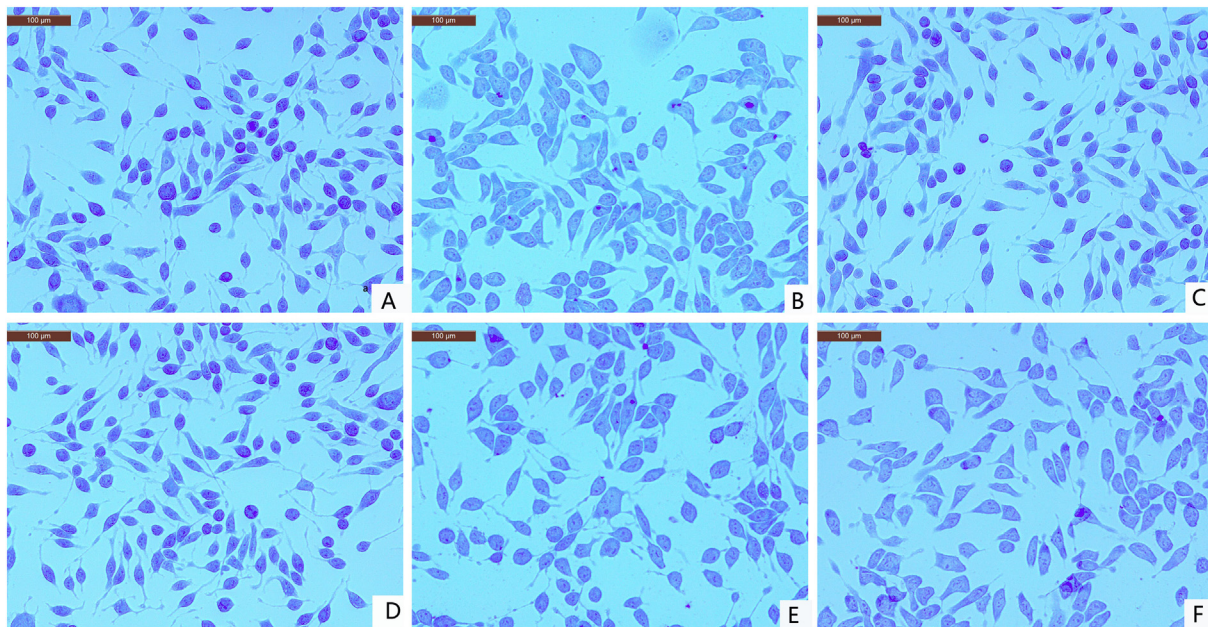


Fig. 3. Effects of BDMC on toluidine blue staining in DNP-IgE/HSA-sensitised cells. After DNP-IgE/HSA sensitisation, the cells were stained with toluidine blue dye for 30 min. Images of the stained cells were examined and captured using an inverted microscope. A: Normal RBL-2H3 cells; B: DNP-IgE/HSA-sensitised RBL-2H3 cells; C: ketotifen fumarate-pretreated, DNP-IgE/HSA-sensitised RBL-2H3 cells; D–F: BDMC-pretreated RBL-2H3 cells sensitised with 30 μ M, 20 μ M, and 10 μ M DNP-IgE/HSA.

conducted by Matsuda et al. has also proved the inhibitory effects of curcumin and BDMC on calcium ionophores (A23187 and ionomycin)-induced RBL-2H3 cells degranulation [34]. And Ryong Kong et al. has shown that BDMC decrease the release of inflammatory mediators in phorbol-12-myristate-13 acetate and A23187-induced human mast cells [13].

We next elucidated the effect of BDMC in model mice, including OVA-induced allergic rhinitis mice, Compound 48/80 induced anaphylactic shock, and OVA induced systemic anaphylaxis. After OVA challenge sensitised the mice, it crosslinks with the IgE-Fc ϵ RI

complexes, which lead mast cells to release mediators, resulting in symptoms of allergic rhinitis, such as nasal rubbing. In contrast, administration of BDMC relieved the nasal rubbing and decreased the levels of OVA-specific IgE. This result is consistent with the pervious study, which has confirmed that curcumin treatment significantly reduced the symptoms, improved the histopathological alterations of nasal mucosa, and inhibited the elevation of serum IgE in allergic rhinitis [35]. Compound 48/80 is a mixed polymer of *p*-methoxy-*N*-methyl phenylethylamine crosslinked by formaldehyde and, as histamine releaser, stimulates mast cell degranulation. We explored the anti-allergic

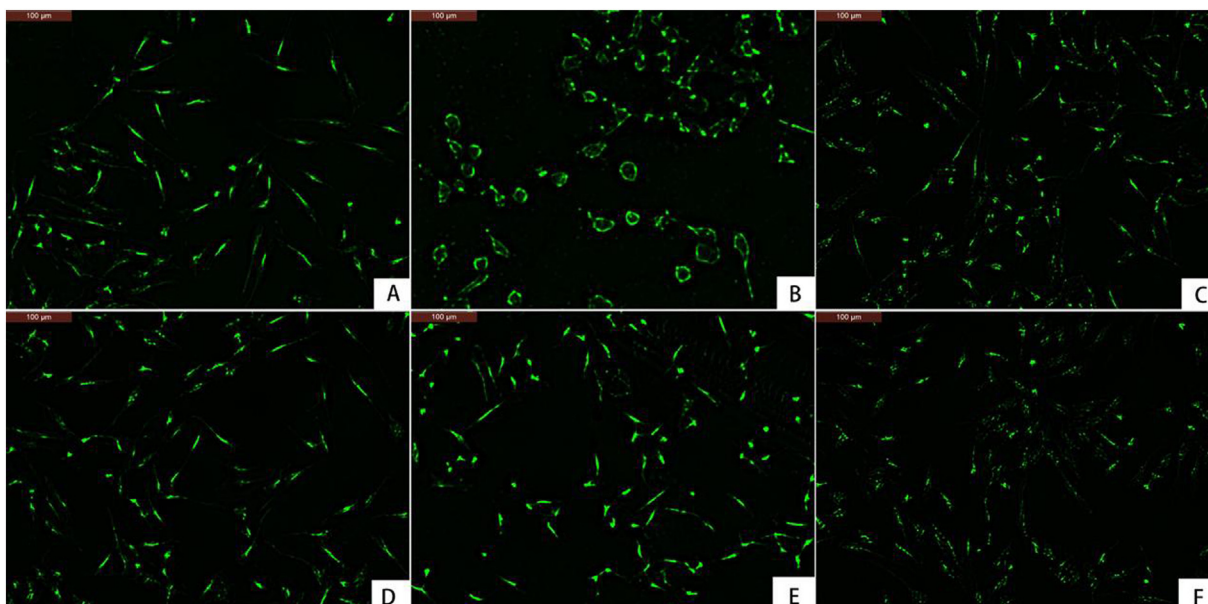


Fig. 4. Effects of BDMC on Alexa Fluor-488 phalloidin staining in DNP-IgE/HSA-sensitised cells. After DNP-IgE/HSA sensitisation, the cells were stained with Alexa Fluor-488 phalloidin dye for 30 min. Images of the stained cells were examined and captured using an inverted microscope. A: Normal RBL-2H3 cells; B: DNP-IgE/HSA-sensitised RBL-2H3 cells; C: ketotifen fumarate-pretreated, DNP-IgE/HSA-sensitised RBL-2H3 cells; D–F: BDMC-pretreated RBL-2H3 cells sensitised with 30 μ M, 20 μ M, and 10 μ M DNP-IgE/HSA.

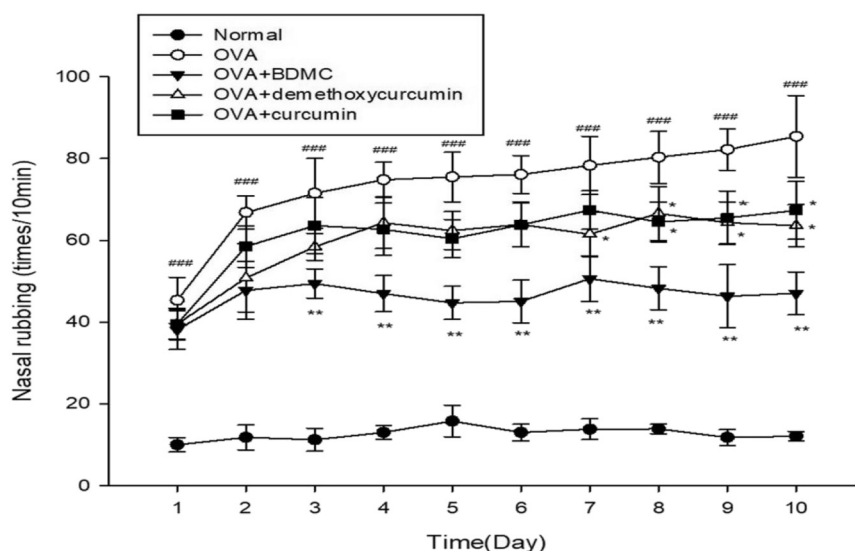


Fig. 5. Effect of BDMC, demethoxycurcumin, curcumin on OVA-induced AR. The number of occurrences of nasal rubbing counted in the 10 min after OVA intranasal provocation. $###p < 0.001$, $##p < 0.01$ were compared to the normal group; $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ were compared to AR mice, $n = 10$ /group.

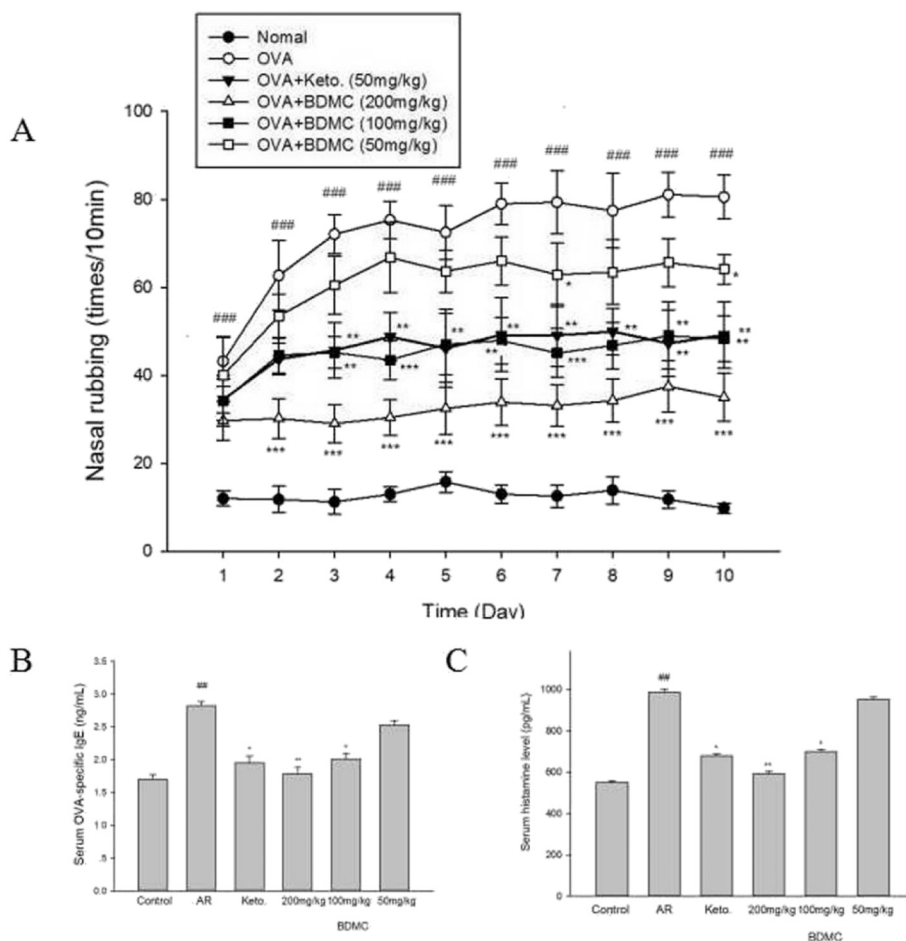


Fig. 6. Effect of BDMC on OVA-induced AR. The number of occurrences of nasal rubbing (A) counted in the 10 min after OVA intranasal provocation. The levels of OVA-specific IgE (B) The levels of histamine (C) $###p < 0.001$, $##p < 0.01$ were compared to the normal group; $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ were compared to AR mice, $n = 10$ /group.

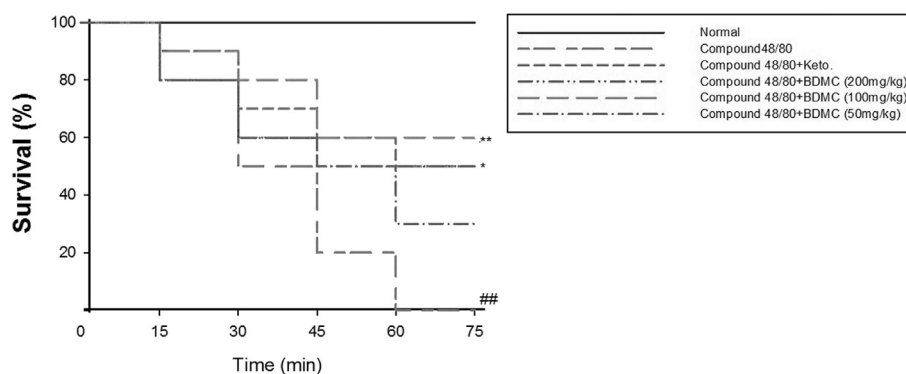


Fig. 7. Effect of BDMC on survival in compound 48/80-induced anaphylaxis shock mice. The survival was measured 1 h after injection of compound 48/80. Each data value is represented as a %, $n = 10/\text{group}$.

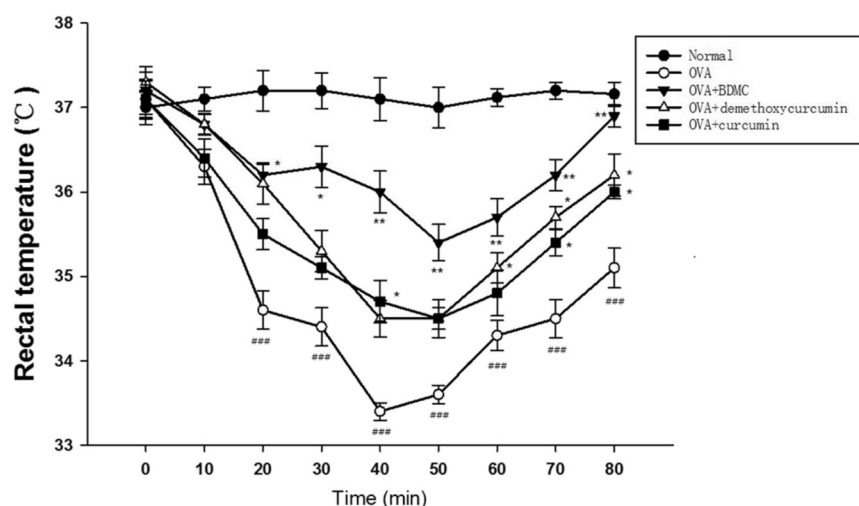


Fig. 8. Effects of BDMC, demethoxycurcumin, curcumin on ovalbumin (OVA)-induced active systemic anaphylaxis. After OVA sensitisation, rectal temperature was measured every 10 min for 80 min. The values are expressed as the mean $\pm$ SE. $###p < 0.001$ and $##p < 0.01$ were compared to the normal group; $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ were compared to the active systemic anaphylaxis mice, $n = 10/\text{group}$.

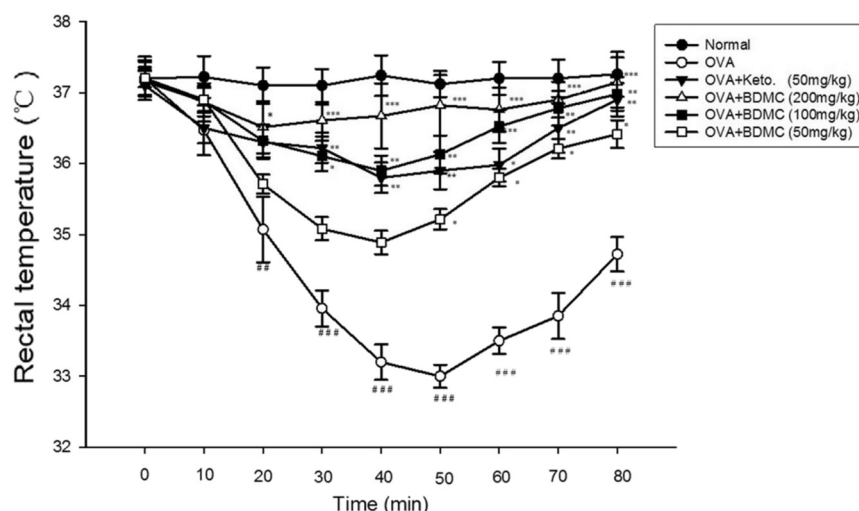


Fig. 9. Effects of BDMC on ovalbumin (OVA)-induced active systemic anaphylaxis. After OVA sensitisation, rectal temperature was measured every 10 min for 80 min. The values are expressed as the mean $\pm$ SE. $###p < 0.001$ and $##p < 0.01$ were compared to the normal group; $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ were compared to the active systemic anaphylaxis mice, $n = 10/\text{group}$.

effect of BDMC on anaphylaxis shock mice induced by intraperitoneal injection of compound 48/80. The results demonstrated that BDMC dramatically increased the survival of anaphylaxis shock mice. Active systemic anaphylaxis induced by intraperitoneal injection of OVA is a widely used mast cell-induced allergic disease model. The degree of rectal temperature reduction, as an allergic symptom, was evaluated in this mouse model. Oral administration of BDMC elevated rectal temperature of anaphylaxis shock mice. As expected, the results of the in vivo experiments are consistent with the in vitro experiments.

Additionally, it is well known that BDMC is one of the polyphenols. Various studies of polyphenols in allergic models such as allergic rhinitis and asthma demonstrated their beneficial effect, such as in our previous study, we found that green tea has anti-allergic properties, and the major polyphenolic compound, epigallocatechin gallate, which can suppress the number of sneezes and the occurrence of nasal rubbing on ovalbumin-induced allergic rhinitis by attenuating the levels of IgE and histamine in serum, and the levels of IL-1 β , IL-4, IL-6 in nasal lavage fluid, and the levels of COX-2, IL-1 β , IL-4, IL-6 in the nasal mucosa

tissue [36]. A study shows that polyphenols contained in fermented grape marc from and Koshu *Vitis vinifera* (K-FGM) have been shown to exhibit anti-allergic activities in murine asthma by reducing IgE production and eosinophil number in bronchial alveolar lavage fluid [37]. BDMC suppressed the above mentioned mast cell-induced allergic disease. This suggests that BDMC can be used as a new medicinal candidate to treat allergic diseases.

Author contributions

Tie Hong, Meng Fu, ShuiLian Fu, Saihong Ni, and Danni Wang conceptualized and designed this study and wrote the paper. Tie Hong, Meng Fu, and ShuiLian Fu performed most of the experiments. Saihong Ni and Danni Wang analyzed the data. All authors read and approved the final manuscript.

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