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# *Phellinus baumii* Inhibits Immediate-type Allergic Reactions

**Tae-Yong Shin*, Hye-Young Shin, Sang-Hyun Kim[1], Dae-Keun Kim, Byeong-Suk Chae, Chan-Ho Oh[2], Moon-Gu Cho[2], Suk-Heung Oh[2], Jong-Hwa Kim[3], Tae-Kyoo Lee[4], Jeong-Suk Park[5], and Sang-Yong Kim[6]**

*Dept. of Pharmacy, College of Pharmacy, Woosuk University, Jeonbuk 565-701, Korea*
*[1]Dept. of Pharmacology, School of Medicine, Kyungpook National University, Daegu 700-422, Korea*
*[2]Dept. of Food Biotechnology, Woosuk University, Jeonbuk 565-701, Korea*
*[3]Dept. of Pharmaceutical Engineering, Woosuk University, Jeonbuk 565-701, Korea*
*[4]Dept. of Food Industry & Management, Woosuk University, Jeonbuk 565-701, Korea*
*[5]Dept. of Oriental Alternative Medicine, Nambu University, Gwangju 506-706, Korea*
*[6]Division of Specimen and Genetic Resources, Korea National Arboretum, Gyeonggi 487-821, Korea*

**Abstract** – In this study, we investigated the effect of *Phellinus baumii* (PB) on immediate-type allergic reaction and inflammatory cytokine secretion. PB inhibited compound 48/80-induced systemic reactions in mice. PB inhibited compound 48/80-induced plasma histamine release. In addition, PB also inhibited the immunoglobulin (Ig) E-mediated local allergic reaction. Furthermore, PB decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated tumor necrosis factor-α and interleukin-6 secretion in human mast cells. These results indicate that PB may be beneficial in the treatment of immediate-type allergic reactions.
**Keywords** – *Phellinus baumii*, allergic reaction, mast cells, tumor necrosis factor-α, interleukin-6

## Introduction

Mast cells are important mediators of inflammatory responses such as allergy and hypersensitivity. Immediate-type hypersensitivity (anaphylaxis), an acute systemic allergic reaction, is mediated by histamine released in response to the antigen cross-linking of immunoglobulin E (IgE) bound to the Fcε receptor I (FcεRI) on mast cells. Mast cell activation causes the process of degranulation that result in releasing of mediators, such as histamine and an array of inflammatory cytokines (Metacalf *et al.*, 1981; Church *et al.*, 1997; Miyajima *et al.*, 1997). Among the inflammatory substances released from mast cells, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Petersen *et al.*, 1996). Mast cell activation is initiated upon interaction of multivalent antigen with its specific IgE antibody attached to the cell membrane *via* FcεRI (Metzger *et al.*, 1986; Alber *et al.*, 1991). Anti-dinitrophenyl (DNP) IgE antibody and antigen have been established to induce passive cutaneous anaphylaxis (PCA) reactions as a typical *in vivo* model for immediate hypersensitivity. Mast cell degranulation also can be elicited by non-immunologic stimulators such as neuropeptides, basic compounds, complement components, and certain drugs (Lagunoff *et al.* 1983). Compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent stimulators of mast cells. Thus, an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Ennis *et al.*, 1980). Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases, and several pro-inflammatory and chemotactic cytokines such as tumor necrosis factor (TNF-α), interleukins (IL-6, IL-4, IL-13), and transforming growth factor-β (Burd *et al.*, 1989; Plaut *et al.*, 1989; Galli *et al.*, 1991; Bradding *et al.*, 1993). Therefore, modulation of secretion of these cytokines from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease. In experimental allergic models, disodium cromoglycate (DSCG : a mast cell stabilizer) shows an obvious inhibitory effect on the immediate-type allergic reactions. This effect of DSCG is thought to be based on the inhibition of the mediator release from mast cells (Thomson and Evans, 1973; Wells and Mann, 1983). We evaluated the inhibitory effects of *Phellinus baumii* in comparison with those of DSCG. *Phellinus baumii*, together

*Author for correspondence
Fax: +82-63-290-1812; E-mail: tyshin@ woosuk.ac.kr

with *Phellinus linteus*, is a mushroom used as a folk medicine for a variety of human diseases in Korea. Jang *et al.* (2004) report that *Phellinus baumii* might be useful in preventing acute pulmonary inflammation in human diseases. The antioxidant and free radical scavenging activity of *Phellinus baumii* were also documented by Shon *et al.* (2003). In this study, we evaluated the effect of *Phellinus baumii* (PB) on compound 48/80-induced systemic reaction and anti-dinitrophenyl (DNP) IgE antibody-induced local allergic reaction. Additionally, the effect of PB on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187 (A23187)-induced TNF-α and IL-6 secretion in a human mast cells (HMC-1) was also investigated.

## Experimental

**Animals** – The original stock of male ICR mice were purchased from Dae-Han Biolink Co. Ltd. (Chungbuk, Korea), and the mice were maintained in the College of Pharmacy, Woosuk University. The mice were housed ten per cage in a laminar air flow room maintained at a temperature of 22 ± 2 °C and relative humidity of 55 ± 5% throughout the study. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

**Reagents** – Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), α-minimal essential medium (α-MEM), o-phthaldialdehyde, phorbol 12-myristate 13-acetate, calcium ionophore A23187 were purchased from Sigma Chemical Co. (St Louis, MO). rTNF-α and rIL-6 were purchased from R & D Systems Inc. (Minneapolis, MN).

**Preparation of PB** – *Phellinus baumii* were purchased from the Sansu Sangwhang Co. (Imsil, Korea). A voucher specimen (number WSP-06-01) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The sample was extracted with purified water at 70 °C for 5 h. The extract was filtered, and lyophilized. The yield of dried extract from starting crude materials was about 6.7%. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

**Compound 48/80-induced systemic reaction** – Mice were given an intraperitoneal injection of 0.008 g/kg body weight (BW) of the mast cell degranulator, compound 48/80. PB was dissolved in saline and administered intraperitoneally ranging from 0.01 to 1 g/kg BW 1 h before the injection of compound 48/80 (n = 10/group). In the time dependent experiment, PB (1 g/kg) was administered intraperitoneally at 5, 10, and 20 min after injection of compound 48/80 (n = 10/group). Mortality was monitored for 1 h after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each mouse.

**PCA reaction** – The mice were injected intradermally with 0.5 μg of anti-DNP IgE into each of 2 dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. After 48 h, each mouse was received an injection of 1 μg of DNP-HSA in PBS containing 4% Evans blue (1 : 4) via the tail vein. PB (0.01 to 1 g/kg BW) was intraperitoneally administered 1 h before the challenge. Thirty minutes after the challenge, the mice were killed and the dorsal skin was removed for measurement of the pigment area. The amount of dye was determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of a mixture of acetone and phosphoric acid (5 : 13) based on the previous report (Katayama *et al.*, 1978). The absorbent intensity of the extraction was measured at 620 nm by using a spectrophotometer.

**Preparation of plasma and histamine determination** – The blood was centrifuged at 400 × *g* for 10 min. The plasma was withdrawn and histamine content was measured by the o-phthaldialdehyde spectrofluorometric procedure (Shore *et al.*, 1959). The fluorescent intensity was measured at a 438 nm emission and a 353 nm excitation using a spectrofluorometer.

**Assay of TNF-α and IL-6 secretion** – TNF-α and IL-6 secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously (Scuderi *et al.*, 1986). HMC-1 cells were cultured with α-MEM plus 10% FBS and resuspended in Tyrode buffer A. The cells were sensitized with PMA (20 nM) plus A23187 (1 μM) for 6 h in the absence or presence of PB. The ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF-α and IL-6 respectively. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. For the standard curve, rTNF-α and rIL-6 were added to serum previously determined to be negative to endogenous TNF-α and IL-6. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human TNF-α and IL-6, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablets substrates. Optical density readings were made within 10

**Table 1.** Effect of PB on compound 48/80-induced systemic allergic reaction

| treatment (g/kg BW) | compound 48/80 (0.008 g/kg BW) | mortality (%) | |
|---|---|---|---|
| | | PB | DSCG |
| none (saline) | + | 100 | 100 |
| 0.01 | + | 100 | 100 |
| 0.05 | + | 60 | 80 |
| 0.1 | + | 30 | 40 |
| 0.5 | + | 10 | 0 |
| 1 | + | 0 | 0 |
| 1 | – | 0 | 0 |

Groups of mice ($n$ = 10/group) were intraperitoneally pretreated with 200 μl of saline or PB or DSCG. Various doses of PB or DSCG were given 1 h before the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

**Table 2.** Time-dependent effect of PB on compound 48/80-induced systemic allergic reaction

| treatment (g/kg BW) | time (min) | compound 48/80 (0.008 g/kg BW) | mortality (%) | |
|---|---|---|---|---|
| | | | PB | DSCG |
| none (saline) | | + | 100 | 100 |
| 1 | 5 | + | 20 | 0 |
| 1 | 10 | + | 50 | 60 |
| 1 | 20 | + | 100 | 100 |

Groups of mice ($n$ = 10/group) were intraperitoneally pretreated with 200 μl of saline or PB or DSCG. PB or DSCG (1 g/kg) was given at 5, 10, and 20 min after the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

min of the addition of the substrate with a 405 nm filter.

**Statistical analysis** – Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed using one-way ANOVA, followed by Duncan's multiple range tests. $p < 0.05$ was used to indicate significance.

## Results

**PB inhibits compound 48/80-induced systemic reaction** – To assess the contribution of PB in systemic reaction, an *in vivo* model of systemic anaphylaxis was used. Compound 48/80 (0.008 g/kg) was used as a model of induction of systemic fatal allergic reaction. After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 h, after which the mortality rate was determined. Compound 48/80 induced fatal shock in 100% of animals. When PB was administered intraperitoneally at a concentrations ranging from 0.01 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was dose-dependently reduced (Table 1). In addition, the mortality of mice administered with PB (1 g/kg) 5, 10, and 20 min after compound 48/80 injection increased time-dependently (Table 2). In this same *in vivo* experiment, DSCG also had an inhibitory effect on compound 48/80-induced systemic reaction.

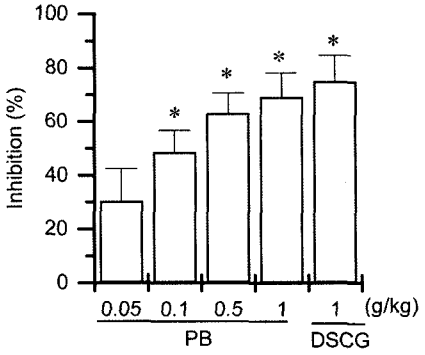


**Fig 1.** Effect of PB on compound 48/80-induced plasma histamine release. Groups of mice ($n$ = 10/group) were intraperitoneally pretreated with 200 μl of saline or PB or DSCG. PB or DSCG was given 1 h before the intraperitoneal injection of compound 48/80. Each date represents the mean ± SEM of three independent experiments. *$p < 0.05$; significantly different from the saline value.

**PB inhibits compound 48/80-induced plasma histamine release** – The effect of PB on compound 48/80-induced plasma histamine release was investigated. PB was given from 0.01 to 1 g/kg BW 1 h before compound 48/80 injection. PB (0.1 to 1 g/kg) and DSCG (1 g/kg) significantly inhibited compound 48/80-induced plasma histamine release (Fig. 1).

**PB inhibits the IgE-mediated local allergic reaction** – PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reactions and, in part, mediated by histamine in the blood stream (Mican *et al.*, 1992). Local extravasation is induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Intraperitoneal administration of PB (0.1 and 1 g/kg) and DSCG (1 g/kg) showed a marked inhibition rate in PCA reaction (Fig. 2).

**PB inhibits pro-inflammatory cytokine secretion in HMC-1 cells** – We examined whether PB could regulate pro-inflammatory cytokines such as TNF-α and IL-6 in HMC-1 cells. HMC-1 cell line is a useful cell for studying cytokine activation pathway (Sillaber *et al.*, 1993; Moller *et al.* 1998). Stimulation of HMC-1 cells with PMA plus A23187 induced the secretion of both cytokines. However,

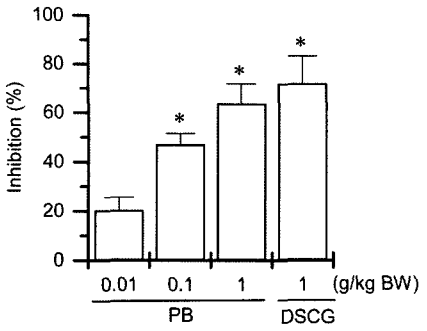


**Fig. 2.** Effect of PB on the PCA reaction. PB or DSCG was intraperitoneally administered 1 h prior to the challenge with antigen. Each date represents the mean ± SEM of three independent experiments. *p < 0.05; significantly different from the saline value.

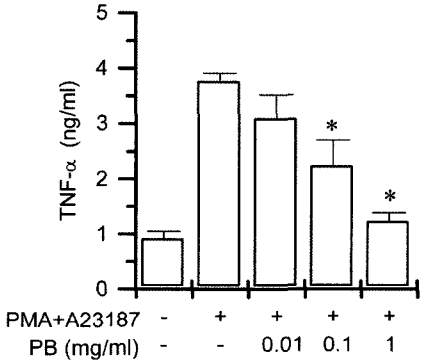


**Fig 3.** Effect of PB on the TNF-α secretion. PMA plus A23187-stimulated HMC-1 cells were incubated for 8 h in the absence or presence of PB. TNF-α secreted into the medium are presented as the mean ± SEM of three independent experiments. *p < 0.05; significantly different from the PMA + A23187 value.

pretreatment with PB decreased PMA plus A23187-induced TNF-α and IL-6 secretion (Fig. 3, Fig. 4).

## Discussion

Numerous reports established that stimulation of mast cells with compound 48/80 or IgE initiates the activation of signal-transduction pathway, which leads to histamine release. Several studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al.*, 1990a; Mousli *et al.*, 1990b; Chahdi *et al.*, 2000). Tasaka *et al.* (1986) reported that compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. These reports indicates that the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable. The results of our study demonstrated

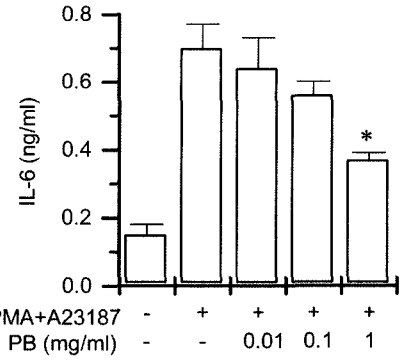


**Fig 4.** Effect of PB on the IL-6 secretion. PMA plus A23187-stimulated HMC-1 cells were incubated for 8 h in the absence or presence of PB. IL-6 secreted into the medium are presented as the mean ± SEM of three independent experiments. *p < 0.05; significantly different from the PMA + A23187 value.

that PB has anti-allergic properties. PB and DSCG inhibited compound 48/80-induced systemic reaction and anti-DNP IgE-mediated local allergic reaction. These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by PB. Taken together, we could speculate that PB might stabilize the lipid bilayer membrane, thus preventing the perturbation induced by compound 48/80. In addition, mice administered PB are protected from anti-DNP IgE-mediated PCA, one of the most important *in vivo* models of anaphylaxis in local allergic reaction. This finding suggests that PB might be useful in the treatment of allergic skin reactions. Mast cell-derived cytokines, especially TNF-α and IL-6 have a critical biological role in the allergic reaction. Mast cells are a principal source of TNF-α in human dermis, and degradation of mast cells in the dermal endothelium is abrogated by the anti-TNF-α antibody (Walsh *et al.*, 1991). IL-6 is also produced from mast cells and its local accumulation is associated with a PCA reaction (Mican *et al.*, 1992). These reports indicate that reduction of pro-inflammatory cytokines from mast cell is a one of the key indicator of reduced allergic symptom. In the present study, PB inhibited the secretion of TNF-α and IL-6 in PMA plus A23187-stimulated HMC-1 cells. This result suggests that the anti-allergic effect of PB results from its reduction of TNF-α and IL-6 release from mast cells.

In conclusion, the results obtained in the present study provide evidence that PB contributes importantly to the prevention or treatment of mast cell-mediated allergic diseases. Also, it suggests that PB may contain compounds with actions that inhibit mast cell-mediated allergic reactions *in vivo* and *in vitro*. The effort for identify active components from PB in the immediate-type allergic reaction is ongoing in our laboratory.

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