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비만세포 매개 즉시형 과민반응에 대한 표고버섯 추출물의 보호 효과

연광해[1] · 최윤호[2*]
[1]중국 연변대학교 해부조직발생학교실, [2]전북대학교 의과대학 해부학교실

The Protective Effect of *Lentinus Edodes* on Mast Cell-Mediated Immediate-Type Hypersensitivity

Guanghai Yan[1] and Yun Ho Choi[2*]
[1]*Department of Anatomy, Histology and Embryology, Yanbian University Medical College, Yanji 133002, P.R. China*
[2]*Department of Anatomy, Medical School, Chonbuk National University, Jeonju, Jeonbuk 561-180, Korea*

Abstract – Mast cells are crucial as effector cells in the immediate-type allergic reaction. *Lentinus edodes* has been the popular edible mushroom in oriental countries and reported to have immunomodulatory, anti-tumor, anti-atherogenic, anti-viral, and anti-allergic activities. However, the roles of *L. edodes* in mast cell-mediated anaphylactic reaction have not been fully elucidated. In this research, we have demonstrated the effects of the methanol extract of *L. edodes* (MELE) on mast cell-mediated anaphylaxis-like and anaphylactic reactions. MELE suppressed systemic anaphylaxis-like reaction, plasma histamine levels, and ear swelling response in mice treated with compound 48/80. MELE also suppressed passive systemic and cutaneous anaphylaxis mediated by anti-dinitrophenyl IgE. In accordance with these findings, MELE dose-dependently decreased histamine release from RPMC evoked by compound 48/80 or the antigen-antibody reaction. To clarify the mechanism of degranulation system, intracellular cAMP levels as well as calcium influx in RPMC was evaluated. In compound 48/80-treated RPMC, MELE blocked calcium uptake into the cells. In addition, MELE elevated the intracellular cAMP content and significantly attenuated compound 48/80-induced cAMP reduction in RPMC. Taken together, we propose the clinical use of MELE in mast cell-mediated immediate-type allergic diseases.

Keywords – Mast cell, Compound 48/80, Histamine, Calcium, cAMP

Anaphylaxis, a life-threatening syndrome, represents the symptom complex such as breathing difficulties and hypotension or shock which affects multiple systems in the body. Especially, food anaphylaxis (allergy) is the leading single known cause of anaphylaxis treated in emergency departments in many countries. The prevalence of anaphylaxis in the general population of South Korea has been known to be at least 0.05-2% and probably higher. However, several studies show that there has been an elevation in its prevalence during the last five years.[1)] Although anaphylaxis occurs partly through immunoglobulin E (IgE)-independent mechanisms or direct mast cell activation, an overview of the mechanisms and triggers of anaphylaxis includes consideration of common IgE-dependent triggers, such as foods, stinging insect venoms, and medications. It is therefore important to understand “immediate” IgE-mediated hypersensitivity response manifested by an abrupt onset of symptoms within minutes to hours of exposure to causative agents.

Mast cells are known to be central effectors in the immediate-type allergic response. The secretory function of mast cells can be elicited by the aggregation of high-affinity receptor (FcεRI) for IgE and the corresponding antigen, which results in immediate release of various biological mediators, including histamine, leukotrienes, proteases, prostaglandins, and cytokines.[2)] Among the bioactive mediators released from mast cells, histamine is the most powerful vasoactive substance implicated in the acute phase reaction of immediate hypersensitivity.[3)]

*교신저자(E-mail) : why76@jbnu.ac.kr
(Tel) : +82-63-270-3082

Mast cell degranulation can be also triggered by the basic secretagogues such as the synthetic compound 48/80 and mastoparans. An appropriate dose of compound 48/80 has been considered as a direct and convenient tool to elucidate the mechanism of non-IgE-dependent anaphylaxis-like (anaphylactoid) responses.[4] As previously reported, compound 48/80 promotes the generation of superoxide anion by attenuating A-kinase through decreasing the intracellular cAMP concentration in mast cells. Superoxide anion produced by compound 48/80 results in the increase of intracellular calcium levels, which bring about histamine release from mast cells.[5]

Lentinus edodes is one of the most widely produced edible mushrooms in eastern Asian nations such as Korea, China, and Japan. It contains several constituents that have a wide range of beneficial actions, including immunopotentiation, liver protection, cholesterol and blood pressure reduction, antiatherogenic, and anti-viral activities.[6-8] Several investigations into its immunomodulatory and anti-allergic activities have been performed. Lentinan, a (1-3) β-D-glucan isolated from this mushroom, has been shown to act as an immunomodulatory and anti-tumor drug.[9]

Previously, we have revealed that *L. edodes* represses allergic airway inflammation in a murine model of asthma.[10] Moreover, *L. edodes* extract significantly reduced the severity of the typical histopathological phenomena associated with atopic dermatitis (AD) and the number of mast cells infiltrating the skin lesions of AD mice, suggesting that *L. edodes* may directly inactivate mast cells associated with AD.[11] Furthermore, Lee et al. have reported that several mushrooms extracts including the water extract from *L. edodes* exert the inhibitory capacity on the IgE-dependent degranulation in rat basophilic leukemia cell line (RBL-2H3 cell).[12] Concretely, the aqueous extract of *L. edodes* significantly showed the suppressive effect on β-hexosaminidase release from RBL-2H3 cells as 23%. Meanwhile, *L. edodes* is known to have the ability to break down plant materials into smaller, more digestible and bioactive compounds. The mycelia extract of *L. edodes*-cultured Glycyrrhiza radix has been demonstrated to have anticancer and anti-allergic activities.[13] Similarly, it has also been shown that elm tree (*Ulmus parvifolia*) bark bioprocessed with mycelia of *L. edodes* has the potential to prevent and/or treat allergic asthma in mice.[14]

Despite these results, the suppressive effects of *L. edodes* on IgE-dependent/independent activation of rat peritoneal mast cells (RPMC) and anaphylaxis *in vivo* have not yet been identified. Earlier report dwells on illuminating the *in vitro* inhibitory capacity of *L. edodes* on release of histamine or β-hexosaminidase from RBL-2H3 cells. Although RBL-2H3 cells may be useful as a model for IgE-mediated degranulation, they may share similarities with basophils rather than with other histamine-releasing cell types.[15] Therefore, our study has attempted to evaluate the protective effect of *L. edodes* against immunologic or non-immunologic activation of murine mast cells *in vitro and in vivo*, thus indicating that *L. edodes* may prevent the mast cell-mediated anaphylaxis-like and anaphylactic reactions. To the best of our knowledge, the present work is the first to establish the *in vitro* and *in vivo* anti-anaphylactic activities of *L. edodes* using laboratory animals such as rats and mice.

Materials and Methods

Materials and Reagents – 4-(2-Hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) , disodium cromoglycate (DSCG), compound 48/80, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), and bovine serum albumin (BSA) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Percoll stock solution was obtained from Pharmacia (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Laboratory Animals – Male ICR mice (6-week-old, 25-30 g) and Sprague-Dawley rats (8-week-old, 230-280 g) were obtained from Central Lab. Animal Inc. (Daejeon, Korea). Animals were housed and maintained 4~6 per cages under laminar air-flow cage at 23±1°C and relative humidity of 52±10% during the study. Animal Research Committee of Chonbuk National University approved the animal study in according to the guidelines of the National Institutes of Health (NIH publication #85-23, 1985).

Preparation of the Methanol Extract of *L. edodes* (MELE) – The dried fruiting bodies of *L. edodes* used in this study were purchased from Jangsu Oriental Pharmacy (Jeonbuk, Korea). A voucher specimen (number 2011-LEMw705025) was deposited in its herbarium of Research Center, Medical School, Chonbuk National University. The air-dried fruiting bodies (120 g) were immersed in 600 mL of 70% methanol, followed by keeping overnight in a refrigerator (4°C), and boiled under reflux for 2 h. The methanol extraction was performed twice. The resultant

extract was filtered through a 0.45-μm filter and concentrated to about 120 mL under reduced pressure. The concentrated extract was finally lyophilized, yielded 24.8 g dried powder and kept at 4 °C until use. The dried extract of fruiting bodies was diluted in saline or HEPES-Tyrode buffer (5.4 mM HEPES, 136 mM NaCl, 11 mM $NaHCO_3$, 5 mM KCl, 2 mM $CaCl_2$, 2.75 mM $MgCl_2$, 0.6 mM NaH_2PO_4, 1.0 mg/mL glucose, 1.0 mg/mL BSA, 0.1 mg/mL heparin, pH 7.4) prior to usage.

Systemic Anaphylaxis-like Reaction Evoked by Compound 48/80 – Mice (n=10/group) were intraperitoneally given compound 48/80 [8 mg/kg body weight (BW)] or sham saline injection as previously reported.[3] MELE or DSCG (0.01~1 g/kg BW) was diluted with saline and ingested orally 1 h before the intraperitoneal challenge with compound 48/80. As a positive control drug, DSCG was administered. Mortality was estimated for 30 min after the induction of anaphylactic shock. After the mortality evaluation, the blood was acquired from the heart of each mouse.

Determination of Plasma Histamine Content – The blood sample was drawn and centrifuged at 150×*g* for 12 min at 4°C. The plasma was withdrawn and the histamine level was calculated by the radioenzymatic method as previously mentioned.[16]

Ear Edema Evoked by Compound 48/80 – Mouse ear edema could be evaluated using the basic secretagogue compound 48/80.[17] Compound 48/80 was freshly diluted with saline (5 mg/mL) and administered intradermally in the anterior aspect of the left ear of each mouse (100 μg/site, 20 μL) using a 30-gauge fine-needle. Sham saline was administered intradermally in the anterior aspect of the right ear of each mouse. The thickness of the ears in all animals was measured using a dial thickness micrometer gauge (Mitutoyo, No. 7326, Japan) under ether anesthesia. Mice remained immobile during the measurement. Ear swelling response, which represents an increment of thickness above baseline, was assessed 1 h after the treatment of compound 48/80 or vehicle (saline). MELE (0.01~1 g/kg BW) was orally ingested 1 h before the intradermal challenge with compound 48/80.

Passive Systemic Anaphylaxis (PSA) Mediated by IgE – PSA mediated by anti-DNP IgE was evaluated according to our modified protocol.[18] Mice (*n*=10/group) were intravenously administered with 3 μg anti-DNP IgE or phosphate-buffered saline (PBS). Twenty-four hours later, mice were treated with intravenously injection of 500 μg of DNP-HSA. At least 1.5 min later, mice were killed by cervical dislocation and blood samples were then collected by cardiac puncture. Plasma was isolated from blood samples and plasma histamine concentration was measured by the radioenzymatic method. Oral administration of MELE (0.01~1 g/kg BW) was carried about 1 h before antigenic challenge.

Passive Cutaneous Anaphylaxis (PCA) Mediated by IgE – Anti-DNP IgE-mediated PCA was examined as previously reported.[19] Male Sprague-Dawley rats were sensitized in the left back skin by intradermal inoculation of 400 ng anti-DNP IgE in 20 μL PBS and were administered a sham PBS injection in the right back skin. Approximately 200 μL of PBS containing 100 μg DNP-HSA with 2% Evans blue was injected into the penile vein of rats after 48 hours later. MELE was orally administered 1 h before the challenge. Thirty minutes after the challenge, the rats were killed, tissue specimens around the intradermally injected site severed and weighed, followed by extraction of extravasated Evans blue dye by incubation of biopsy specimens in 1 mL formamide at 55°C for 24 h and measurement of absorbance at 620 nm. The concentration of dye in the extracts was calculated from a standard curve of dye content in the 0.01 to 30 μg/mL range.

Isolation and Purification of Rat Peritoneal Mast Cells (RPMC) – To obtain RPMC, anesthetized rats received intraperitoneal injection of 10 mL of HEPES-Tyrode buffer not containing calcium and the rat's abdomen was carefully massaged for about 1 min 30 s. The incision into the peritoneal cavity was left completely open and the fluid was acquired using a sterile Pasteur pipette, and then highly purified and viable RPMC can be isolated through Percoll purification as described previously.[20] RPMC preparations were approximately 98% pure as evaluated by toluidine blue staining and about 97% of these cells were viable as observed by trypan blue exclusion. Purified RPMC (1×10^6 cells/mL) were subsequently resuspended in HEPES-Tyrode buffer.

RPMC Viability Test – To analyze the viability of RPMC, the tetrazolium (MTT) based colorimetric assay was conducted as defined in detail elsewhere.[3] RPMC (1×10^6 cells/well) were pretreated with variable MELE concentrations (0.01~1 mg/mL) for 4 h time intervals. Afterwards, 10 μL of MTT were added in each well and incubated for additional 1 h, with a final MTT concentration into each well-plate of 0.5 mg/mL. Living cells reduce the yellow

MTT to the purple formazan crystals. After removing the supernatant fraction, cells were incubated with 100 μL of dimethylsulfoxide (Sigma, USA) while shaking for 5–10 min. This method measures the MTT reduction in mitochondria of living cells, which results in intensity of the colored product as quantified by a spectrophotometer at 550 nm. The intensity of the colored product formed is positively proportionate to the number of intact cells present in a sample. The absorbance of the untreated group (control) was taken as 100% viability, thus the values of treated groups were calculated as a percentage compared to control.

Assay of Histamine Release – Purified RPMC suspensions (2×10^5 cells in 200 μL) were preincubated with MELE (0.01~1 mg/mL) or DSCG at 37°C for 10 min followed by stimulation with compound 48/80 (0.25 μg/mL) for 20 min. To elucidate the effect of MELE on IgE-mediated mast cell activation, RPMC were also treated with anti-DNP IgE (10 μg/mL) for 6 h and preincubated with MELE or DSCG at 37°C for 10 min before antigenic challenge with DNP-HSA (100 ng/mL). The samples were centrifuged at 150×*g* for 12 min at 4°C, and then the content of histamine in the supernatant was estimated by the radioenzymatic method. The inhibition percentage of the histamine secretion was defined by the following formula: % Inhibition = [(histamine release without MELE – histamine release with MELE) / histamine release without MELE] × 100.

Determination of ^{45}Ca Uptake into RPMC – The amount of calcium uptake into RPMC was analyzed according to the protocol suggested elsewhere.[21] Percoll-purified RPMC were resuspended in modified HEPES-Tyrode buffer containing ^{45}Ca (1.5 mCi/mL; 1 Ci = 3.7×10^{10} becquerels; PerkinElmer Life Sciences, MA, USA). RPMC suspensions were preincubated with MELE (0.01~1 mg/mL) at 37°C for 10 min followed by stimulation with compound 48/80 (0.25 μg/mL) for 20 min. The reaction was stopped by adding 1 mM lanthanum chloride. Following centrifugation 3 times at 150×*g* for 12 min at 4°C, and then RPMC were treated with the detergent 10% Triton X-100 followed by vigorous shaking. Radioactivity of the solution was calculated in a scintillation β-counter (Liquid Scintillation Analyzer, A Canberra Company, Australia).

Measurement of cAMP Level – The level of cyclic adenosine-3', 5' monophosphate (cAMP) was measured by the protocol reported previously.[22] In brief, the cell suspension was mixed to an equivalent content (200 μL) of prewarmed buffer containing variable MELE concentrations (0.01~1 mg/mL) in a sample tube. The reaction was allowed to proceed for discrete time intervals, stopped by centrifugation at 150×*g* for 12 min at 4°C, and then each sample was added to 250 μL of sodium acetate buffer (50 mM, pH 6.2) followed by vigorous vortexing. Each mixed sample was immediately snap frozen in liquid nitrogen. The frozen samples were thawed and vortexed, and then the debris were sedimented by a centrifugation at 1200×*g* for 12 min at 4°C. The cAMP content in the supernatant was measured by radioimmunoassay employing a Rianen assay system (PerkinElmer Life Sciences, MA, USA).

Statistical Analysis – All quantitative data presented are mean ± standard error of the mean (S.E.M.). A statistical assessment for data was in accordance with the ANOVA followed by Dunnett's test; The threshold for defining statistical significance is $p < 0.05$.

Results

Effect of MELE on Systemic Anaphylaxis-like Reaction Evoked by Compound 48/80 – To investigate the effect of MELE in anaphylaxis-like reaction, we used an *in vivo* murine model of systemic anaphylaxis-like reaction evoked by compound 48/80. Following the intraperitoneal administration of compound 48/80 (8 mg/kg BW) into mice, a mortality rate was measured for 30 min. As revealed in Table I,

Table I. Inhibitory effect of the methanol extract of *L. edodes* (MELE) on compound 48/80-stimulated systemic anaphylaxis-like reaction in mice

Treatment (g/kg BW)		Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)		+	100
MELE	0.01	+	70
	0.1	+	40
	1	+	20
	1	–	0
DSCG	0.01	+	80
	0.1	+	60
	1	+	20

Groups of mice (n=10/group) were orally ingested with 300 μL of saline or drugs (MELE or DSCG) 1 h before the challenge of compound 48/80. The intraperitoneal administration of compound 48/80 solution was performed in the groups of mice. Mortality (%) within 30 min following compound 48/80 injection was denoted as the number of dead mice × 100/total number of experimental mice.

the administration of compound 48/80 causes 100% death of mice. Oral administration of MELE (0.01~1 g/kg BW) dose-dependently attenuated compound 48/80-stimulated mortality. Likewise, DSCG (positive control drug) also suppressed compound 48/80-stimulated mortality in a dose-dependent manner.

Effect of MELE on Plasma Histamine Concentrations – After mortality test, the effect of MELE on the quantity of histamine released into plasma was also investigated. As mentioned above, variable concentration (0.01~1 g/kg BW) of MELE was ingested 1 h before compound 48/80 injection. The inhibition rate of plasma histamine level due to treatment with MELE was significant at doses of 0.01-1.0 g/kg BW (Fig. 1).

Effect of MELE on Ear Edema Evoked by Compound 48/80 – Ear edema (swelling) was caused by stimulation with compound 48/80 (100 μg/site) as described (Choi *et al*., 2006). Orally ingested MELE dose-dependently blocked the ear swelling response evoked by compound 48/80 (Table II). In agreement with plasma histamine levels of anaphylactoid mice, the inhibition rate due to treatment with MELE was significant at all dose levels.

Effect of MELE on anti-DNP IgE-mediated PSA – To confirm the ameliorative effect of MELE on IgE-mediated anaphylaxis *in vivo*, we also used a PSA model. PSA can be induced in mice by an immunological pathway that depends on IgE-mediated mast cell activation and release of histamine. Thus, study on PSA was done with quantitative histamine determination on plasma. As expected, oral administration of MELE dose-dependently mitigated anti-DNP IgE-mediated systemic reactions in experimental mice (Fig. 2).

Table II. Inhibitory effect of the methanol extract of *L. edodes* (MELE) on compound 48/80-stimulated ear edema in mice

MELE (g/kg BW)	Compound 48/80 (100 μg/site)	Increment in ear thickness (×100 μm)	Inhibition (%)
0	+	1.443 ± 0.264	
0.01	+	0.933 ± 0.210	35.30*
0.1	+	0.699 ± 0.160	51.58*
1	+	0.651 ± 0.138	54.89*
1	–	0.465 ± 0.127	

Groups of mice (n=10/group) were orally ingested with 300 μL of saline or MELE 1 h before compound 48/80 challenge. Twenty microliters of compound 48/80 (100 μg/site) were administered to the ears of mice. Each value denotes the mean ± S.E.M. of three independent experiments. Inhibition (%) = [(increment in ear thickness without MELE - increment in ear thickness with MELE)/increment in ear thickness without MELE] × 100. *p<0.05 (significantly different from the saline value).

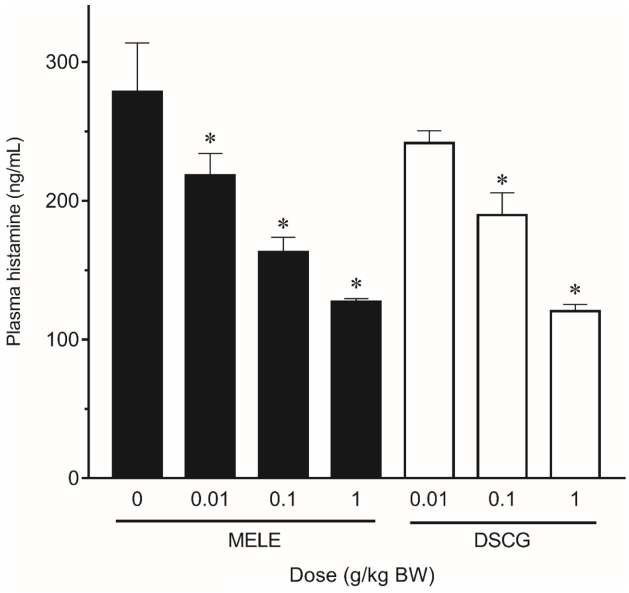

Fig. 1. Effect of the methanol extract of *L. edodes* (MELE) on compound 48/80-stimulated plasma histamine levels in mice. Groups of mice (n=10/group) were orally ingested with 300 μL of saline or drugs (MELE or DSCG) 1 h before the challenge of compound 48/80. The intraperitoneal administration of compound 48/80 solution was performed in the groups of mice. Each bar denotes the mean ± S.E.M. of three independent experiments. *p<0.05 (significantly different from the saline value).

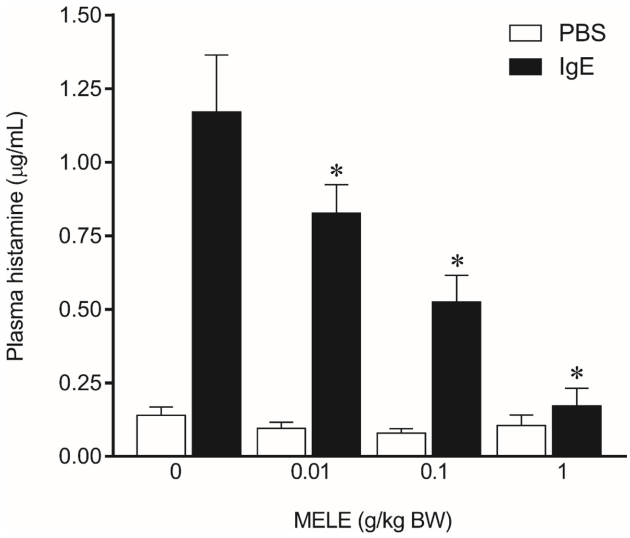

Fig. 2. Effect of the methanol extract of *L. edodes* (MELE) on anti-DNP IgE-mediated passive systemic anaphylaxis in mice. MELE was orally ingested 1 h prior to the challenge with antigen. Each bar denotes the mean ± S.E.M. of three independent experiments. * p<0.05 (significantly different from the saline value).

Effect of MELE on anti-DNP IgE-mediated PCA – Similar to ear swelling response evoked by compound 48/80, local extravasation could also be elicited by a local allergic reaction between anti-DNP IgE and DNP-HSA. After 48 hours, as revealed by extravasation of Evans blue, the PCA reaction was significantly reduced by 0.01 to 1 g/kg BW of MELE (Fig. 3).

Effect of MELE on Mast Cell Viability – MTT colorimetric test was used to quantify the viability of RPMC exposed to MELE. The viable cells were approximately 100% after treatment to variable concentrations (0.01~1 mg/mL) of MELE for 24 h. Thus, MELE had no cytotoxicity on RPMC (data not shown).

Effect of MELE on Histamine Release from RPMC – In consideration of the inhibitory capacity of MELE on anaphylaxis-like reactions *in vivo*, we examined the effect of MELE on release of preformed mediators from mast cells. The histamine secretion from compound 48/80-stimulated RPMC was remarkably reduced in a dose-dependent manner of MELE (Fig. 4). DSCG also showed a significant inhibition rate. In addition, the inhibitory effect of MELE on IgE-mediated histamine release from RPMC was shown in Fig. 5. MELE dose-dependently suppressed IgE-mediated histamine secretion from RPMC. These results suggest that MELE contains the active compounds, which mitigate compound 48/80-induced or IgE-mediated anaphylaxis-like responses by blocking histamine secretion from RPMC.

Effect of MELE on Calcium Uptake into RPMC – To date, it is well recognized that the augmentation in calcium uptake contributes to the elevation of intracellular calcium levels, which promotes histamine liberation.[23] Therefore, we measured calcium uptake. Treatment with MELE repre-

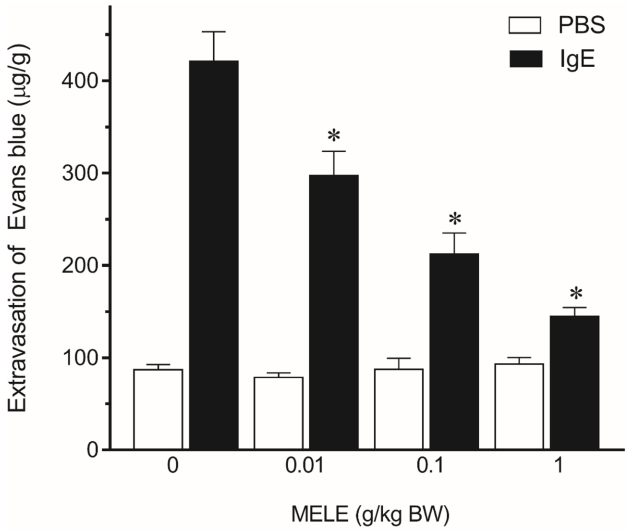

Fig. 3. Effect of the methanol extract of *L. edodes* (MELE) on anti-DNP IgE-mediated passive cutaneous anaphylaxis in rats. MELE was orally ingested 1 h prior to the antigenic challenge. Each bar denotes the mean ± S.E.M. of three independent experiments. *p<0.05 (significantly different from the saline value).

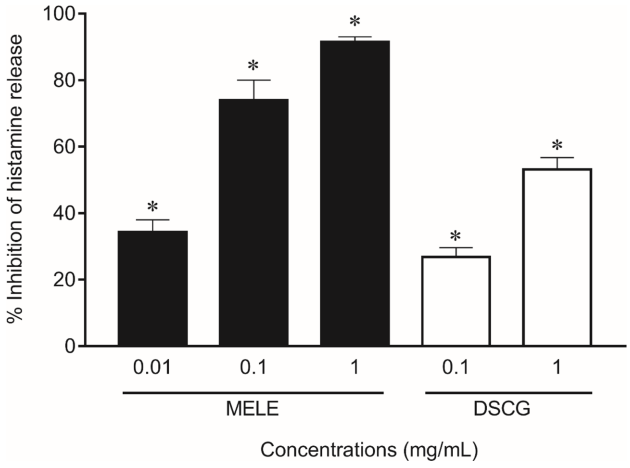

Fig. 4. Inhibitory effect of the methanol extract of *L. edodes* (MELE) on compound 48/80-stimulated histamine release from rat peritoneal mast cells (RPMC). RPMC were pretreated with MELE at 37°C for 10 min prior to the stimulation with compound 48/80. Each bar denotes the mean ± S.E.M. of three independent experiments. *p<0.05 (significantly different from the saline value).

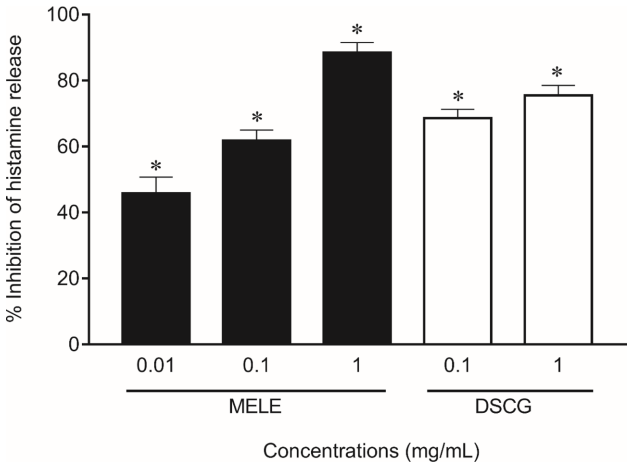

Fig. 5. Inhibitory effect of the methanol extract of *L. edodes* (MELE) on anti-DNP IgE-mediated histamine release from rat peritoneal mast cells (RPMC). RPMC were pretreated with MELE at 37°C for 10 min prior to the challenge with DNP-HSA. Each bar denotes the mean ± S.E.M. of three independent experiments. *p<0.05 (significantly different from the saline value).

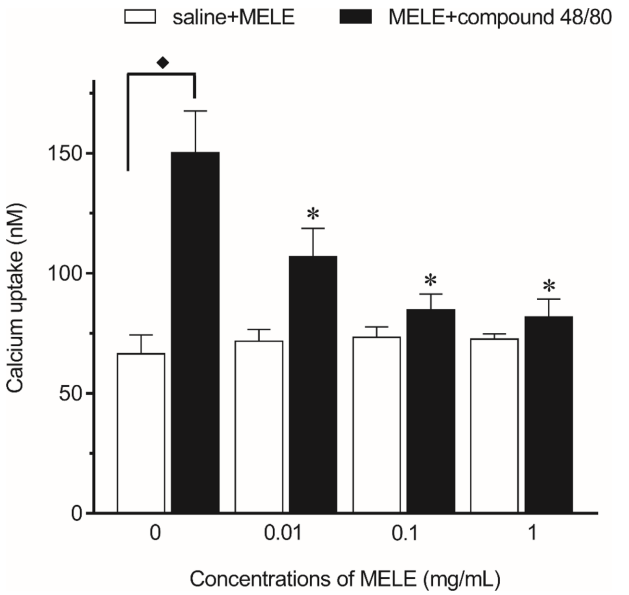

Fig. 6. Inhibitory effect of the methanol extract of *L. edodes* (MELE) on compound 48/80-stimulated calcium uptake in rat peritoneal mast cells (RPMC). RPMC were pretreated with MELE at 37°C for 10 min prior to the stimulation with compound 48/80. Each bar denotes the mean ± S.E.M. of three independent experiments. ♦$p<0.05$ (when compared between the saline and saline plus compound 48/80 value); *$p<0.05$ (significantly different from the saline plus compound 48/80 value).

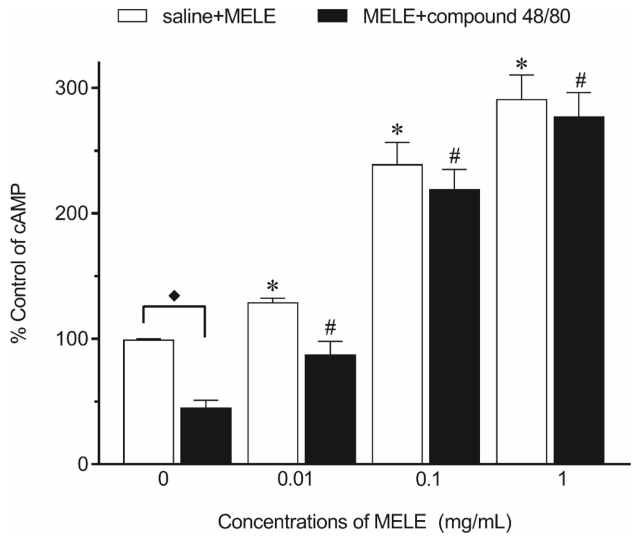

Fig. 7. Inhibitory effect of the methanol extract of *L. edodes* (MELE) on compound 48/80-stimulated decrease of cAMP level in rat peritoneal mast cells (RPMC). Various concentrations of MELE were added into the RPMC suspension for 10 min, and cAMP levels were estimated. Each bar denotes the mean ± S.E.M. of three independent experiments. ♦$p<0.05$ (when compared between the saline and saline plus compound 48/80 value); *$p<0.05$ (significantly different from the saline value); #$p<0.05$ (significantly different from the saline plus compound 48/80 value).

sented no change in calcium uptake. However, calcium uptake was greatly enhanced by the challenge of RPMC with compound 48/80. The compound 48/80-stimulated calcium uptake was hindered in a concentration-dependent manner of MELE (Fig. 6). From these results, it is inferred that MELE may reduce histamine liberation through blocking calcium influx into RPMC.

Effect of MELE on cAMP Level of RPMC – To examine whether MELE exhibits any effects on the compound 48/80-induced decline of cAMP in RPMC, we also assessed the intracellular cAMP level. Treatment of MELE increased the cAMP level in a concentration-dependent manner. Treatment of RPMC with compound 48/80 represented a significant decrease in the cAMP level as compared with those treated with buffer only. Pretreatment with MELE not only inhibited the compound 48/80-induced decline of cAMP in RPMC but also elevated the cAMP level over those treated with buffer only (Fig. 7). These data imply that MELE mitigates histamine liberation by increasing the cAMP level in RPMC.

Discussion

The results of this study demonstrate that MELE has anti-anaphylactic and anti-allergic properties. The murine models of anaphylaxis-like reaction were developed to assess the contribution of MELE in mast cell-dependent anaphylactoid reactions. MELE significantly mitigated systemic anaphylactoid reaction, plasma histamine level, and ear swelling response *in vivo* in mice treated with compound 48/80. To further investigate the mechanism by which MELE inhibits the anaphylaxis-like reaction, an *in vitro* study has been performed. The inhibitory effect of MELE on compound 48/80-stimulated histamine release from RPMC was concentration-dependently significant. The liberation of histamine and other inflammatory mediators from mast cells is a prominent feature of acute inflammatory response such as the immediate-type anaphylaxis.[24] Therefore, our findings suggest that MELE inhibits mast cell-mediated anaphylaxis-like reaction by blocking histamine release from RPMC.

PSA and PCA represent *in vivo* models of acute allergic

reactions in which IgE-mediated mast cell activation appears to be a key modulatory element.[25] In these models, IgE of defined allergen specificity is injected into the caudal vein or skin, and 48 h thereafter, the specific antigen is administered intravenously. The ensuing FcεRI aggregation of mast cells results in the secretion of multiple preformed mediators. These products in turn produce the systemic effects such as cardiopulmonary changes and death as well as the local effects including increased cutaneous blood flow, enhanced vascular permeability leading to tissue swelling and itching due to the stimulation of cutaneous sensory nerves by histamine. Our result has revealed that physiological responses associated with PSA and PCA are less severe in MELE-treated mice. In view of the inhibitory effect of MELE on these reactions *in vivo*, we examined the effect of MELE on RPMC triggered by IgE. MELE substantially inhibited antigen-induced histamine release from RPMC, which is in agreement with *in vivo* effect. Although different types of cells were used in the different experimental condition, it has been shown that the water extract from *L. edodes* (WELE) also exhibits significant inhibitory potency on IgE-mediated β-hexosaminidase production from RBL-2H3 cells at 10 μg/mL.[12] However, to compare the efficacy of MELE with WELE, it is considered that experiments should be carried out using the same types of cells and parameters. Accordingly, it is reasonable to presume that MELE attenuates IgE-mediated anaphylaxis by down-regulating mast cell activation. Future study will be necessary to elucidate the detailed mechanism of MELE inhibition in FcεRI-induced degranulation of mast cells.

cAMP and calcium-related pathways are critical to the mast cell activation. As previously reported, agents that augment intracellular cAMP level have been shown to lessen histamine secretion from mast cells.[26,27] Several data have revealed an algorithm between cAMP and calcium influx. In compound 48/80-stimulated RPMC, cAMP-elevating agents decrease the generation of superoxide anion *via* cAMP-dependent protein phosphorylation.[4] Decreased superoxide anion as well as increased cAMP reduces calcium release from the endoplasmic reticulum (ER).[5,28] Accordingly, calcium-filling state in the ER blocks calcium uptake into RPMC, which brings about the decline of intracellular calcium content.[29] Consequently, decreased intracellular calcium levels prevent histamine release from RPMC.[23] Interestingly, treatment of MELE increased cAMP level beyond the baseline value. Although the mechanism of MELE-elicited cAMP production has not fully been clarified, MELE may activate the adenylate cyclase directly or indirectly, otherwise inactivate cAMP phosphodiesterase. Moreover, MELE dose-dependently prevented the compound 48/80-induced cAMP decline and calcium uptake of RPMC. Taken together, the mechanism of inhibitory action of MELE on histamine liberation from compound 48/80-stimulated RPMC may be due to the elevation of intracellular cAMP. Subsequently, it is plausible that MELE-induced cAMP elevation mitigates calcium influx into RPMC *via* a cascade of intracellular events described above and then decreased intracellular calcium content prevents histamine liberation from RPMC.

This research has a few limitations, however, probably originating from the functional compartments in RPMC for cAMP and calcium uptake on histamine release.[30] Unlike other mast cells, RPMC have been known not to require the calcium influx into the cells for anaphylactic histamine release. Presumably, the histamine release with compound 48/80 does not require calcium influx, which may have been a secondary reaction in this experiment. Additionally, it has been demonstrated earlier that the histamine liberation from RPMC is not diminished at all by the elevation of cAMP, such as the augmentation of cAMP through β stimulation by isoproterenol.[31] Therefore, the possibility that the elevation of cAMP by MELE may not inhibit histamine liberation from RPMC cannot be excluded. Taken together, future work will be required to determine the detail relationship among histamine liberation, calcium uptake, and cAMP in RPMC.

Our results obtained in this study have proved that MELE inhibits both compound 48/80-stimulated anaphylaxis-like reaction and IgE-mediated anaphylactic reaction *in vivo* and *in vitro* in a murine mast cell model. However, some previous reports have introduced that the consumption of raw or undercooked *L. edodes* could be the cause of allergic disorders such as food allergy, allergic contact dermatitis, and hypersensitivity pneumonitis.[32] Similarly, moderate allergenicity was observed to extracts of *L. edodes* hyphae in a guinea pig maximization test.[33] These results seem to contradict directly what we have demonstrated. In view of that overall adverse reaction may occur after ingestion of uncooked *L. edodes* in certain people, allergy-eliciting substance is likely to be temperature-sensitive. Interestingly, a thermally induced conformation transition of lentinan has been demonstrated previously.[34] After its treatment for can-

cer, some patients represented cutaneous side-effects similar to dermatitis induced by *L. edodes*. Thus, although not directly proven, lentinan has been implicated as the causative agent for allergic reaction induced by *L. edodes*.[35] Therefore, allergy-inducing and anti-allergic effects of *L. edodes* should be further investigated in the subsequent study.

As we used the whole methanol extract from the fruiting body of *L. edodes*, not a purified component in the present study, the active components that are largely responsible for the biological property are not clear at this time. According to the previous study regarding the chemical characteristics of *L. edodes* ethanolic extract, polyphenols, flavonoids, β-carotene, and lycopene were determined to be the major phytochemical component in the extract.[11] These phytochemicals are known to modulate allergic response due to their antioxidant activity and anti-inflammatory action.[36] Although the different extraction method has been employed in our study, MELE is also likely to contain the above-mentioned phytochemicals, suggesting that phytochemicals might be active components attributed to the anti-anaphylactic activity of *L. edodes*. Given this, the additional effort to identify active ingredients from MELE should be performed in subsequent work. Furthermore, future studies will be required to elucidate the possibility that MELE may also be effective in the human mast cells and in the treatment of human allergic disorders such as asthma and allergic rhinitis.

In conclusions, our present data indicate that *L. edodes* extract may be an effective tool in the prevention or treatment of mast cell-mediated immediate-type allergic diseases.

Conclusion

MELE suppressed not only anaphylaxis-like reaction and ear swelling response evoked by compound 48/80 but also IgE-mediated PSA and PCA reaction in the *in vivo* murine model. Moreover, MELE significantly attenuated compound 48/80-stimulated or IgE-mediated histamine release from RPMC, which is in agreement with *in vivo* effect. As expected from inhibition of degranulation by MELE, MELE dose-dependently prevented the compound 48/80-induced calcium uptake and cAMP reduction of RPMC. Taken together, the inhibition of histamine release by MELE appears to be mediated partly through the suppression of calcium uptake as well as the augmentation of cAMP levels. *L. edodes* may therefore be suitable for preventing and treating mast cell-mediated anaphylactic disorders.

References

1. Jin, H. J. (2016) Anaphylaxis: diagnosis, management, and current barriers. *Allergy Asthma Respir. Dis.* **4**: 79-81.
2. Fukao, T., Yamada, T., Tanabe, M., Terauchi, Y., Ota, T., Takayama, T., Asano, T., Takeuchi, T., Kadowaki, T., Hata Ji, J. and Koyasu, S. (2002) Selective loss of gastrointestinal mast cells and impaired immunity in PI3K-deficient mice. *Nat. Immunol.* **3**: 295-304.
3. Choi, Y. H., Yan, G. H., Chai, O. H., Lim, J. M., Sung, S. Y., Zhang, X., Kim, J. H., Choi, S. H., Lee, M. S., Han, E. H., Kim, H. T. and Song, C. H. (2006) Inhibition of anaphylaxis-like reaction and mast cell activation by water extract from the fruiting body of *Phellinus linteus*. *Biol. Pharm. Bull.* **29**: 1360-1365.
4. Fukuishi, N., Sakaguchi, M., Matsuura, S., Nakagawa, C., Akagi, R. and Akagi, M. (1997) The mechanisms of compound 48/80-induced superoxide generation mediated by A-kinase in rat peritoneal mast cells. *Biochem. Mol. Med.* **61**: 107-113.
5. Akagi, M., Katakuse, Y., Fukuishi, N., Kan, T. and Akagi, R. (1994) Superoxide anion-induced histamine release from rat peritoneal mast cells. *Biol. Pharm. Bull.* **17**: 732-734.
6. Suzuki, H., Okubo, A., Yamazaki, S., Suzuki, K., Mitsuya, H. and Toda, S. (1989) Inhibition of the infectivity and cytopathic effect of human immunodeficiency virus by water-soluble lignin in an extract of the culture medium of *Lentinus edodes* mycelia (LEM). *Biochem. Biophys. Res. Commun.* **160**: 367-373.
7. Wojtas, M., Bienkowski, T., Tateyama, S., Sagami, H., Chojnacki, T., Danikiewicz, W. and Swiezewska, E. (2004) Polyisoprenoid alcohols from the mushroom *Lentinus edodes*. *Chem. Phys. Lipids* **130**: 109-115.
8. Yamada, T., Oinuma, T., Niihashi, M., Mitsumata, M., Fujioka, T., Hasegawa, K., Nagaoka, H. and Itakura, H. (2002) Effects of *Lentinus edodes* mycelia on dietary-induced atherosclerotic involvement in rabbit aorta. *J. Atheroscler. Thromb.* **9**: 149-156.
9. Wang, X. E., Wang, Y. H., Zhou, Q., Peng, M., Zhang, J., Chen, M., Ma, L. J. and Xie, G. M. (2018) Immunomodulatory effect of lentinan on aberrant t subsets and cytokines profile in non-small cell lung cancer patients. *Pathol. Oncol. Res*. Nov 20. [Epub ahead of print]
10. Yan, G. H. and Choi, Y. H. (2014) *Lentinus edodes* suppresses allergen-induced airway hyperresponsiveness and inflammation by downregulating nuclear factor-kappa B activity in a

murine model of allergic asthma. *Korean J. Phys. Anthropol.* **27**: 79-90.
11. Choi, E. J., Park, Z. Y. and Kim, E. K. (2016) Chemical composition and inhibitory effect of *Lentinula edodes* ethanolic extract on experimentally induced atopic dermatitis *in vitro* and *in vivo*. *Molecules* **21**.
12. Lee, S. E., Noh, H. J., Choi, J., Kim, G. S., Lee, D. Y. and Kim, S. Y. (2014) Study on the anti-allergy activity of mushrooms in IgE-sensitized RBL-2H3 cells. *J. Mushrooms* **12**: 324-329.
13. Bae, M. J. and Ye, E. J. (2007) Effect of mycelia extracts from *Lentinus edodes* mushroom-cultured *Lonicera japonica* thunberg on anticancer and antiallergy activities. *J. Korean Soc. Food Sci. Nutr.* **36**: 424-430.
14. Kim, S. P., Lee, S. J., Nam, S. H. and Friedman, M. (2016) Elm tree (*Ulmus parvifolia*) bark bioprocessed with mycelia of shiitake (*Lentinus edodes*) mushrooms in liquid culture: composition and mechanism of protection against allergic asthma in mice. *J. Agric. Food Chem.* **64**: 773-784.
15. Passante, E., Ehrhardt, C., Sheridan, H. and Frankish, N. (2009) RBL-2H3 cells are an imprecise model for mast cell mediator release. *Inflamm. Res.* **58**: 611-618.
16. Harvima, R. J., Harvima, I. T. and Fraki, J. E. (1988) Optimization of histamine radio enzyme assay with purified histamine-N-methyltransferase. *Clin. Chim. Acta* **171**: 247-256.
17. Wang, J. P., Raung, S. L., Chen, C. C., Kuo, J. S. and Teng, C. M. (1993) The inhibitory effect of magnolol on cutaneous permeability in mice is probably mediated by a nonselective vascular hyporeactivity to mediators. *Naunyn Schmiedebergs Arch. Pharmacol.* **348**: 663-669.
18. Choi, Y. H. and Yan, G. H. (2009) Silibinin attenuates mast cell-mediated anaphylaxis-like reactions. *Biol. Pharm. Bull.* **32**: 868-875.
19. Neel, N. F., Creasy, B. M., Rankin, J. N., Pierce, E. M., McCoy, M. E., Daner, R. H., Fowler, J. A., Daniel, J. C. and Lantz, C. S. (2004) Absence of interleukin-3 does not affect the severity of local and systemic anaphylaxis but does enhance eosinophil infiltration in a mouse model of allergic peritonitis. *Immunol. Lett.* **95**: 37-44.
20. Hachisuka, H., Nomura, H., Sakamoto, F., Mori, O., Okubo, K. and Sasai, Y. (1988) Effect of antianaphylactic agents on substance-P induced histamine release from rat peritoneal mast cells. *Arch. Dermatol. Res.* **280**: 158-162.
21. Choi, Y. H., Song, C. H. and Mun, S. P. (2018) Proanthocyanidin-rich *Pinus radiata* bark extract inhibits mast cell-mediated anaphylaxis-like reactions. *Phytother. Res.* **32**: 290-297.
22. Holmegaard, S. N. (1982) Measurement of cyclic AMP in clinical investigations. *Acta Endocrinol. Suppl. (Copenh)* **249**: 1-47.
23. Izushi, K. and Tasaka, K. (1989) Histamine release from beta-escin-permeabilized rat peritoneal mast cells and its inhibition by intracellular Ca2+ blockers, calmodulin inhibitors and cAMP. *Immunopharmacology* **18**: 177-186.
24. Jutel, M., Blaser, K. and Akdis, C. A. (2006) The role of histamine in regulation of immune responses. *Chem. Immunol. Allergy* **91**: 174-187.
25. Reber, L. L., Marichal, T. and Galli, S. J. (2012) New models for analyzing mast cell functions *in vivo*. *Trends Immunol.* **33**: 613-625.
26. Tasaka, K., Mio, M. and Okamoto, M. (1986) Intracellular calcium release induced by histamine releasers and its inhibition by some antiallergic drugs. *Ann. Allergy* **56**: 464-469.
27. Weston, M. C. and Peachell, P. T. (1998) Regulation of human mast cell and basophil function by cAMP. *Gen. Pharmacol.* **31**: 715-719.
28. Yoshii, N., Mio, M. and Tasaka, K. (1988) Ca uptake and Ca releasing properties of the endoplasmic reticulum in rat peritoneal mast cells. *Immunopharmacology* **16**: 107-113.
29. Hoth, M. and Penner, R. (1993) Calcium release-activated calcium current in rat mast cells. *J. Physiol.* **465**: 359-386.
30. Alfonso, A., Cabado, A. G., Vieytes, M. R. and Botana, L. M. (2000) Functional compartments in rat mast cells for cAMP and calcium on histamine release. *Cell Signal.* **12**: 343-350.
31. Marquardt, D. L. and Wasserman, S. I. (1982) Characterization of the rat mast cell beta-adrenergic receptor in resting and stimulated cells by radioligand binding. *J. Immunol.* **129**: 2122-2127.
32. Kopp, T., Mastan, P., Mothes, N., Tzaneva, S., Stingl, G. and Tanew, A. (2009) Systemic allergic contact dermatitis due to consumption of raw shiitake mushroom. *Clin. Exp. Dermatol.* **34**: e910-e913.
33. Ueda, A., Obama, K., Aoyama, K., Ueda, T., Xu, B. H., Li, Q., Huang, J., Kitano, T. and Inaoka, T. (1992) Allergic contact dermatitis in shiitake (*Lentinus edodes* (Berk) Sing) growers. *Contact Dermatitis* **26**: 228-233.
34. Wang, X., Xu, X. and Zhang, L. (2008) Thermally induced conformation transition of triple-helical lentinan in NaCl aqueous solution. *J. Phys. Chem. B* **112**: 10343-10351.
35. Nakamura, T. (1992) Shiitake (*Lentinus edodes*) dermatitis. *Contact Dermatitis* **27**: 65-70.
36. Bellik, Y., Hammoudi, S. M., Abdellah, F., Iguer-Ouada, M. and Boukraa, L. (2012) Phytochemicals to prevent inflammation and allergy. *Recent Pat. Inflamm. Allergy Drug Discov.* **6**: 147-158.

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