Inhibitory Effects of *Agaricus blazei* on Mast Cell-Mediated Anaphylaxis-Like Reactions

Yun Ho CHOI, a,b Guang Hai YAN, a,b Ok Hee CHAI, a,b Yung Hyun CHOI, c Xin ZHANG, a,b Jung Min LIM, a,b Ji-Hyun KIM, a,b Moo Sam LEE, a Eui-Hyeog HAN, a Hyoun Tae KIM, a and Chang Ho SONG∗, a,b

a Department of Anatomy, Chonbuk National University Medical School; b Research Center for Allergic Immune Diseases, Chonbuk National University; Jeonju, Jeonbuk, 561–756, Republic of Korea and c Department of Biochemistry, Dongeui University College of Oriental Medicine; Busan, 614–052, Republic of Korea.

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* To whom correspondence should be addressed. e-mail: asch@chonbuk.ac.kr

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*Agaricus blazei* is a medically important mushroom widely eaten and prescribed around the world. It is belonged to the *Agaricaceae* family and is popularly known as “Himematsutake” in Japan. In Brazil, this mushroom is used as a traditional medicine for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis, and chronic hepatitis. Recently, it has been reported that *Agaricus blazei* has the anti-tumor, immunomodulator, anti-mutagenic, anti-oxidant, and anti-bacterial activities. However, it has not been cleared whether it prevents allergic diseases in experimental model.

In general, immediate-type hypersensitivity reactions that involve asthma, allergic rhinitis, urticaria, and anaphylaxis are mediated by mast cells. In response to the IgE-dependent and IgE-independent stimuli, mast cells elicit degranulation, which results in releasing various mediators, such as histamine and an array of inflammatory cytokines. Histamine, a main content of granules in mast cells, exerts many effects related to the immediate phase of allergic inflammation including vasodilatation, increased vascular permeability, tissue edema, constriction of bronchial and intestinal smooth muscle, and increased mucus production.7) Mast cell degranulation can also be elicited by the basic secretagogues. The most potent secretagogues include the compound 48/80, which is a mixed polymer of phenethylamine cross-linked by formaldehyde and a high dose of which induces almost a 90% release of histamine from mast cells.8) Thus, compound 48/80 has been used as a direct and convenient reagent to study the mechanism of allergy and anaphylaxis.7)

In this study, we demonstrate the *Agaricus blazei* water extract (ABWE) inhibits compound 48/80-induced systemic anaphylaxis-like reaction, ear swelling response, and passive cutaneous anaphylaxis-like reaction in mice. We also show that ABWE reduces anti-dinitrophenyl (DNP) IgE-mediated passive cutaneous anaphylaxis. Afterward, the inhibitory effect of ABWE on compound 48/80-induced mast cell activation is elucidated, as reported previously.9)

**Materials and Methods**

**Materials** Compound 48/80, disodium cromoglycate (DSCG) theophylline, anti-DNP IgE, DNP-human serum albumin (HSA), bovine serum albumin (BSA) and HEPES were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Percoll solution was purchased from Pharmacia (Uppsala, Sweden).

**Experimental Animals** Male ICR mice (6 week old, 25—30 g) and Sprague-Dawley rats (8 week old, 230—280 g) were purchased from Damool Science (Daejeon, Korea). Animals were housed 3—5 per cages in laminar airflow cabinet maintained at 22±1 °C and relative humidity of 55±10% throughout the study. Animal Research Committee of Chonbuk National University approved the animal study in accordance with the guidelines of the National Institutes of Health (NIH publication #85-23, 1985).

**Preparation of Agaricus blazei Water Extract (ABWE)** *Agaricus blazei* water extract used in this study was provided from Dr. Yung Hyun Choi (Department of Biochemistry, Dongeui University, Busan, Korea). The method of preparation of ABWE is as follows; the dried *Agaricus blazei* (100 g) was immersed in 1000 ml of distilled water, kept overnight in a refrigerator (10°C), and boiled under reflux for 3 h. This hot-water extraction was conducted twice. The resulting extract was filtered through a 0.45-μm filter, and concentrated to approximately 100 ml under reducing pressure. The concentrated extract was finally lyophilized,
yielded 11.5 g dried powder and kept at −20 °C until use. The dried extract was dissolved in saline or HEPES-Tyrode buffer (136 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 11 mM NaHCO₃, 0.6 mM NaH₂PO₄, 2.75 mM MgCl₂, 5.4 mM HEPES, 1.0 mg/ml BSA, 1.0 mg/ml glucose, 0.1 mg/ml heparin, pH 7.4) before use.

**Compound 48/80-Induced Systemic Anaphylaxis-Like Reaction** Mouse (n=10/group) were intraperitoneally received compound 48/80 [8 mg/kg body weight (BW)] as described previously.10) ABWE or DSCG (0.01 to 1 g/kg BW) was dissolved in saline and orally administered 24, 12 and 1 h before the injection of compound 48/80. Mortality was monitored for 1 h after the induction of anaphylactic shock.

**Compound 48/80-Induced Ear Swelling Response** Ear swelling response was investigated by the method described previously.11) Compound 48/80 was freshly dissolved in saline (5 mg/ml) and intradermally injected in the ventral aspect of the left side of mouse ear (100 μg/site, 20 μl) using a 30-gauge hypodermic needle. Sham saline was intradermally injected in the ventral aspect of the right side of mouse ear. Ear thickness was measured with a digital micrometer (Mitutoyo, No. 7326, Japan) under mild anesthesia induced by the intraperitoneal injection of 1:1 mixture (50 μl) of ketamin (1 mg/ml) and xylazine hydrochloride (23.32 mg/ml). Mice were kept in immobility state during the measurement. Ear swelling response represented an increment in thickness above baseline control values. Ear swelling response was determined 1 h after the injection of compound 48/80 or vehicle. ABWE (0.01 to 1 g/kg BW) was orally administered 24, 12 and 1 h before the injection of compound 48/80.

**Compound 48/80-Induced or Anti-Dinitrophenyl (DNP) IgE-Mediated Passive Cutaneous Anaphylaxis (PCA)-Like Reaction** After the intradermal injection of 0.5 μg/20 μl compound 48/80 in the dorsal skin of each mouse, 2% Evans blue solution was intravenously injected into the lateral tail vein of each mouse. The next steps were conducted following the procedures outlined in the next paragraph. Anti-DNP IgE-mediated PCA was examined as reported previously.12) Mice were sensitized in the right dorsal skin by the intradermal injection of 200 ng anti-DNP IgE in 20 μl phosphate-buffered saline (PBS) and were given a sham PBS injection in the left dorsal skin. Twenty-four hours later, the mice received into the lateral tail vein an injection of 200 μl of PBS containing 100 μg DNP-HSA with 2% Evans blue. ABWE was orally administered 24, 12 and 1 h before the injection of compound 48/80 or challenge.

Thirty minutes after the 2% Evans blue injection or challenge, the mice were killed by terminal anaesthesia, tissue sections around the intradermal injection site excised and weighed, followed by extraction of extravasated Evan’s blue dye by incubation of biopsies in 1 ml formamide at 55 °C for 24 h and measurement of absorbance at 620 nm using a spectrophotometer (Spectra MAX PLUS, Molecular Devices, CA, U.S.A.).13) Tissue Evans blue concentrations were quantified by interpolation on a standard curve of dye concentrations in the range of 0.01 to 30 μg/ml.

**Preparation of Mast Cell Suspension** Rat peritoneal mast cells (RPMC) were isolated as described previously.14) In brief, rats were anesthetized with ether and injected with 10 ml of calcium-free HEPES-Tyrode buffer into the peritoneal cavity, and the abdomen was gently massaged for about 90 s. The peritoneal cavity was opened, and the fluid was aspirated using a Pasteur pipette, and RPMC were purified by using a percoll density gradient as described in detail elsewhere.15) RPMC preparations were about 95% pure as assessed by toluidine blue staining and at least 98% of these cells were viable as assessed by trypan blue exclusion.16) Purified mast cells (1×10⁶ cells/ml) were resuspended in HEPES-Tyrode buffer.

**Mast Cell Viability Assay** To test the viability of cells, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was performed as described previously.17) Briefly, RPMC (2×10⁵ cells/well) were incubated with various concentrations (10—1000 μg/ml) of ABWE at 37 °C for 2 h. After addition of MTT (100 μg in 100 μl saline), cells were incubated at 37 °C for 1 h. The crystallized MTT was dissolved, and the absorbance was measured at 570 nm with a spectrophotometer.

**Assay of Histamine Release** RPMC suspensions (2×10⁵ cells in 200 μl) were preincubated with ABWE (10—1000 μg/ml) or DSCG at 37 °C for 10 min and then incubated with compound 48/80 (0.25 μg/ml) for 20 min. In addition, RPMC were sensitized with 10 μg/ml anti-DNP IgE for 18 h and preincubated with ABWE or DSCG at 37 °C for 10 min prior to challenge with DNP-HSA (100 ng/ml). Following centrifugation at 150×g for 10 min at 4 °C, the amount of histamine in the supernatant was determined by the radioenzymatic method.18) The inhibition percentage of histamine release was calculated using the following formula: % inhibition=[(histamine release without ABWE−histamine release with ABWE)/histamine release without ABWE]×100.

**Measurement of ⁴⁵Ca Uptake** The calcium uptake of mast cells was measured according to the method described by Chai et al.19) Purified RPMC were resuspended in HEPES-Tyrode buffer containing ⁴⁵Ca (1.5 mCi/ml; 1 Ci=3.7×10¹⁰ becquerels; PerkinElmer Life Sciences, MA, U.S.A.), and incubated at 4 °C for 10 min. Mast cell suspensions were preincubated with ABWE (10—1000 μg/ml) at 37 °C for 10 min and then incubated with compound 48/80 (0.25 μg/ml) at 37 °C for 20 min. The reaction was stopped by the addition of 1 mM lanthanum chloride. The samples were centrifuged 3 times at 150×g for 10 min at 4 °C, and then RPMC were lysed with 10% Triton X-100 and vigorous shaking. Radioactivity of the solution was measured in a scintillation β-counter (Liquid Scintillation Analyzer, A Canberra Company, Australia).

**Measurement of cAMP Level** The cyclic adenosine-3',5'-monophosphate (cAMP) level was measured by the method described by Holmegaard.20) In brief, RPMC suspensions were added to an equivalent volume (200 μl) of pre-warmed buffer containing ABWE (10—1000 μg/ml) or theophylline (1000 μg/ml) in an Eppendorf tube. The reaction was allowed to proceed for discrete time intervals, terminated by centrifugation at 150×g for 10 min at 4 °C, and then each sample was added to 250 μl of 50 mM sodium acetate buffer (pH 6.2) under vigorous vortexing, followed by snap frozen in liquid nitrogen. The frozen samples were thawed and vortexed, and then the debris were sedimented by centrifugation at 1200×g for 10 min at 4 °C. The cAMP level in the supernatant was determined by radioimmunoassay using a Rianen assay system (PerkinElmer Life Sciences, MA, U.S.A.).
Statistical Analysis  The results obtained were expressed as mean±S.E.M. for the number of experiments. Student’s t-test was used for a statistical comparison between groups. Results with p<0.05 were considered statistically significant.

RESULTS

Effect of ABWE on Compound 48/80-Induced Systemic Anaphylaxis-Like Reaction  To investigate the effects of ABWE in anaphylactic reactions, we first used an in vivo model of systemic anaphylaxis-like reaction using compound 48/80. After the intraperitoneal injection of compound 48/80 (8 mg/kg BW) into mice, a mortality rate was examined for 1 h. As shown in Table 1, the injection of compound 48/80 resulted in 100% death of animals. Oral administration of ABWE (0.01 to 1 g/kg BW) reduced the mortality induced by compound 48/80 in a dose-dependent manner. Moreover, DSCG (reference drug) dose-dependently inhibited compound 48/80-induced mortality.

Effect of ABWE on Compound 48/80-Induced Ear Swelling Response  Ear swelling was induced by the injection of compound 48/80 (100 µg/site) as described.11) Oral administration of ABWE reduced the ear swelling response induced by compound 48/80 in a dose-dependent fashion (Table 2).

Effect of ABWE on Compound 48/80-Induced or Anti-DNP IgE-Mediated PCA-Like Reaction  It has been previously demonstrated that the intradermal injection of compound 48/80 into the dorsal skin of mice provokes the increase of mast cell-dependent vascular permeability documented by Evans blue extravasation.21) As shown in Table 3, oral administration of ABWE dose-dependently reduced the vascular permeability changes induced by compound 48/80. In addition, as described in Materials and Methods, local extravasation was also induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Oral administration of ABWE dose-dependently inhibited anti-DNP IgE-mediated PCA (Table 4).

Mast Cell Viability Assay  MTT conversion assay was used to determine the viability of RPMC exposed to ABWE. As shown in Fig. 1, the viable cells were almost 100% after exposure to 1000 µg/ml ABWE for 2 h, and ABWE had no

Table 1. Inhibitory Effect of Agaricus blazei Water Extract (ABWE) on Compound 48/80-Induced Systemic Anaphylaxis-Like Reaction in Mice

<table>
<thead>
<tr>
<th>Treatment (g/kg BW)</th>
<th>Compound 48/80 (8 mg/kg BW)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (saline)</td>
<td>+</td>
<td>100</td>
</tr>
<tr>
<td>ABWE 0.01</td>
<td>+</td>
<td>80</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>50</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>DSCG 0.01</td>
<td>+</td>
<td>80</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>60</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2. Inhibitory Effect of Agaricus blazei Water Extract (ABWE) on Compound 48/80-Induced Ear Swelling Response in Mice

<table>
<thead>
<tr>
<th>ABWE (g/kg BW)</th>
<th>Compound 48/80 (100 µg/site)</th>
<th>Ear thickness (x100 µm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>2.146±0.034</td>
<td>—</td>
</tr>
<tr>
<td>0</td>
<td>—</td>
<td>0.543±0.071</td>
<td>—</td>
</tr>
<tr>
<td>0.01</td>
<td>+</td>
<td>1.384±0.089</td>
<td>35.50***</td>
</tr>
<tr>
<td>0.01</td>
<td>—</td>
<td>0.492±0.141</td>
<td>—</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>1.195±0.037</td>
<td>44.37***</td>
</tr>
<tr>
<td>0.1</td>
<td>—</td>
<td>0.511±0.066</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>0.827±0.013</td>
<td>61.46***</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>0.561±0.132</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 3. Inhibitory Effect of Agaricus blazei Water Extract (ABWE) on Compound 48/80-Induced Passive Cutaneous Anaphylaxis-Like Reaction in Mice

<table>
<thead>
<tr>
<th>ABWE (g/kg)</th>
<th>Compound 48/80 (0.5 µg/site)</th>
<th>Amount of Evans blue (µg/g)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>299.47±8.08</td>
<td>—</td>
</tr>
<tr>
<td>0.01</td>
<td>+</td>
<td>247.02±7.13</td>
<td>17.51**</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>198.34±4.78</td>
<td>33.76***</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>147.13±3.24</td>
<td>50.87***</td>
</tr>
</tbody>
</table>

Table 4. Inhibitory Effect of Agaricus blazei Water Extract (ABWE) on Anti-DNP IgE-Mediated Passive Cutaneous Anaphylaxis in Mice

<table>
<thead>
<tr>
<th>ABWE (g/kg)</th>
<th>Anti-DNP IgE</th>
<th>Amount of Evans blue (µg/g)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>451.17±28.36</td>
<td>—</td>
</tr>
<tr>
<td>0.01</td>
<td>+</td>
<td>308.87±22.55</td>
<td>31.54**</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>267.68±8.41</td>
<td>40.67***</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>184.65±5.71</td>
<td>59.07***</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of Agaricus blazei Water Extract (ABWE) on Rat Peritoneal Mast Cells (RPMC) Viability

RPMC were treated with the various concentrations of ABWE for 2 h. RPMC viability was determined by MTT assay and the percentage of viability was calculated as a ratio of A₅₇₀ of control cells (treated with HEPES-Tyrode buffered solution). Each value is the mean±S.E.M. of five independent experiments.
cytotoxicity on RPMC.

**Effect of ABWE on Histamine Release from RPMC**
The effect of ABWE on compound 48/80-induced histamine release from RPMC was shown (Fig. 2). The histamine release from compound 48/80-treated RPMC was reduced in a dose-dependent manner of ABWE. The histamine release from compound 48/80-treated RPMC after pretreatment of 0, 10, 100, or 1000 mg/ml of ABWE amounted to 2.34±0.23, 1.71±0.08, 1.49±0.09, or 0.99±0.02 μg/ml, respectively. DSCG also showed a significant inhibition rate. In addition, the inhibitory effect of ABWE on anti-DNP IgE-mediated histamine release from RPMC was shown in Fig. 3. ABWE dose-dependently inhibited anti-DNP IgE-mediated histamine release. These results indicate that ABWE contains the active compounds, which inhibit compound 48/80-induced or IgE-mediated anaphylaxis-like reactions by blocking histamine release from RPMC.

**Effect of ABWE on Calcium Uptake into RPMC** It is well established that an augmentation of intracellular calcium content by increased calcium uptake into RPMC contributes to histamine release.22,23) Thus, we measured calcium uptake. ABWE alone did not affect calcium uptake into RPMC (Fig. 4). The calcium uptake into RPMC induced by 0, 10, 100, or 1000 μg/ml of ABWE amounted to 44.2±2.0, 46.8±1.4, 42.2±0.3, or 43.7±2.6 nm, respectively. However, ABWE inhibited compound 48/80-induced calcium uptake into RPMC in a concentration-dependent manner. The calcium uptake into compound 48/80-treated RPMC after pretreatment of 0, 10, 100, or 1000 μg/ml of ABWE amounted to 2.34±0.23, 1.71±0.08, 1.49±0.09, or 0.99±0.02 μg/ml, respectively. DSCG also showed a significant inhibition rate. In addition, the inhibitory effect of ABWE on anti-DNP IgE-mediated histamine release from RPMC was shown in Fig. 3. ABWE dose-dependently inhibited anti-DNP IgE-mediated histamine release. These results indicate that ABWE contains the active compounds, which inhibit compound 48/80-induced or IgE-mediated anaphylaxis-like reactions by blocking histamine release from RPMC.

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level as compared to those treated with buffer only. But ABWE inhibited the compound 48/80-induced cAMP reduction of RPMC in a dose-dependent way (Fig. 5). The cAMP level in compound 48/80-treated RPMC after pretreatment of 0, 10, 100, or 1000 μg/ml of ABWE amounted to 0.81±0.02, 1.14±0.07, 1.30±0.04, or 1.37±0.03 pmol/ml, respectively. Theophylline (phosphodiesterase inhibitor) also increased the level of intracellular cAMP and significantly inhibited compound 48/80-induced cAMP reduction in RPMC. These results suggest that ABWE may block histamine release by increasing the cAMP level in RPMC.

**DISCUSSION AND CONCLUSIONS**

Here we have demonstrated that ABWE pretreatment profoundly attenuates compound 48/80-induced systemic anaphylaxis-like reaction, ear swelling response, and PCA-like reaction in mice. ABWE also inhibits anti-DNP IgE-mediated PCA. ABWE dose-dependently reduces histamine release from RPMC activated by compound 48/80 or anti-DNP IgE. These results suggest that ABWE contains the active compounds to inhibit both compound 48/80-induced anaphylaxis-like reaction and IgE-mediated anaphylactic reaction by blocking histamine release from RPMC.

Stimulation of mast cells with compound 48/80 is believed to initiate the activation of a signal transduction pathway, which leads to histamine release. In general, compound 48/80 initiates the generation of superoxide anion by A-kinase inactivation through decreasing the intracellular cAMP concentration in RPMC.24) Generated superoxide anion results in the inositol 1,4,5-triphosphate (IP3) or GTP-induced calcium release from the endoplasmic reticulum (ER).25) Accordingly, depletion of intracellular calcium store (ER) activates a calcium influx into RPMC, which leads to the increase of the intracellular calcium content.26) Consequently, increased intracellular calcium levels cause histamine release from RPMC.22,23) Interestingly, treatment of ABWE increases cAMP level beyond the basal level. Although the mechanism of ABWE-induced cAMP production has not been elucidated, ABWE may activate the adenylate cyclase directly or increasing the cAMP level in RPMC.19,22) ABWE may inhibit the compound 48/80-induced anaphylaxis-like reaction and IgE-mediated anaphylactic reaction by blocking histamine release from RPMC.22,23)

Comically, treatment of ABWE increases cAMP level beyond the basal level. Although the mechanism of ABWE-induced cAMP production has not been elucidated, ABWE may activate the adenylate cyclase directly or indirectly, otherwise inhibit cAMP phosphodiesterase. In addition, ABWE prevents the compound 48/80-induced cAMP reduction and calcium uptake of RPMC in a dose-dependent fashion. Taken together, ABWE may block the generation of superoxide anion by A-kinase activation through increasing the intracellular cAMP in compound 48/80-treated RPMC. Subsequently, we speculate that decreased superoxide anion as well as increased cAMP impedes histamine release by decreasing intracellular calcium content via inhibiting not only IP3 or GTP-induced calcium release from ER but also calcium uptake into compound 48/80-treated RPMC.25,27)

PCA represents one model of acute allergic reactions in which mast cells appear to be essential.22) ABWE significantly inhibits anti-DNP IgE-mediated PCA. The mechanism of the protection against anti-DNP IgE has been suggested in part by the activity of ABWE to inhibit several protein tyrosine kinases in RPMC. Alternatively, ABWE may antagonize the effects of chemical mediators. Therefore, the protective mechanism of ABWE remains to be further studied.

In summary, the results from this study demonstrate the evidence that ABWE inhibits the immediate-type allergic reactions in experimental murine models. Therefore, we suggest that ABWE has a potent anti-allergic effect. However, further work should be directed toward the purification and identification of active constituents of *Agaricus blazei*. In addition, further studies are needed to elucidate the possibility that ABWE may also be effective in the human mast cell and in the treatment of human allergic disorders.

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