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Anti-allergic actions of rottlerin from *Mallotus philippinensis* in experimental mast cell-mediated anaphylactic models

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ABSTRACT

Allergy is an acquired hypersensitivity reaction of the immune system mediated by cross-linking of the allergen-specific IgE-bound high-affinity IgE receptors, leading to immediate mast cell degranulation. Rottlerin is an active molecule isolated from *Mallotus philippinensis*, a medicinal plant used in Ayurvedic Medicine System for anti-allergic and anti-helminthic treatments. The present study investigated potential anti-allergic effects of rottlerin in animal models of IgE-dependent anaphylaxis and the anti-allergic mechanisms of action of rottlerin in mast cells. Anti-allergic actions of rottlerin were evaluated in passive cutaneous anaphylaxis and passive systemic anaphylaxis mouse models, and in anaphylactic contraction of bronchial rings isolated from sensitized guinea pigs. Direct mast cell-stabilizing effect of rottlerin was examined in RBL-2H3 mast cell line. Anti-allergic signaling mechanisms of action of rottlerin in mast cells were also examined. Rottlerin prevented IgE-mediated cutaneous vascular extravasation, hypothermia, elevation in plasma histamine level and tracheal tissue mast cell degranulation in mice in a dose-dependent manner. In addition, rottlerin suppressed ovalbumin-induced guinea pig bronchial smooth muscle contraction. Furthermore, rottlerin concentration-dependently blocked IgE-mediated immediate release of β -hexosaminidase from RBL-2H3 mast cells. Rottlerin was found to inhibit IgE-induced PLC γ 1 and Akt phosphorylation, production of IP₃ and rise in cytosolic Ca²⁺ level in mast cells. We report here for the first time that rottlerin possesses anti-allergic activity by blocking IgE-induced mast cell degranulation, providing a foundation for developing rottlerin for the treatment of allergic asthma and other mast cell-mediated allergic disorders.

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Introduction

Allergy is an acquired hypersensitivity reaction of the immune system in response to normally innocuous environmental substances (Galli et al. 2008). It displays in various forms ranging from urticaria, allergic rhinitis and conjunctivitis, asthma, to life-threatening anaphylaxis (Galli et al. 2008; Simons 2010), causing an enormous socioeconomic burden. The most common clinical signs for cutaneous allergy as a result of wheal-and-flare reaction are hives and angioedema. In allergic asthma, inhaled aeroallergen can trigger off acute bronchospasm and shortness of breath (Bradding et al. 2006). Systemic anaphylaxis is the most severe form of allergic reaction depicted by cardiovascular and pulmonary

dysfunctions with severe hypotension and hypothermia as the clinical signs (Kalesnikoff and Galli 2010; Simons 2010).

Mast cells play a central role in allergic responses. Development of allergy commences with allergen sensitization which promotes elevation of IL-4 and IL-13 levels, leading to immunoglobulin class-switching in B cells to IgE production. Re-exposure to allergen triggers aggregation of the allergen-specific IgE-bound high-affinity IgE receptors (Fc ϵ RI) on mast cells and basophils, activation of phospholipid enzyme phospholipase C γ (PLC γ) leading to stimulation of protein kinase C (PKC) and increases in intracellular inositol (1,4,5)-triphosphate (IP₃) and Ca²⁺ levels, and degranulation and releases of mediators leading to immediate hypersensitivity reaction (Metcalf et al. 2009). Increase in intracellular Ca²⁺ is the single most critical signal required to induce mast cell degranulation (Nishida et al. 2005). More recently, cross-linking of Fc ϵ RI has also been shown to activate phosphoinositide 3-OH kinase (PI3K)/Akt pathway, leading to granule translocation and mast cell degranulation (Metcalf et al. 2009; Nishida et al. 2011). Among the known mast cell-derived mediators, histamine is essential for nearly every aspect of allergic responses

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including angioedema, vascular hyperpermeability, vasodilatation, bronchoconstriction, mucus production and hypothermia (Yoko et al. 2002).

Mallotus philippinensis is an important traditional Indian medicinal plant in Ayurvedic Medicine System (Chopra et al. 1956). It possesses many therapeutic values including anti-filarial (Singh et al. 1997), anti-allergic (Daikonya et al. 2002), and anti-helminthic activities (Gupta et al. 1984). The most important constituent of this Kamala plant is the dark brownish red adhering resin known as rottlerin (1-[6-[(3-acetyl-2,4,6-trihydroxy-5-methylphenyl)methyl]-5,7-dihydroxy-2,2-dimethyl-2H-1-benzopyran-8-yl]-3-phenyl-2-propen-1-one). Originally identified as a specific inhibitor of PKC δ , rottlerin was later found ineffective in inhibiting PKC δ but rather potent in blocking other kinases including Akt/PKB and p38 MAPK (Kan et al. 2004; Soltoff 2007; Choi et al. 2009). Rottlerin could inhibit human T cell responses (Springael et al. 2007), reduce MUC5AC expression in human epithelial cells (Choi et al. 2011), abrogate reactive oxygen species production in hepatic stellate cells (Guimarães et al. 2010), and prevent histamine-induced H₁-receptor gene expression in HeLa cells (Mizuguchi et al. 2011).

In this study, we explored potential anti-allergic effects of rottlerin in both *in vitro* and *in vivo* animal models of IgE-mediated anaphylaxis. We reveal for the first time that rottlerin prevented passive cutaneous anaphylaxis (PCA) and passive systemic anaphylaxis (PSA) in mice, ovalbumin (OVA)-induced anaphylactic contraction of bronchial rings from guinea pigs, and IgE-induced mast cell degranulation, probably via inhibition of the activation of PLC γ 1 and Akt, and reduction of the levels of IP₃ and intracellular Ca²⁺.

Materials and methods

Animals

Female BALB/c mice, 6–8 week-old, and female Hartley guinea pigs, weighing 350–450 g, were obtained from the Animal Resources Center (Canning Vale, Western Australia, Australia). Animal experiments were performed according to the Institutional Animal Care and Use Committee of the National University of Singapore.

IgE-mediated PCA

Mice were anesthetized and injected intradermally in the left dorsal skin with mouse anti-dinitrophenol (DNP)-specific IgE (100 ng in 40 μ l PBS, Sigma–Aldrich, St. Louis, MO, USA). PBS alone was used as vehicle control injected in the right dorsal skin. Mice were challenged 24 h later via intravenous injection of 100 μ g DNP-human serum albumin (DNP-HSA; Sigma–Aldrich) in 100 μ l of 0.5% Evan's blue dye. Rottlerin (0.3, 1 and 3 mg/kg, >85% purity, Sigma–Aldrich) or vehicle (6% DMSO) in 0.1 ml saline was given intravenously 2 h before DNP-HSA challenge. Vascular permeability was visualized 60 min after the allergen challenge by the blue staining at the intradermal injection sites. Skin samples were harvested and Evan's blue was extracted from the skin tissue using 0.5 mL of formamide at 55 °C for 48 h, and quantified by a spectrophotometer infinite F200 (TECAN, Männedorf, Switzerland) at 610 nm.

IgE-mediated PSA

Mice were sensitized by an intravenous injection of 20 μ g of anti-DNP IgE in 100 μ l PBS, and challenged 24 h later with 200 μ g of DNP-HSA in 50 μ l PBS via intravenous injection. Rottlerin (0.3, 1, and 3 mg/kg) or vehicle in 100 μ l saline was given intravenously 2 h

before the allergen challenge. Drop in body temperature associated with systemic anaphylaxis were measured at time 0, 1 and subsequently every 5 min up to 90 min using a rectal probe coupled to a digital thermometer (AD Instruments, Castle Hill, Australia). At the end of the experiment, lung and tracheal tissues were excised and fixed in 10% buffered formalin solution (Sigma–Aldrich) for histological analysis. To measure plasma histamine level, a separate set of mice was sacrificed and blood collected 3 min after allergen challenge, due to the short half-life of histamine. Histamine level was determined using ELISA (IBL International, Hamburg, Germany).

Histological analysis of tracheal mast cell degranulation

Paraffinized trachea samples were cut into 4.5- μ m sections, stained with toluidine blue, and mounted with HistoMount medium (National Diagnostics, Atlanta, GA, USA). The stained sections were examined under a light microscope for numeration of mast cells in the trachea of different treatment groups. The level of degranulated mast cells with extruded granules in the each section was expressed as percentage of total number of mast cells in each tracheal section (Begueret et al. 2007).

OVA-induced bronchial ring anaphylactic contraction

Guinea pigs were actively sensitized by OVA, and bronchial rings were prepared and contractile studies were monitored using force-displacement transducers coupled to a PowerLab/4SP data-recording system (AD Instruments) as previously described (Tsang and Wong 2000; Duan et al. 2003). After a 90-min equilibration in Krebs-bicarbonate solution, sensitized bronchial rings were contracted to 60 mM KCl to obtain maximum contractile responses to which all subsequent OVA-induced anaphylactic contractions were compared. To evaluate inhibitory effects of rottlerin on OVA-induced anaphylactic contraction, rottlerin (30 μ M) or vehicle control (0.1% DMSO) was pre-incubated with the ring preparations for 1 h before challenge with 1 μ g/ml OVA. Anaphylactic contractions were monitored over a 60-min period.

IgE-induced mast cell degranulation

Rat basophilic leukemia cells (RBL-2H3) (ATCC, Rockville, MD, USA) were seeded on 6-well (1×10^6 cells/well) plates and primed with anti-DNP IgE (200 ng/ml) overnight and then challenged with DNP-HSA (300 ng/ml) for 30 min for mast cell degranulation analysis. Rottlerin (3, 10, and 30 μ M) or vehicle (0.1% DMSO) was pre-incubated with RBL-2H3 cells for 2 h before DNP-HSA challenge. Cells and supernatants were collected, and incubated with 50 μ l of *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide (1.3 mg/ml, Sigma–Aldrich) for 1 h at 37 °C before measuring the optical density at 405 nm (Victor3V Multilabel Plate Reader, PerkinElmer, Waltham, MA, USA). The level of released β -hexosaminidase was expressed as percentage of total β -hexosaminidase recovered in both supernatants and cells (Chan et al. 2007).

Immunoblotting

IgE-primed RBL-2H3 cells were challenged with DNP-HSA for indicated times and then lysed in M-PER mammalian protein extraction reagent (Thermo Scientific, Waltham, MA, USA). Proteins were separated by 10% SDS-PAGE, and immunoblots were probed with anti-PLC γ 1, anti-phospho-PLC γ 1, anti-Akt or anti-phospho-Akt (1:1000; Cell Signaling, Boston, MA, USA), followed by HRP-conjugated anti-rabbit secondary antibody (DAKO, Glostrup,

Denmark). Blots were developed using the enhanced chemiluminescence reagent (PerkinElmer).

Measurement of cellular IP_3

RBL-2H3 cells were seeded in 75 cm² cell culture flasks at a density of 6×10^6 cells/flask. Cells were allowed to adhere for 3 h and then primed with 180 ng/mL of DNP-IgE overnight. Four hours later, primed cells were treated with rottlerin (3, 10, and 30 μ M) or vehicle for 12 h. Cells were then re-suspended in 96-well plates at 6×10^4 cells/well in 30 μ L PBS containing 1% BSA, and stimulated with 300 ng/mL DNP-HSA for 20 s. Cellular IP_3 levels were measured using the Hit Hunter IP_3 Fluorescence Polarization Assay Kit (DiscoverRx, Fremont, CA, USA). Polarized fluorescence signals were read at excitation 485 nm and emission 530 nm using the Victor3 V Multilabel Plate Reader (PerkinElmer).

Imaging of intracellular Ca^{2+} in single cells

RBL-2H3 cells were grown on coverslips and primed with 1 μ g/mL DNP-IgE overnight. Cells were pre-incubated with 30 μ M rottlerin or vehicle for 2 h before Ca^{2+} signal induction. RBL-2H3 cells were replenished in 1 mL Ca^{2+} -containing EBSS (Sigma-Aldrich) and loaded with 4 μ L of Ca^{2+} -activated fluorescent dye fura 2-AM (2 μ M, Molecular Probes, Eugene, OR, USA) for 30 min. The coverslips were transferred to the stage of an inverted microscope (Eclipse TS100, Nikon, Japan) in a superfusion chamber perfused with EBSS. Cytosolic Ca^{2+} was measured in individual single cells stimulated with DNP-HSA using the InCyt dual wavelength fluorescence imaging system (Intracellular Imaging, Cincinnati, OH, USA). Fluorescence intensity was monitored with alternating excitation at wavelengths 340 nm (F340) and 380 nm (F380). Recordings were made via the Image Master (Photon Technology, Birmingham, NJ, USA), and the relative intracellular Ca^{2+} level was depicted as the average ratio of fluorescence (340/380 nm) (Cheng et al. 2013).

Statistical analysis

Data are presented as means $\pm$ SEM. One-way ANOVA followed by Dunnett's test was used to determine significant differences between treatment groups. Significant levels were set at $p < 0.05$.

Results

Rottlerin suppresses IgE-mediated PCA

PCA is a localized anaphylactic reaction characterized by an increase in cutaneous vascular permeability (Inagaki and Nagai 2009). Mice sensitized with intradermal anti-DNP IgE and challenged with intravenous DNP-HSA with Evan's blue dye for 1 h developed intense blue staining at the intradermal injection sites as compared to PBS vehicle-sensitized mice (Fig. 1a). Rottlerin (0.3, 1 and 3 mg/kg) significantly suppressed the IgE-mediated vascular permeability ($p < 0.05$) in a dose-dependent manner as compared to the DMSO control (Fig. 1b). Taken together, these data clearly indicate that rottlerin is able to suppress IgE-mediated PCA reactions *in vivo*.

Rottlerin inhibits IgE-mediated PSA

Drop in body temperature is one of the major hallmarks of PSA (Yoko et al. 2002; Finkelman 2007). Mice sensitized with intravenous anti-DNP IgE antibody and then challenged with intravenous DNP-HSA developed a drastic drop in body temperature from 37 °C to the lowest at 32.3 °C within 20 min and slowly

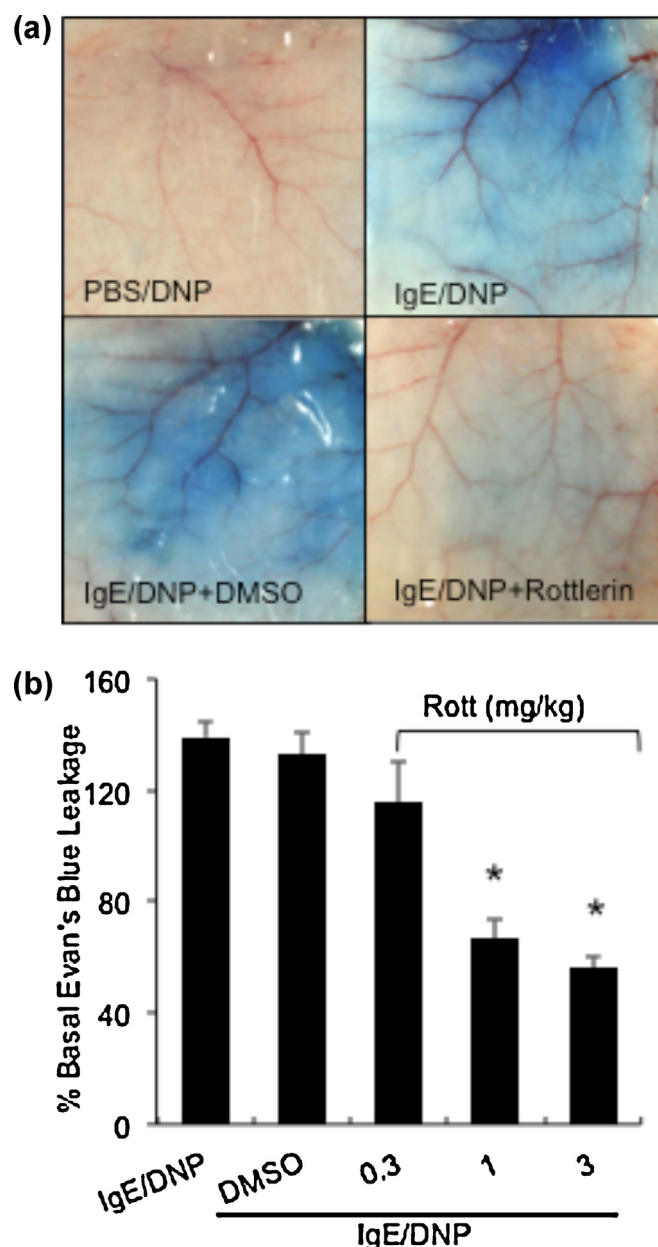


Fig. 1. Effects of rottlerin on PCA. (a) DNP-HSA challenge induced localized vascular hyperpermeability and plasma extravasation together with Evan's blue dye as visualized as intense blue staining in the skin. PBS/DNP (negative control), IgE/DNP (positive control), IgE/DNP + DMSO (vehicle control) and IgE/DNP + rottlerin (3 mg/kg). Photomicrographs shown are representatives of 6 separate experiments. (b) Rottlerin dose-dependently blocked DNP-HSA-induced plasma extravasation. Values shown are the means $\pm$ SEM for 6 mice per treatment group. *Significant difference from DMSO control, $p < 0.05$.

returned to 36.3 °C at 90 min. Rottlerin markedly halted the drop in rectal temperature at 20 min in a dose-dependent manner, and facilitated recovery to normal body temperature by 50 min dose-dependently as compared to vehicle control mice (Fig. 2a and b). Histamine is believed to play a major role in vasodilation, vascular hyperpermeability and drop in body temperature during systemic anaphylaxis (Yoko et al. 2002; Finkelman 2007). It is rapidly released into systemic circulation upon IgE-induced mast cell degranulation. We detected a substantial rise in plasma histamine level in just 3 min after DNP-HSA challenge of the sensitized mice (Fig. 2c). Pretreatment with rottlerin significantly reduced plasma histamine levels in a dose-dependent manner as compared

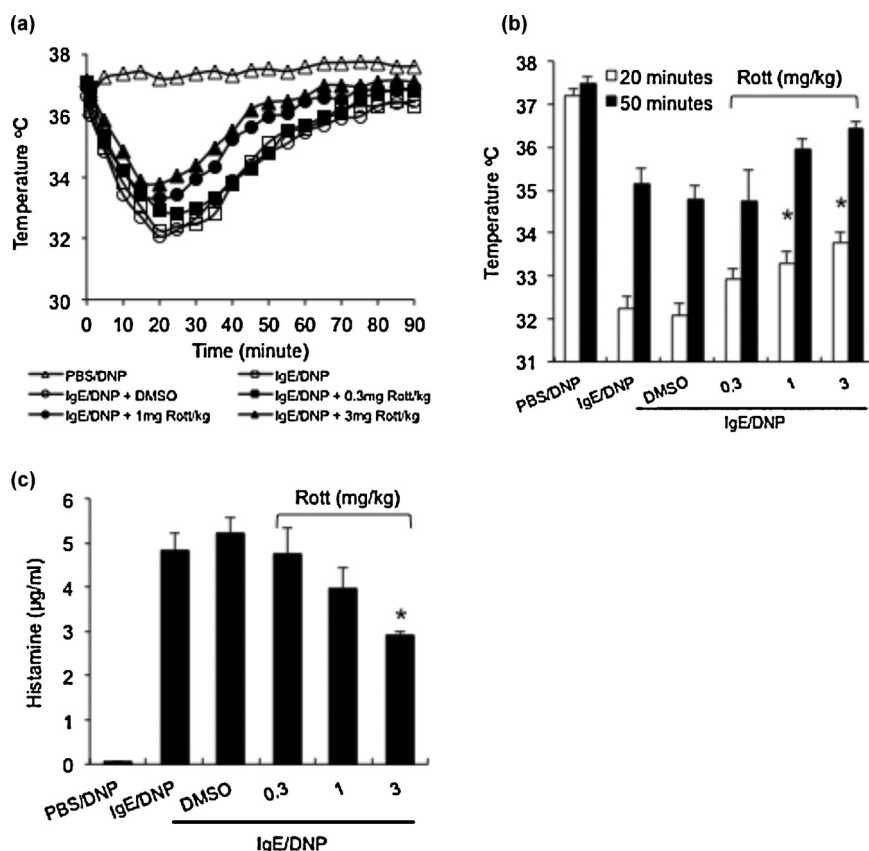


Fig. 2. Effects of rottlerin on PSA. Anti-DNP IgE-sensitized mice were challenged with an intravenous injection of DNP-HSA to produce PSA. (a) Mice developed hypothermia and rectal temperature was measured at time 0, 1 and subsequently every 5 min up to 90 min. (b) Rottlerin prevented the drop of body temperature and temperature measurements at 20 and 50 min are presented as mean $\pm$ SEM ($n=6$ mice for each group). (c) Plasma histamine levels at 3 min after DNP-HSA challenge. Values presented are the mean $\pm$ SEM for 6 mice per treatment group. *Significant difference from DMSO control, $p < 0.05$.

to vehicle control. These results clearly indicate the ability of rottlerin in attenuating systemic anaphylactic reactions *in vivo*.

Rottlerin prevents tracheal mast cell degranulation

To directly capture mast cell stabilizing effect of rottlerin, we examined mast cell degranulation in tracheal histological sections obtained from the PSA mouse model. DNP-HSA challenge of sensitized mice triggered off extensive mast cell degranulation in the mouse airway as manifested by conspicuous mast cell granule extrusion (Fig. 3a). Pre-treatment with rottlerin (0.3, 1 and 3 mg/kg) markedly prevented IgE-mediated mast cell degranulation and increased number of intact mast cells in the airways as compared to vehicle control (Fig. 3b).

Rottlerin attenuates OVA-induced bronchial anaphylactic contraction

Sensitized guinea pig bronchial rings contracted to 60 mM KCl with an active force of 1.95 ± 0.18 g ($n=27$). All subsequent OVA-induced anaphylactic contractions were expressed as percentage of the KCl responses. OVA, at 1 µg/ml, induced strong and sustaining anaphylactic contractions reaching 2.65 ± 0.18 g ($n=8$) and, over the time course of 60 min, producing an area-under-curve (AUC) of 6360.56 ± 611.66 KCl contraction min (Fig. 4). Rottlerin significantly suppressed OVA-induced bronchial contractions as reflected by the reduction in AUC by 27.1% as compared to DMSO control (Fig. 4). These findings further demonstrated the inhibitory effect

of rottlerin on blocking IgE-induced mast cell degranulation in an *in vitro* model of anaphylaxis.

Rottlerin suppresses IgE-induced mast cell degranulation

DNP-IgE-primed RBL-2H3 cells released significant amount of pre-formed mediator β -hexosaminidase upon DNP-HSA challenge as compared to un-primed mast cells. Rottlerin (3, 10 and 30 µM) significantly ($p < 0.05$) prevented IgE-induced β -hexosaminidase release in a concentration-dependent manner (Fig. 5a). To address the mechanism of action of rottlerin on blocking the IgE-induced immediate mast cell degranulation, we have examined the phosphorylation state of PLC γ 1 and Akt upon Fc ϵ RI stimulation by DNP-HSA in RBL-2H3 cells. PLC γ 1 and Akt (Ser 473) phosphorylation could be observed within 20 s upon Fc ϵ RI activation and lasted for at least 5 min. Rottlerin (30 µM) totally blocked IgE-induced PLC γ 1 and Akt phosphorylation in a time-dependent manner as compared to vehicle control (Fig. 5b). Activation of PLC γ is essential for the production of IP $_3$ and the increase in intracellular Ca $^{2+}$ level, leading to mast cell degranulation (Metcalfe et al. 2009; Ishijima et al. 2012). For the IP $_3$ assay, DNP-HSA challenge induced an immediate and massive IP $_3$ production from primed RBL-2H3 cells. Pretreatment with rottlerin markedly suppressed the upsurge of DNP-HSA-induced IP $_3$ production in a concentration-dependent manner (Fig. 5c). Similarly, IgE-primed RBL-2H3 cells responded with a rapid surge in cytosolic Ca $^{2+}$ level to DNP-HSA challenge. In contrast, the level of cytosolic Ca $^{2+}$ in un-primed cells remained stable upon allergen challenge (Fig. 5d). Rottlerin (30 µM) significantly

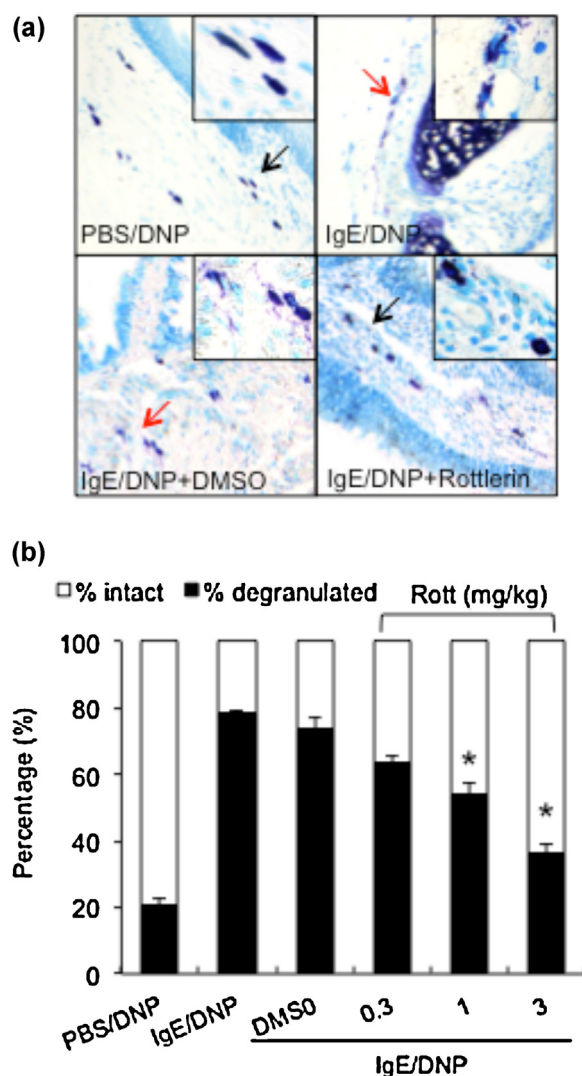


Fig. 3. Effects of rottlerin on PSA-mediated tracheal mast cell degranulation. (a) Tracheal tissue sections were stained with toluidine blue. Black arrows point to intact mast cells and red arrows point to degranulated mast cells. Stained sections were examined under light microscopy (magnification at 200 $\times$ and 1000 $\times$ for the insets). (b) Numeration of intact and degranulated mast cells in tracheal sections and data are expressed as mean $\pm$ SEM for 6 mice per treatment group. *Significant difference from DMSO control, $p < 0.05$.

($p < 0.05$) blocked DNP-HSA-induced Ca^{2+} rise in RBL-2H3 cells (Fig. 5d).

Discussion

$\text{Fc}\epsilon\text{RI}$ aggregation-induced mast cell degranulation is pivotal to the development of allergic diseases (Galli et al. 2008). Mast cell-derived vasoactive mediators such as histamine, serotonin and peptidoleukotrienes are rapidly released into extracellular milieu (Roquet et al. 1997; Yoko et al. 2002; Finkelman 2007; Inagaki and Nagai 2009). Histamine appears to be most crucial to the manifestation of vasodilation, increased vascular permeability, a drop in body temperature and bronchial smooth muscle contraction in allergic anaphylaxis (Yoko et al. 2002; Finkelman 2007; Inagaki and Nagai 2009). Strategy to prevent IgE-induced mast cell degranulation is central to the drug discovery for allergic diseases. In this study, we report for the first time that rottlerin possesses anti-allergic activity in mast cell-dependent *in vivo* and *in vitro* models of anaphylaxis. Rottlerin blocked IgE-induced mast cell degranulation likely via

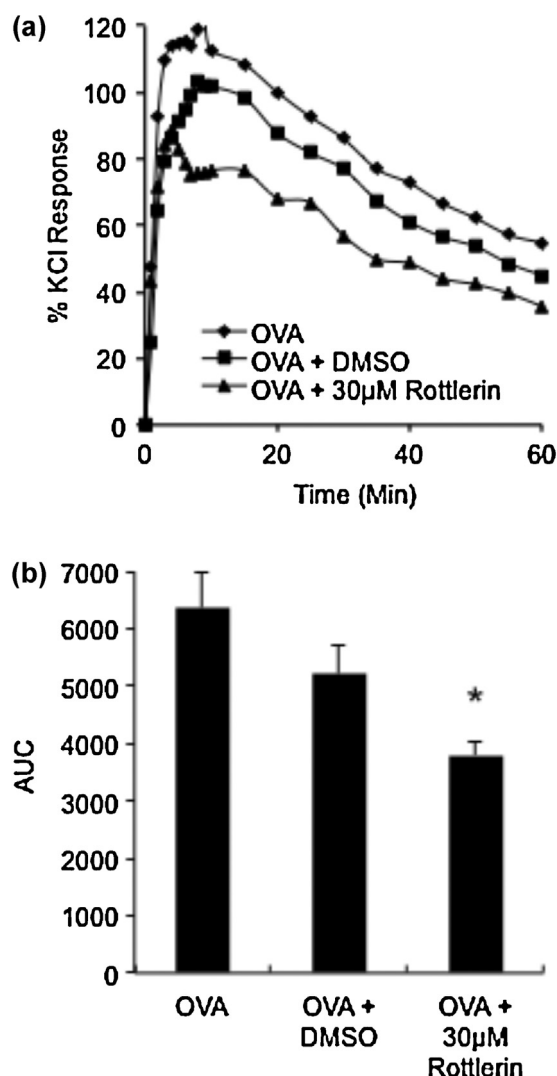


Fig. 4. Effects of rottlerin on OVA-induced anaphylactic contraction of guinea pig bronchi. (a) Time-course study of OVA-induced anaphylactic bronchial contraction in the presence and absence of 30 μM rottlerin. (b) Area-under-curve (AUC) for OVA-induced anaphylactic bronchial contraction over 60-min. Each point represents the mean $\pm$ SEM of four experiments. *Significant difference from DMSO vehicle control, $p < 0.05$.

inhibition of $\text{PLC}\gamma 1$ and Akt phosphorylation, IP_3 production and cytosolic Ca^{2+} level.

PCA and PSA are commonly used *in vivo* animal models to demonstrate IgE-mediate mast cell degranulation and to evaluate potential anti-allergic compounds (Finkelman 2007; Inagaki and Nagai 2009). PCA is a localized cutaneous allergic response as a result of allergen-induced vascular hyperpermeability and plasma extravasation, representing clinical features of urticaria (Inagaki and Nagai 2009). PSA is a generalized allergic reaction manifested as hypotension, bronchoconstriction and hypothermia, bearing resemblance to clinical features of anaphylaxis (Yoko et al. 2002; Finkelman 2007). Histamine, a preformed mediator rapidly released upon mast cell degranulation, plays a critical role in anaphylaxis by binding to histamine receptors (e.g. H_1 and H_2), leading to widening of intercellular gap junctions via endothelial cell retraction and vasodilation via increased production of nitric oxide (Evora and Simon 2007; Kumar et al. 2009). Rottlerin prevented DNP-HSA-induced cutaneous vascular hyperpermeability and hypothermia in mice, elevation of plasma histamine level, and tracheal tissue mast cell degranulation. Allergen-induced anaphylactic airway

smooth muscle contraction is a widely used *in vitro* animal model to demonstrate immediate mast cell degranulation and rapid release of spasmogens like histamine and peptidoleukotrienes, causing bronchial contraction (Roquet et al. 1997; Tsang and Wong 2000). Rottlerin significantly suppressed OVA-induced guinea pig bronchial anaphylactic contraction over a 60-min interval.

To uncover its anti-allergic mechanisms of action, we investigated the direct effects of rottlerin on RBL-2H3 mast cell line. RBL-2H3 cell line possesses functional and biochemical characteristics similar to mast cells and basophils, and has been used as a surrogate to study mast cell biology. It expresses FcεRI and degranulates upon crossing linking of IgE-bound FcεRI by multivalent

allergens, leading to rapid release of histamine, β-hexosaminidase, serotonin and peptioeukotrienes (Passante and Frankish 2009). Rottlerin significantly abated DNP-HSA-induced RBL-2H3 release of β-hexosaminidase in a concentration-dependent manner. These findings clearly indicate that rottlerin can block IgE-bound FcεRI cross-linking-induced mast cell degranulation.

FcεRI aggregation triggers off a cascade of signaling pathways culminating in immediate mast cell degranulation. Syk tyrosine kinase can be activated instantaneously upon FcεRI stimulation, followed by rapid phosphorylation of PLCγ and PI3K/Akt pathway (Kim et al. 2008; Metcalfe et al. 2009; Nishida et al. 2011; Ishijima et al. 2012). In this study, we observed that DNP-HSA induced rapid

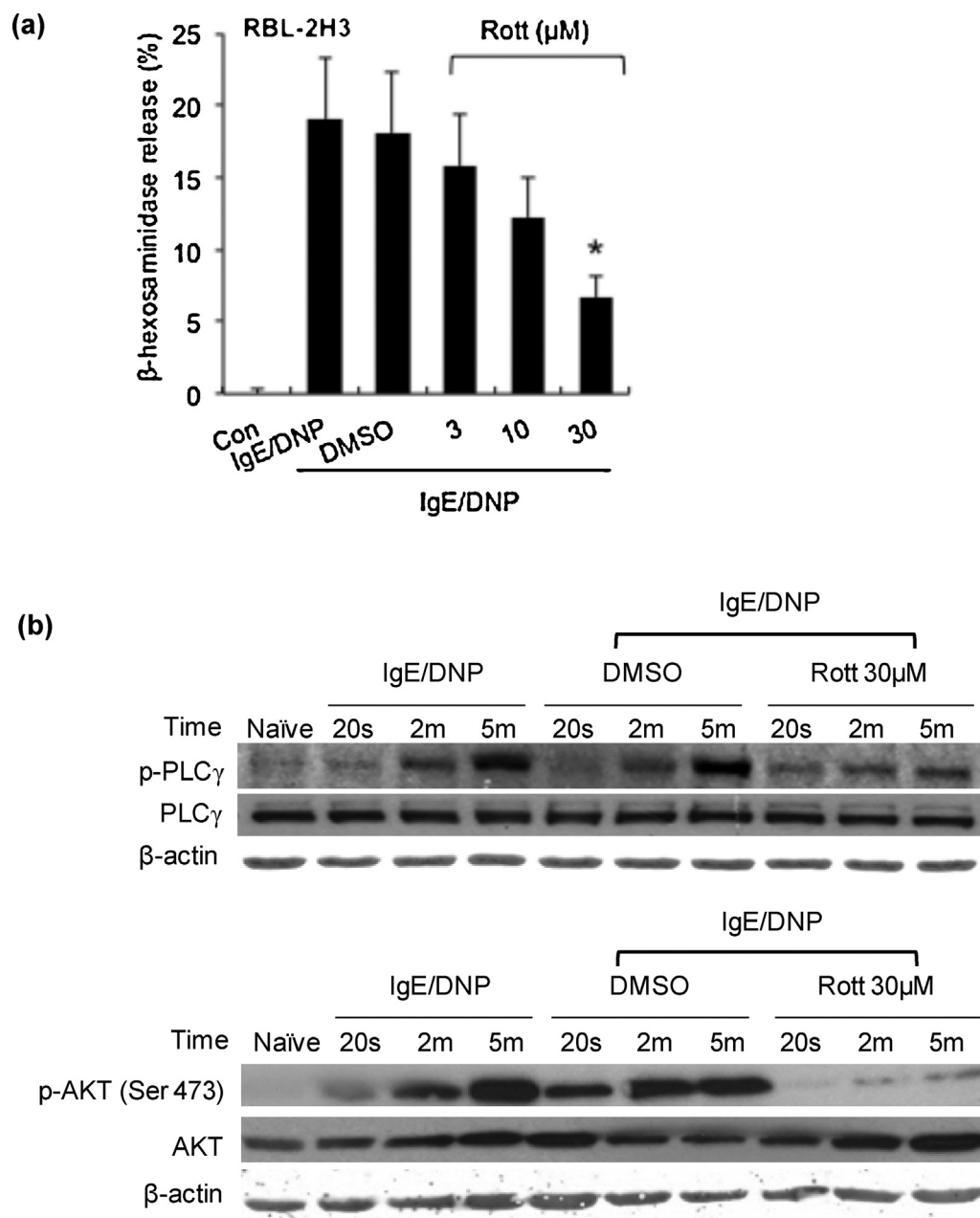


Fig. 5. Effects of rottlerin on FcεRI-mediated mast cell degranulation. (a) β-Hexosaminidase release from RBL-2H3 cells was measured as described (Chan et al. 2007). β-Hexosaminidase data are expressed as percentage of total cellular contents. Values shown are means ± SEM from triplicate samples of 4 separate experiments. (b) Rottlerin markedly blocked DNP-HSA-induced phosphorylation of PLCγ1 and Akt. β-Actin was used as internal control. Immunoblots shown are representatives of 3 separate experiments with similar patterns of results. (c) Rottlerin markedly prevented DNP-HSA-induced IP₃ production in a concentration-dependent manner. Values shown are means ± SEM from triplicate samples of 3 separate experiments. (d) Rottlerin attenuated cytosolic Ca²⁺ levels in DNP-HSA-stimulated mast cells. Left panel displays typical tracings of Ca²⁺ levels of different treatment groups in single mast cells. Right panel presents the group data showing the effect of artesunate on cytosolic Ca²⁺. Data are expressed as means ± SEM of 20–46 individual cells. *Significant difference from DMSO control, *p* < 0.05.

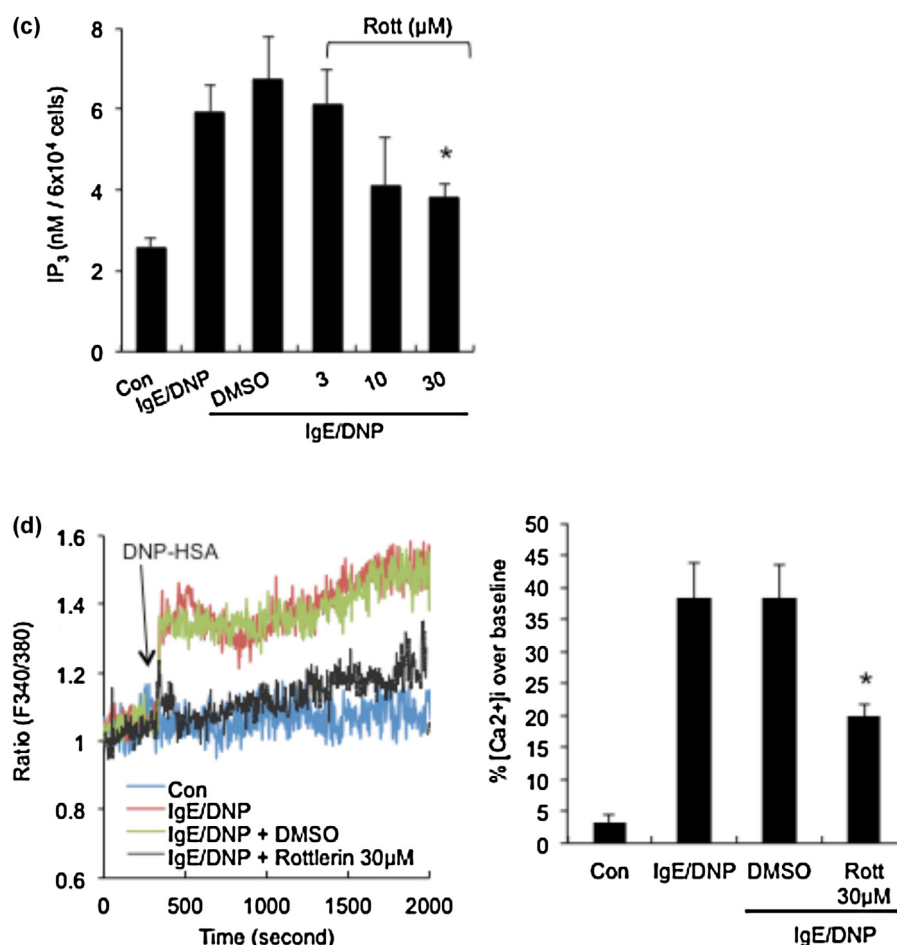


Fig. 5. (Continued.)

PLCγ1 phosphorylation in RBL-2H3 cells in a time-dependent manner, and rottlerin markedly blocked the PLCγ1 phosphorylation. A recent genetic analysis study has linked enhanced PLCγ2 activity to the development of cold urticaria as a consequence of mast cell degranulation (Ombrello et al. 2012). Inhibition of PLCγ1 activation has been shown to abolish FcεRI-mediated Ca²⁺ mobilization and mast cell degranulation (Kageyama-Yahara et al. 2011). PLCγ plays an important role in the production of IP₃ and ensuing rise in cytosolic Ca²⁺ level (Kuehn et al. 2008; Ishijima et al. 2012). An increase in intracellular Ca²⁺ levels in mast cell provides an indispensable and ubiquitous mechanism for exocytotic release of granules upon FcεRI aggregation (Nishida et al. 2005). Rottlerin markedly inhibited FcεRI-induced increase in IP₃ production and in cytosolic Ca²⁺ level in RBL-2H3 cells. On the other hand, Nishida et al. (2011) have elucidated an essential role of PI3K/Akt pathway in IgE-mediated granule translocation and mast cell degranulation. Inhibition of PI3K by SP600125 has been shown to block IgE-induced mast cell degranulation (Tanemura et al. 2009). We observed that rottlerin completely blocked DNP-HSA induced Akt phosphorylation in RBL-2H3 cells. Taken together, the anti-allergic actions of rottlerin may start from PLCγ1 and Akt inhibition, leading to suppression of IP₃ production and Ca²⁺ mobilization, and prevention of IgE-mediated mast cell degranulation.

We report here for the first time that rottlerin possesses anti-allergic activity by stabilizing mast cells in different animal models of anaphylaxis, providing a foundation for developing rottlerin for mast cell-mediated allergic disorders including urticaria and allergic asthma.

Conflict of interest

None.

Disclosure statement

There was no significant financial support for this work that could have influenced its outcome.

Role of funding source

None.

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