Regular Article

Effect of Royal Jelly and Brazilian Green Propolis on the Signaling for Histamine H₁ Receptor and Interleukin-9 Gene Expressions Responsible for the Pathogenesis of the Allergic Rhinitis

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The significant correlation between nasal symptom scores and level of histamine H₁ receptor (H1R) mRNA in nasal mucosa was observed in patients with pollinosis, suggesting that H1R gene is an allergic disease sensitive gene. We demonstrated that H1R and interleukin (IL)-9 gene are the allergic rhinitis (AR)-sensitive genes and protein kinase C δ (PKC δ) signaling and nuclear factor of activated T-cells (NFAT) signaling are involved in their expressions, respectively. Honey bee products have been used to treat allergic diseases. However, their pathological mechanism remains to be elucidated. In the present study, we investigated the mechanism of the anti-allergic effect of royal jelly (RJ) and Brazilian green propolis (BGPP). Treatment with RJ and BGPP decreased in the number of sneezing on toluene 2,4-diisocyanate (TDI)-stimulated rats. The remarkable suppression of H1R mRNA in nasal mucosa was observed. RJ and BGPP also suppressed the expression of IL-9 gene. RJ and BGPP suppressed phorbol-12-myristate-13-acetate-induced Tyr³¹¹ phosphorylation of PKC δ in HeLa cells. In RBL-2H3 cells, RJ and BGPP also suppressed NFAT-mediated IL-9 gene expression. These results suggest that RJ and BGPP improve allergic symptoms by suppressing PKC δ and NFAT signaling pathways, two important signal pathways for the AR pathogenesis, and suggest that RJ and BGPP could be good therapeutics against AR.

Key words Brazilian green propolis; royal jelly; histamine H₁ receptor; nuclear factor of activated T-cells; protein kinase C δ

Nasal hypersensitivity is a representative incurable disease. Histamine is a prime molecule responsible for allergic and inflammatory reactions mediated mainly through histamine H₁ receptor (H1R).^{1,2} Alleviation of acute allergic symptoms using antihistamines is suggested by the suppression of H1R gene expression in nasal mucosa. Allergic rhinitis (AR) patients expressed high level of H1R mRNA in their nasal mucosa.^{3,4} We reported that neuron mediated inflammation assists to release histamine from mast cells after the application of toluene 2,4-diisocyanate (TDI) at the nostril of guinea pigs.^{5,6} Antihistamines are the first choice for the therapy of this disease and *d*-chlorpheniramine suppressed both H1R mRNA and H1R protein in TDI-sensitized rats.⁷ Furthermore, the correlation between the expression of H1R gene with intensity of allergic symptoms in TDI-model rats and in pollinosis patients has been revealed.^{8,9} However, suppression of H1R signaling could not completely improve nasal symptoms.¹⁰ And, recently, we demonstrated that interleukin (IL)-9 gene is the additional allergy sensitive gene in TDI-sensitized rats and nuclear factor of activated T-cells (NFAT) signaling is involved in this cytokine gene expression.¹⁰ Helper T cell type 2 (Th2) cytokines are also considered as important elements of allergic development. IL-4, IL-5, and IL-13 are involved in the initiation and maintenance of allergic reaction.^{11–13}

Since ancient times, honey and its products have been utilized as food and medicine to heal many diseases.^{14,15} AR

patients showed improvement of allergic symptoms after the ingestion of honey.¹⁶ Anti-allergic activities in royal jelly (RJ) were reported *in vivo* study, in which major royal jelly protein 3 suppressed IL-4 production in ovalbumin-immunized mice.¹⁷ It was also reported that RJ suppressed antigen-specific immunoglobulin E (IgE) production and histamine release from mast cells.¹⁸ Brazilian green propolis (BGPP) yield in Southeast region of Brazil has been highly used as a nutrient, especially in Japan, and its main origin is *Baccharis dracunculifolia*.^{19,20} It did not show any side effects in mice, rats, and human after administration.^{21,22} Randomized double-blind placebo-controlled trials were demonstrated the efficacy and safety of propolis supplement on management of Japanese cedar pollinosis.^{23,24} It was reported that the suppression of cysteinyl-leukotriene release from mast cells is one of the mechanism of anti-AR effect of BGPP.²⁵ It was also reported that Brazilian propolis down-regulate type-1 allergy by the inhibition of mast cell degranulation.²⁶ However, effect of RJ and BGPP on the histamine signaling remains unknown.

In the current study, we investigated the effect of RJ and BGPP on the H1R gene expression. Next, we investigated the effect of RJ and BGPP on the gene expression of histamine signaling related Th2 cytokines, IL-4 and IL-5. Then, we investigated their effect on the expression of IL-9 gene, an additional allergy sensitive gene. Our investigation proved that orally pretreated RJ and BGPP improved the allergic

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Fig. 1. Experimental Protocol

10 μ L of 10% TDI in ethyl acetate was used to sensitize the rats applying through the nostrils for 2 weeks. Further the nasal symptoms were provoked using the same TDI preparation after 1 week. Ethyl acetate only used to sensitize the control rats. Royal jelly and Brazilian green propolis were administered orally as doses 40 and 80 mg/kg/d (described in Materials and Methods).

nasal symptoms and had also H1R mRNA suppressive activity in TDI-sensitized rats. We also showed that suppression of H1R-mediated activation of protein kinase C δ (PKC δ) is the underlying mechanism of their effect. They also suppressed NFAT-mediated IL-9 gene expression. These studies suggest that RJ and BGPP suppress both PKC δ and NFAT signaling responsible for the development of AR.

MATERIALS AND METHODS

Animals Brown Norway rats (male, six-week-old, 200–250 g; Japan SLC, Hamamatsu, Japan) were used for the present study. Rats were allowed free access to water and food. Room temperature and humidity was maintained at 25 $\pm$ 2°C and 55 $\pm$ 10%, respectively, with a 12-h light/dark cycle. The animals were divided into 6 groups (*i.e.*, control, sensitized with TDI (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and test groups) with 5 rats in each group. To perform this experiment, we followed the guidelines of the Animal Research Committee of Tokushima University.

TDI Sensitization and Provocation and Administration of RJ and BGPP Rats provocation was performed with TDI by the technique described by Kitamura *et al.*²⁷⁾ Briefly, 10 μ L of a 10% solution of TDI in ethyl acetate (Wako Pure Chemical Industries, Ltd.) was applied bilaterally to the nasal vestibule once a day for 5 consecutive days. After a 2-d interval, this sensitization procedure was repeated. Nine days after the second sensitization, nasal allergic-like symptoms was provoked by the application of 10 μ L of 10% TDI to the nasal vestibule. Control rats were treated with ethyl acetate only according to the same schedule (Fig. 1). Enzyme-treated (protease-degraded) RJ and ethanol extract of BGPP were obtained from Yamada Bee Company, Inc. (Okayama, Japan). RJ and ethanol extract of BGPP (40 and 80 mg/kg) was suspended in 0.5% carboxymethyl cellulose on the day of the experiments and orally administered once daily for 3 weeks. RJ and BGPP were administered orally 1 h before the TDI sensitization (Fig. 1). It was reported that 100 mg/kg of RJ and propolis were used to evaluate their anti-inflammatory effect in rats,^{28,29)} therefore, we used 40 mg/kg as low dose and 80 mg/kg as high dose in this study. The number of sneezes and the ex-

Table 1. Criteria for Grading the Severity of TDI-Induced Nasal Responses in Rats

Nasal response	Score			
	0	1	2	3
Watery rhinorrhea	(–)	At the nostril	Between 1 and 3	Drops of discharges from nose
Swelling and redness	(–)	Slightly swollen	Between 1 and 3	Strong swelling with redness

tent of watery rhinorrhea considered as the indicator of nasal allergic-symptoms and were determined using the protocol of Abe *et al.*⁶⁾ After TDI provocation, the number of sneezes and watery rhinorrhea severity were examined for 10 min. Scaling from 0 to 3 was used as the basis to estimate the level of watery rhinorrhea described in the Table 1.

For standardization of RJ and BGPP, HPLC analyses were conducted. The enzyme-treated RJ powder (Lot: YRP-M-140906) was extracted with methanol and analyzed using an HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a Sunniet C18 column (ChromaNik Technologies Inc., Osaka, Japan) at 40°C with the gradient solvent system of methanol in 0.1% trifluoroacetic acid (TFA). The HPLC chemical fingerprint of the extract was recorded by evaporative light scattering detector (ELSD, 30°C; Supplementary Fig. 1A). The BGPP ethanol extract powder (Lot: 140605) was re-extracted with methanol and analyzed using an HPLC system (Shimadzu Corporation) equipped with a Cosmosil 5C₁₈-AR-II column (Nacalai Tesque, Kyoto, Japan) at 40°C under a constant flow rate of 70% methanol in 0.1% TFA. The HPLC chemical fingerprint of the extract was recorded by photo diode array (PDA) detector at 275 nm (Supplementary Fig. 1B). All HPLC data were collected at Yamada Bee Company, Inc. Standardization analyzes revealed that enzyme-treated RJ powder contains 3.5% (*E*)-10-hydroxy-2-decenoic acid and 0.6% 10-hydroxydecanoic acid, and methanol extract of BGPP ethanol extract powder contains 8% artemisinin C.

Real-Time Quantitative RT-PCR Rats were sacrificed at 4 h after TDI provocation and nasal mucosa was collected from the nasal septum, accumulated in RNAlater (Applied

Table 2. Nucleotide Sequences of Primers and Probes Used in This Study

Primer/probe name	Sequence
Human H1R mRNA	
Sense primer	5'-CAGAGGATCAGATGTTAGGTGATAGC-3'
Anti-sense primer	5'-AGCGGAGCCTCTTCCAAGTAA-3'
Probe	FAM-CTTCTCTCGAACGGACTCAGATACCACC-TAMRA
Rat H1R mRNA	
Sense primer	5'-TATGTGTCCGGGCTGCACT-3'
Anti-sense primer	5'-CGCCATGATAAAAACCAACTG-3'
Probe	FAM-CCGAGAGCGGAAGGCAGCCA-TAMRA
Rat IL-4 mRNA	
Sense primer	5'-CAGGGTGCTTCGCAAATTTTAC-3'
Anti-sense primer	5'-CACCGAGAACCCAGACTTG-3'
Probe	FAM-CCCACGTGATGTACCTCCGTGCTTG-TAMRA
Rat IL-5 mRNA	
Sense primer	5'-CAGTGGTGAAAGAGACCTTGATACAG-3'
Anti-sense primer	5'-GAAGCCTCATCGTCTCATTGC-3'
Probe	FAM-TGTCACCTACCGAGCTCTGTTGACG-TAMRA
Rat IL-9 mRNA	
Sense primer	5'-CAGAGGATCAGATGTTAGGTGATAGC-3'
Anti-sense primer	5'-AGCGGAGCCTCTTCCAAGTAA-3'
Probe	FAM-CTTCTCTCGAACGGACTCAGATACCACCT-TAMRA

Biosystems, U.S.A.), and stocked at -80°C for further analysis. Polytron (Model PT-K; Kinematica) was used as homogenizer to homogenize the collected nasal mucosa. Total RNA was isolated using RNAiso Plus reagent (TaKaRa Bio Inc., Kyoto, Japan). HeLa cells were cultured at 37°C under a humidified 5% CO_2 , 95% air atmosphere in MEM- α containing 8% fetal calf serum and 1% antibiotic-antimycotic (Invitrogen, Carlsbad, CA, U.S.A.). HeLa cells cultured to 70% confluency in 6-well dishes were serum-starved for 24 h and treated with RJ and BGPP for 12 and 3 h, respectively, before histamine or phorbol-12-myristate-13-acetate (PMA) stimulation. After a 3-h treatment with histamine or PMA, the cells were harvested and total RNA was prepared as reported previously.³⁰⁾ For RBL-2H3 cells culture we used in MEM containing 10% fetal bovine serum (FBS), 120 IU/mL penicillin, and 120 $\mu\text{g}/\text{mL}$ streptomycin. 70% confluent RBL-2H3 cells in 6-well dishes were treated with RJ and BGPP for 12 and 3 h, respectively, before stimulation with 1 μM ionomycin. After a 2-h stimulation, the cells were harvested and total RNA was prepared. RNA sample (5 μg) was reverse transcribed to cDNA using PrimerScript RT reagent Kit (TaKaRa Bio Inc.). Real-time PCR was conducted using a GeneAmp 7300 sequence detection system (Applied Biosystems). The nucleotide sequences of the primers and probes used in this study are listed in Table 2. To standardize the starting material, rodent or human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers and probe reagents (Applied Biosystems) were used.

Immunoblot Analysis Immunoblot analysis was conducted as previously reported.³¹⁾ Protein sample (10 μg) was separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad). The membrane was briefly rinsed in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and then incubated for 1 h at room temperature in TBS-T containing 5% skim milk (Difco) or 3% bovine serum albumin (BSA) (for detecting phosphoproteins; Sigma, St. Louis, MO, U.S.A.). Total and phosphory-

lated PKC δ was detected using antibodies for PKC δ (C-20) (sc-937; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) and phospho-PKC δ (Tyr³¹¹) (#2055S; Cell Signaling Technology Japan, Tokyo). Immobilon Western Chemiluminescent horseradish peroxidase (HRP) substrate (Millipore) was used to visualize proteins.

Statistical Analysis The results are shown as the mean $\pm$ standard error of the mean (S.E.M.) Statistical analyses were performed with analysis of variance with Dunnett's test using the GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, U.S.A.). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of RJ and BGPP on Manifestation of Allergy in TDI-Model Rats Allergic symptoms such as itching, sneezing and watery rhinorrhea were induced by intranasal TDI application. Orally pre-administration of RJ and BGPP for 3 weeks reduced TDI-provoked sneezing and nasal score (Figs. 2A, B). After only ethyl acetate provocation control rats did not show any allergic-like symptoms.

Effect of RJ and BGPP on H1R mRNA Expression in the Nasal Mucosa of TDI-Sensitized Rats The up-regulation of H1R mRNA in rat nasal mucosa after 4 h of TDI provocation has been reported.²⁷⁾ Following these situations, treatment with RJ and BGPP minimized H1R mRNA level in the nasal mucosa of allergic model rats sensitized by TDI (Fig. 3A).

Effect of RJ and BGPP on Th2-Cytokines Gene Expression in the Nasal Mucosa of TDI-Sensitized Rats TDI-provocation in the allergic model rat nasal mucosa causes up-regulation of Th2-cytokines including IL-4, IL-5, and IL-9.^{8,15)} Oral administration of the RJ and BGPP tended to reduce the TDI-induced IL-4 mRNA upregulation (Fig. 3B). Identically, TDI provocation elevated the mRNA level of IL-5 and pre-treatment with RJ and BGPP tended to decrease its level (Fig. 3C). RJ and BGPP suppressed TDI-induced IL-9 mRNA

Fig. 2. Effect of Royal Jelly and Brazilian Green Propolis on TDI-Induced Nasal Symptoms

A, Rats were sensitized and provoked. Sneezing number were counted for 10min according to the Materials and Methods. In B, nasal score described in Table 1 was scored. RJ, royal jelly; BGPP, Brazilian green propolis. Data are expressed as means $\pm$ S.E.M. ## $p < 0.01$ vs. control; ** $p < 0.01$, * $p < 0.05$ vs. TDI (ANOVA with Dunnett's test, $n = 5$).

Fig. 3. Both Royal Jelly and Brazilian Green Propolis Suppressed the Expression of H1R and Th2 Genes in the Nasal Mucosa of TDI-Stimulated Rats

Following the Materials and Methods, rats were sensitized and provoked and sacrificed at 4h, and amount of H1R (A), IL-4 (B), IL-5 (C), and IL-9 (D) mRNA were determined using real time RT-PCR. RJ, royal jelly; BGPP, Brazilian green propolis. Data are expressed as means $\pm$ S.E.M.; ## $p < 0.01$ vs. control; ** $p < 0.01$, * $p < 0.05$ vs. TDI (ANOVA with Dunnett's test, $n = 5$).

elevation (Fig. 3D).

Effect of RJ and BGPP on H1R Gene Expression in HeLa Cells Stimulated by PMA or Histamine PMA or histamine induced an elevation of H1R mRNA expression in HeLa cells remarkably.³²⁾ RJ and BGPP pretreatment, dose-dependently suppressed PMA-induced (Figs. 4A, B) and histamine-induced (Figs. 4C, D) up-regulation of H1R gene expression in HeLa cells.

Effect of RJ and BGPP on Ionomycin-Induced IL-9 Expression in RBL-2H3 Cells Formerly, we showed that stimulation with ionomycin in RBL-2H3 cells remarkably up-regulated the NFAT signal dependent expression of IL-9 gene.¹⁰⁾ As RJ and BGPP suppressed TDI-induced up-regulation of IL-9 gene expression in the nasal mucosa of TDI-sensitized rats, the effect of RJ and BGPP on NFAT signal-mediated IL-9 gene expression in RBL-2H3 cells was examined. Iono-

Fig. 4. Effects of Royal Jelly and Brazilian Green Propolis on PMA- and Histamine-Induced Upregulation of H1R Expression in HeLa Cells

HeLa cells were serum-starved for 24 h at 37°C before treatment with 100 nM PMA (A and B) and 100 μM histamine (C and D) for 3 h. HeLa cells were preincubated with indicated concentrations of royal jelly (25 to 200 μg/mL; A and C) and Brazilian green propolis (25 to 200 μg/mL; B and D) for 12 and 3 h, respectively. In C and D, rottlerin, a PKCδ signal inhibitor (rott, 10 μM for 1 h) was used as a positive control. H1R mRNA expression was determined using real-time RT-PCR. his, histamine; RJ, royal jelly; BGPP, Brazilian green propolis. Data are expressed as means ± S.E.M.; ^{##}*p* < 0.01 vs. control; ^{**}*p* < 0.01, and ^{*}*p* < 0.05 vs. PMA- or histamine-treated cells (ANOVA with Dunnett's test, *n* = 3–4).

Fig. 5. Effects of Royal Jelly and Brazilian Green Propolis on Ionomycin-Induced Upregulation of IL-9 Expression in RBL-2H3 Cells

RBL-2H3 cells were preincubated with indicated concentrations of royal jelly (A) and Brazilian green propolis (B) for 12 and 3 h, respectively, before stimulation with 1 μM ionomycin. After a 2-h stimulation, the cells were harvested and total RNA was prepared. IL-9 mRNA expression was determined using real-time RT-PCR. IO, ionomycin; RJ, royal jelly; BGPP, Brazilian green propolis. Data are expressed as means ± S.E.M.; ^{##}*p* < 0.01 vs. control; ^{**}*p* < 0.01, and ^{*}*p* < 0.05 vs. ionomycin-treated cells (ANOVA with Dunnett's test, *n* = 3–4).

mycin stimulated IL-9 gene up-regulation was significantly reduced through RJ and BGPP treatment. (Figs. 5A, B).

Effect of RJ and BGPP on Phosphorylation of PKCδ at Tyr³¹¹ in HeLa Cells Phosphorylation and dephosphorylation of PKCs regulate their activities, stabilities, and functions, and Tyr³¹¹ phosphorylation initiates PKCδ.³³⁾ In HeLa cells acceleration by histamine or PMA increased PKCδ phosphorylation at Tyr³¹¹ has been reported.³¹⁾ It also been reported

that histamine or PMA stimulation caused PKCδ translocation to Golgi from the cytosol.³¹⁾ Therefore, we carried out the outcome of RJ and BGPP on PMA-induced Tyr³¹¹ phosphorylation of PKCδ. Pretreatment with RJ and BGPP suppressed PMA-induced phosphorylation (Figs. 6A, B).

Fig. 6. Effect of Royal Jelly and Brazilian Green Propolis on PMA-induced Tyr³¹¹ Phosphorylation of PKC δ

A, HeLa cells were serum-starved for 24 h and were then treated with royal jelly (RJ) and Brazilian green propolis (BGPP) (75 μ g/mL each) for 12 and 3 h, respectively, before stimulation with 100 nM PMA for 10 min. Total cell lysates were prepared, and phosphorylation of PKC δ at Tyr³¹¹ was determined using immunoblot analysis. Representative data from three separate experiments are shown. P-PKC δ , phospho-PKC δ ; T-PKC δ , total PKC δ . B, Quantification of Tyr³¹¹ phosphorylation of PKC δ . Densitometric analysis was performed using Image J software. Data are expressed as means $\pm$ S.E.M.; ## $p < 0.01$ vs. control; ** $p < 0.01$ vs. control (ANOVA with Dunnett's test, $n = 3$).

DISCUSSION

The global population growth has been accompanied by the increasing of allergic disease and the major challenge is to reduce the relative incidence of allergic diseases. The currently available treatments have some limitations regarding efficacy and safety. As the anti-allergic effect of antihistamines is not in satisfactory level, researchers are searching the synthetic as well as natural resources that inhibit histamine signaling. Our previous studies suggested the correlation between allergic symptoms severity and H1R gene expression level.⁹ Moreover, medicinal plants having H1R gene suppressive activity alleviated nasal symptoms in TDI-sensitized rats.^{30,34,35} In HeLa cells, PKC δ signaling was involved in PMA- or histamine-induced H1R up-regulation, and the activation of PKC δ leads to its translocation to Golgi from cytosol, where MEK and extracellular signal-regulated kinase (ERK) phosphorylated by PKC δ .³¹ Thus, in this study, we, first, focus upon the anti-allergic activity of RJ and BGPP mediated through the suppression of histamine signaling using TDI-sensitized rats.

Nasal allergy-like symptoms just after provocation with TDI represents the early phase reaction derived from the release of preformed histamine.²⁷ RJ and BGPP suppressed sneezing and nasal score (Fig. 2). Higher dose of BGPP also tended to decline the nasal symptoms, but not markedly suppressed. Extract could contain not only inhibitors but also activators of histamine signaling. Therefore, it is likely that in higher dose, the effect of activators may not become negligible. Our study demonstrated that both RJ- and BGPP-treated groups showed significant suppression on H1R gene up-regulation in TDI-sensitized rats. The H1R protein increased in the nasal mucosa of TDI-sensitized rats 24 h after TDI-provocation.³² So, it can be proposed that the anti-allergic activity of both RJ and BGPP may be mediated through the decrease in the amount of H1R protein in the nasal mucosa by suppressing its transcription.

There is ample of evidence of concurrence of cytokines and histamine release in which histamine influences the expression and activities of several cytokines.^{36–38} Previously, it was reported that antihistamines suppressed Th2 cytokine gene up-regulations in TDI-sensitized rats.⁹ We also exposed the correlation of H1R expression with those of IL-4 and IL-5 in TDI-sensitized rats and patients with pollinosis and showed the correlation between H1R and Th2 cytokines gene expres-

sions and H1R gene expression decreased Th2 cytokines production.^{9,39} Recently, we have demonstrated that NFAT signaling-mediated IL-9 gene is the additional AR-sensitive gene and suppression of both histamine and NFAT signaling remarkably improved nasal symptoms in allergy model rats.¹⁰ IL-9 is a pleiotropic cytokine produced by activated helper T cells and activated mast cells and it promotes Th2-specific allergic responses, allergic inflammation, and asthmatic symptoms.^{40,41} As IL-9 upregulates IL-4, IL-5, and IL-13, suppression of IL-9 expression affects the expression levels of these Th2 cytokines.⁴² RJ and BGPP inhibited TDI-induced up-regulation of IL-9 gene expression in TDI-sensitized rats and NFAT signal-mediated-IL-9 gene expression in ionomycin-stimulated RBL-2H3 cells. Data suggest that RJ and BGPP inhibited the expressions of H1R gene and IL-9 gene through the inhibition of PKC δ and NFAT signaling, respectively, and consistent with the report showing that nasal rubbing and sneezing were significantly inhibited after repeated administration of Brazilian propolis for 2 weeks, but its single administration caused no significant effect⁴³ although we have no data on direct comparison between single and repeated administration of RJ or BGPP in this study. As the effect of RJ and BGPP on the IL-4 and IL-5 gene up-regulations was not significant, it is likely that RJ and BGPP did not significantly affect the expression level of IL-4 and IL-5 although RJ and BGPP significantly suppressed allergic symptoms.

To unravel the mechanism of action for RJ and BGPP, we studied the impact of RJ and BGPP on PKC δ activation for H1R gene up-regulation in HeLa cells. Pretreatment with RJ and BGPP inhibited PKC δ phosphorylation at Tyr³¹¹ (Fig. 6A), resulting the prevention of PKC δ translocation to the Golgi (data not shown). It was reported that membrane recruiting of PKC δ was considered as the important factor for the phosphorylation of Tyr³¹¹ residue of PKC δ .⁴⁴ Accordingly, inhibition of the translocation of PKC δ to the Golgi from cytosol by RJ and BGPP is likely to be the most important underlying mechanism in their suppressive effect on the up-regulation of H1R gene expression.

In conclusion, data suggest that RJ and BGPP show anti-allergic activity by suppressing H1R mRNA up-regulation induced by TDI in the nasal mucosa of TDI-sensitized rats through the inhibition of histamine signaling pathway. The molecular basis of their suppressive effect involved in the

inhibition of PKC δ translocation to the Golgi that triggers the suppression of PKC δ activation. BGPP has suppressive effect on Th2-cytokine signaling which could influence the histamine-cytokine network. In addition to their suppressive effect on histamine signaling, IL-9 gene suppression by RJ and BGPP suggest that they could suppress NFAT-mediated IL-9 gene expression, the important signaling responsible for the development of AR. Collectively, our data recommend that both RJ and BGPP could be good therapeutics for AR.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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