

# *Phlomis umbrosa* Root Inhibits Mast Cell-Dependent Allergic Reactions and Inflammatory Cytokine Secretion

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**The effect of an aqueous extract of *Phlomis umbrosa* Turcz. (Labiatae) root (PUAE) on mast cell-dependent allergic reactions and inflammatory cytokine secretion were investigated. PUAE (0.01–1 g/kg) inhibited compound 48/80-induced systemic allergic reaction. When PUAE was employed in a systemic allergic reaction test, the plasma histamine levels were reduced in a dose-dependent manner. PUAE (0.1 and 1 g/kg) also significantly inhibited the local allergic reaction activated by anti-dinitrophenyl (DNP) IgE. PUAE (0.001–1 mg/mL) dose-dependently inhibited the histamine release from rat peritoneal mast cells activated by compound 48/80 or anti-DNP IgE. PUAE (0.01–1 mg/mL) inhibited the secretion of interleukin (IL)-1 $\beta$  in phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated human mast cell line (HMC-1) cells. PUAE (1 mg/mL) inhibited the gene expression and production of the main inflammatory cytokine, TNF- $\alpha$ , in HMC-1 cells. These results provide evidence that PUAE may be beneficial in the treatment of allergic diseases. Copyright © 2007 John Wiley & Sons, Ltd.**

**Keywords:** *Phlomis umbrosa*; allergic reaction; compound 48/80; interleukin-1 $\beta$ ; mast cells; TNF- $\alpha$ .

## INTRODUCTION

The dried root of *Phlomis umbrosa* Turcz. (Labiatae), well known as 'Sok-dan' in Korea, has been used for centuries as traditional medicine. This crude drug is used for the management of hemorrhage, common cold and bronchitis; moreover, it has been used for bone fractures in Korea (Lee, 1966; But *et al.*, 1997; Zu, 1998).

Mast cells are bone marrow derived effector cells of the immune system, and are found abundantly in connective tissue, skin, mucosal membranes and tissues interfacing with the external environmental. Recent findings implicate a role for mast cells, in addition to immediate type hypersensitivity reactions and defense against parasitic infections, also in defense against bacterial infections (Echtenacher *et al.*, 1996; Malaviya *et al.*, 1996). Mast cell degranulation can be elicited by nonimmunologic stimulators such as neuropeptides, basic compounds, complement components and certain drugs. Compound 48/80 is one of the most potent secretagogues of mast cells (Ennis *et al.*, 1980). Compared with the natural process, a high concentration of

compound 48/80 induces almost a 90% release of histamine from 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 (Allansmith *et al.*, 1989). The secretory response of mast cells can be induced by aggregation of their cell surface-specific receptors for immunoglobulin E (IgE) by the corresponding antigen (Segal *et al.*, 1977; Metzger *et al.*, 1986; Alber *et al.*, 1991). It has been established that the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA) as a typical *in vivo* model of immediate hypersensitivity. Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases and several proinflammatory and chemotactic cytokines such as TNF- $\alpha$ , interleukins (IL)-6, IL-4, IL-8, IL-13 and transforming growth factor (TGF)- $\beta$  (Bradding *et al.*, 1993; Plaut *et al.*, 1989; Wodnar-Filipowicz *et al.*, 1989; Burd *et al.*, 1989; Gurish *et al.*, 1991; Galli *et al.*, 1991). Therefore, modulation of these cytokines secretion from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease.

This paper deals with an evaluation of the effect of PUAE on the compound 48/80 induced systemic anaphylaxis, anti-dinitrophenyl (DNP) IgE antibody-induced PCA and histamine release from rat peritoneal mast cells (RPMC). The effect of PUAE on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore A23187 (A23187)-induced IL-1 $\beta$  secretion in human mast cell line (HMC-1) cells was investigated. Finally, mRNA expression and the production of TNF- $\alpha$  were studied using HMC-1 cells.

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## MATERIALS AND METHODS

**Reagents.** Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA),  $\alpha$ -minimal essential medium ( $\alpha$ -MEM), *o*-phthalaldehyde, Evans blue, PMA, calcium ionophore A23187 and metrizamide were purchased from Sigma Chemical Co. (St Louis, MO, USA). rIL-1 $\beta$  was purchased from R&D Systems Inc. (Minneapolis, MN, USA).

**Animals.** The original stock of male Sprague-Dawley rats and ICR mice were purchased from Dae-Han Experimental Animal Center (Daejeon, Korea), and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed five to ten per cage in a laminar air flow room maintained under a temperature of  $22 \pm 2$  °C and relative humidity of  $55 \pm 5\%$  throughout the study.

**Preparation of PUAE.** The dried root of *Phlomis umbrosa* was purchased from the oriental drug store, Bohwa Dang (Jeonju, Korea). A voucher specimen (number WSP-00-29) was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with purified water at 70 °C for 5 h. The extract was filtered through Whatman No. 1 filter paper, lyophilized, and kept at  $-4$  °C. The yield of dried extract from starting crude materials was about 10.8%. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 5.6 mM glucose, 0.1% bovine serum albumin) before use.

**Compound 48/80-induced systemic anaphylaxis.** Compound 48/80-induced systemic anaphylaxis was examined as described previously (Shin *et al.*, 1999). Mice were given an intraperitoneal injection of 0.008 g/kg body weight (BW) of the mast cell degranulator, compound 48/80. PUAE was dissolved in saline and administered by intraperitoneal injection ranging from 0.01 to 1 g/kg BW 1 h before the injection of compound 48/80 ( $n = 10/\text{group}$ ). In time dependent experiments, PUAE (1 g/kg BW) was injected intraperitoneally at 5, 10, 20 and 30 min after compound 48/80 injection ( $n = 10/\text{group}$ ). 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.** An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the mouse tail vein. The anti-DNP IgE antibody and DNP-HSA were diluted in phosphate-buffered saline (PBS). The mice were injected intradermally with 0.5  $\mu\text{g}$  of anti-DNP IgE into each of two dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Each mouse, 48 h later, received an injection of 1  $\mu\text{g}$  of DNP-HSA in PBS containing 4% Evans blue (1:4) via the tail vein. PUAE (0.001–1 g/kg BW) was administered intraperitoneally and orally 1 h before the challenge. After 30 min of the challenge, the mice were killed and the dorsal skin was removed for measurement of pigment area. The amount of dye was determined colorimetrically after extraction with 1 mL

of 1 M KOH and 9 mL of mixture of acetone and phosphoric acid (5:13) based on the method of Katayama *et al.* (1978). The absorbent intensity of the extraction was measured at 620 nm in a spectrophotometer (Shimadzu, UV-1201, Japan).

**Preparation of plasma and histamine determination.** The blood was centrifuged at  $400 \times g$  for 10 min. The plasma was withdrawn and histamine content was measured by the *o*-phthalaldehyde spectrofluorometric procedure followed by Shore *et al.* (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer (Shimadzu, RF-5301 PC, Japan).

**Preparation of RPMC.** RPMC were isolated as previously described (Shin *et al.*, 2000). In brief, rats were anesthetized by ether and injected with 20 mL of Tyrode buffer B (137 mM NaCl, 5.6 mM glucose, 12 mM  $\text{NaHCO}_3$ , 2.7 mM KCl, 0.3 mM  $\text{NaH}_2\text{PO}_4$  and 0.1% gelatin) into the peritoneal cavity and the abdomen was gently massaged for about 90 s. The peritoneal cavity was opened carefully and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at  $150 \times g$  for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells, i.e. macrophages and small lymphocytes, according to the method described by Yurt *et al.* (1977). In brief, peritoneal cells suspended in 1 mL of Tyrode buffer B were layered on 2 mL of metrizamide (22.5 w/v %) and centrifuged at room temperature for 15 min at  $400 \times g$ . The cells remaining at the buffer–metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 mL of Tyrode buffer A. Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue uptake.

**Inhibition of histamine release.** Purified RPMC were resuspended in Tyrode buffer A for the treatment with compound 48/80. RPMC suspensions ( $2 \times 10^5$  cells/mL) were preincubated for 10 min at 37 °C before the addition of compound 48/80 (5  $\mu\text{g}/\text{mL}$ ). The cells were preincubated with the PUAE preparations and then incubated (10 min) with the compound 48/80. RPMC suspensions ( $2 \times 10^5$  cells/mL) were also sensitized with anti-DNP IgE (10  $\mu\text{g}/\text{mL}$ ) for 16 h. The cells were preincubated with PUAE at 37 °C for 10 min prior to the challenge with DNP-HSA (1  $\mu\text{g}/\text{mL}$ ). The cells were separated from the released histamine by centrifugation at  $400 \times g$  for 5 min at 4 °C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at  $400 \times g$  for 5 min at 4 °C.

**Assay of IL-1 $\beta$  secretion.** IL-1 $\beta$  secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously (Scuderi *et al.*, 1986). HMC-1 cells ( $3 \times 10^5$  cells/mL) were cultured with  $\alpha$ -MEM plus 10% FBS and resuspended in Tyrode buffer A. The cells were sensitized with PMA (20 nm) plus A23187 (1  $\mu\text{M}$ ) for 10 h in the absence or presence of PUAE. The ELISA was performed by coating 96-well plates with 6.25 ng/well of murine monoclonal antibody with specificity for murine IL-1 $\beta$ . 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. All reagents used in this assay and the coated wells were incubated for 1 h at room temperature. For the standard curve, rIL-1 $\beta$  was added to serum previously determined to be negative to endogenous IL-1 $\beta$ . After exposure to the medium, the plates were exposed sequentially to biotinylated anti-human IL-1 $\beta$ , 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablet substrates. Optical density readings were made within 10 min of the addition of the substrate on a Titertek Multiscan (Flow Laboratories) with a 405 nm filter.

**RT-PCR analysis.** Total RNA was isolated from HMC-1 cells with Easy-blue according to the manufacturer's instructions, PCR was performed with following primers for TNF- $\alpha$  (5'CGG GAC GTG GAG CTG GCC GAG GAG3'; 5'CAC CAG CTG GTT ATC TCT CAG CTC 3'). The actin (5'GTG GGG CGC CCC AGG CAC CA3'; 5'GTC CTT AAT GTC ACG CAC GAT TTC3') was used to verify that equal amounts of RNA were used for reverse transcription and PCR amplification from different experimental conditions. The annealing temperature was 60 °C for TNF- $\alpha$  and actin. Amplified fragment sizes for TNF- $\alpha$  and actin were 355 bp and 661 bp, respectively. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

**Western blotting of TNF- $\alpha$ .** Cell extracts were prepared by detergent lysis procedure. Cells ( $5 \times 10^6$  cells/mL) were scraped, washed with PBS, and resuspended in lysis buffer. Samples were lysed by vortexing for 1 h at 4 °C and centrifuged at  $400 \times g$  for 5 min at 4 °C. Samples were heated at 95 °C for 5 min, and briefly cooled on ice. Following centrifugation at  $400 \times g$  for 5 min, 50  $\mu$ L aliquots were resolved by 12.5% sodium dodecyl sulfate polyacrylamide. Resolved proteins were electrotransferred overnight to nitrocellulose membranes in 25 mM Tris, 0.2 M glycerol, 20% methanol at 25 V. Blots were blocked for at least 2 h with 1  $\times$  TBST plus 10% nonfat dry milk. IL-1 $\beta$  was analysed essentially according to the manufacturer's instructions.

**Statistical analysis.** The results obtained were expressed as mean  $\pm$  SEM. Student's *t*-test was used to make a statistical comparison between the groups. Results with *p* < 0.05 were considered statistically significant.

## RESULTS

### PUAE inhibition of compound 48/80-induced systemic anaphylaxis

To assess the contribution of PUAE in anaphylaxis, an *in vivo* model of systemic anaphylaxis was used. Compound 48/80 (0.008 g/kg BW) was used as a systemic fatal anaphylaxis inducer. After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 h, after which the mortality rate was determined. As shown in Table 1, an intraperitoneal injection of 200  $\mu$ L saline as a control induced a fatal shock in 100% of each group. When mice were pretreated with PUAE at concentrations ranging from 0.01 to 1 g/kg BW for

**Table 1. Effect of PUAE on compound 48/80-induced systemic anaphylaxis**

| PUAE treatment (g/kg BW) | Compound 48/80 (0.008 g/kg BW) | Mortality (%) |
|--------------------------|--------------------------------|---------------|
| None (saline)            | +                              | 100           |
| 0.01                     | +                              | 100           |
| 0.05                     | +                              | 60            |
| 0.1                      | +                              | 50            |
| 0.5                      | +                              | 10            |
| 1                        | +                              | 0             |
| 1                        | –                              | 0             |

Groups of mice (*n* = 10/group) were intraperitoneally pretreated with 200  $\mu$ L saline or PUAE. PUAE was given at various doses 1 h before the compound 48/80 injections. The compound 48/80 solution was intraperitoneally given to the group of mice. Mortality (%) within 1 h following compound 48/80 injections was represented as the number of dead mice  $\times$  100/total number of experimental mice.

**Table 2. Time-dependent effect of PUAE on compound 48/80-induced systemic anaphylaxis**

| PUAE treatment (g/kg BW) | Time (min) | Compound 48/80 (0.008 g/kg BW) | Mortality (%) |
|--------------------------|------------|--------------------------------|---------------|
| None (saline)            |            | +                              | 100           |
| 1                        | 5          | +                              | 0             |
|                          | 10         | +                              | 10            |
|                          | 20         | +                              | 70            |
|                          | 30         | +                              | 100           |

Groups of mice (*n* = 10/group) were intraperitoneally pretreated with 200  $\mu$ L saline or PUAE. PUAE (1 g/kg) was given at 5, 10, 20 and 30 min after the compound 48/80 injections. The compound 48/80 solution was intraperitoneally given to the group of mice. Mortality (%) within 1 h following compound 48/80 injections was represented as the number of dead mice  $\times$  100/total number of experimental mice.

1 h, the mortality with compound 48/80 was reduced dose-dependently (*n* = 10/group). Especially, PUAE inhibited compound 48/80-induced systemic anaphylactic reaction 100% with a dose of 1 g/kg. In addition, the mortality of mice injected intraperitoneally with PUAE (1 g/kg BW) 5, 10, 20 and 30 min after compound 48/80 injections increased time-dependently (Table 2). Treatment with PUAE (1 g/kg) caused no apparent side effects.

### PUAE inhibition of compound 48/80-induced plasma histamine release

The inhibitory effect of PUAE on compound 48/80-induced plasma histamine release was investigated. PUAE was administered at concentrations ranging from 0.01 to 1 g/kg BW 1 h before (*n* = 10/group) compound 48/80 injections. The correlation results with those of the mortality test were shown when their plasma histamine contents were measured. The inhibition rate of histamine by PUAE was significant at doses of 0.1 to 1 g/kg (Table 3). In time dependent experiments, PUAE (1 g/kg) was given at 5, 10, 20 and 30 min after

**Table 3. Effect of PUAE on compound 48/80-induced plasma histamine release**

| PUAE treatment (g/kg BW) | Compound 48/80 (0.008 g/kg BW) | Amount of histamine ( $\mu\text{g/mL}$ ) |
|--------------------------|--------------------------------|------------------------------------------|
| None (saline)            | +                              | $0.127 \pm 0.021$                        |
| 0.01                     | +                              | $0.121 \pm 0.018$                        |
| 0.05                     | +                              | $0.080 \pm 0.009$                        |
| 0.1                      | +                              | $0.068 \pm 0.006^a$                      |
| 0.5                      | +                              | $0.029 \pm 0.004^a$                      |
| 1                        | +                              | $0.023 \pm 0.002^a$                      |

Groups of mice ( $n = 10/\text{group}$ ) were intraperitoneally pretreated with 200  $\mu\text{L}$  saline or PUAE. PUAE was given at various doses 1 h before the compound 48/80 injections. Each data represents the mean  $\pm$  SEM of three independent experiments.

<sup>a</sup>  $p < 0.05$ : significantly different from the saline value.

compound 48/80 injection. The correlation results with those of the time dependent mortality test were shown when their plasma histamine contents were measured (Table 4).

### PUAE inhibition of PCA

Passive cutaneous anaphylaxis is one of the most important *in vivo* models of anaphylaxis in local allergic reaction (Wershil *et al.*, 1987). As described in the experimental procedures, local extravasations were induced by a skin injection of anti-DNP IgE followed by an antigenic challenge. Both oral and intraperitoneal administrations of PUAE (0.001–1 g/kg) dose-dependently inhibited PCA. PUAE (0.1 and 1 g/kg) showed a marked inhibition rate in the PCA reaction (Fig. 1).

### PUAE inhibition of compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC

The inhibitory effect of PUAE on compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC is shown in Fig. 2. PUAE dose-dependently inhibited compound 48/80-induced or anti-DNP IgE-mediated histamine release at concentrations of 0.001–1 mg/mL. In particular, PUAE significantly inhibited compound 48/80-induced or anti-DNP IgE-mediated histamine release at concentrations of 0.1 and 1 mg/mL.

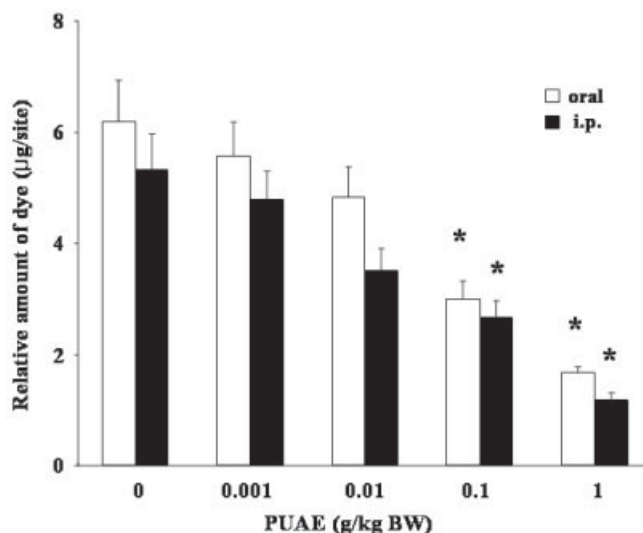


**Figure 1.** Effect of PUAE on 48 h PCA. PUAE was administered orally or intraperitoneally (i.p.) 1 h prior to the challenge with antigen. Each data point represents the mean  $\pm$  SEM of three independent experiments. \*  $p < 0.05$ : significantly different from the saline value.

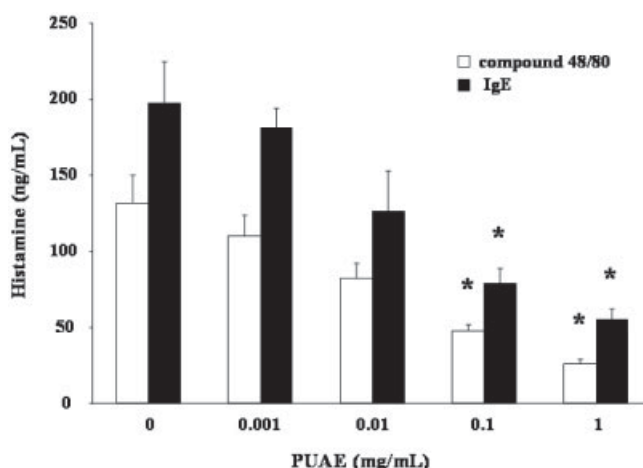


**Figure 2.** Effect of PUAE on compound 48/80 and IgE mediated histamine release from RPMC. The cells ( $2 \times 10^5$  cells/mL) were preincubated with PUAE at 37  $^{\circ}\text{C}$  for 10 min prior to incubation with compound 48/80 or DNP-HSA. Each data point represents the mean  $\pm$  SEM of three independent experiments. \*  $p < 0.05$ : significantly different from the saline value.

**Table 4. Time-dependent effect of PUAE on compound 48/80-induced plasma histamine release**

| PUAE treatment (g/kg BW) | Time (min) | Compound 48/80 (0.008 g/kg BW) | Amount of histamine ( $\mu\text{g/mL}$ ) |
|--------------------------|------------|--------------------------------|------------------------------------------|
| None (saline)            |            | +                              | $0.215 \pm 0.028$                        |
| 1                        | 5          | +                              | $0.060 \pm 0.007^a$                      |
|                          | 10         | +                              | $0.065 \pm 0.007^a$                      |
|                          | 20         | +                              | $0.172 \pm 0.021$                        |
|                          | 30         | +                              | $0.194 \pm 0.018$                        |

Groups of mice ( $n = 10/\text{group}$ ) were intraperitoneally pretreated with 200  $\mu\text{L}$  saline or PUAE. PUAE (1 g/kg) was given at 5, 10, 20 and 30 min after the compound 48/80 injections. Each data represents the mean  $\pm$  SEM of three independent experiments.

<sup>a</sup>  $p < 0.05$ : significantly different from the saline value.

**Table 5.** Effect of PUAE on PMA plus A23187-stimulated IL-1 $\beta$  secretion from HMC-1 cells

| Treatment           | Concentration (mg/mL) | IL-1 $\beta$ content (ng/mL)   |
|---------------------|-----------------------|--------------------------------|
| None (saline)       | –                     | 0.281 $\pm$ 0.098              |
| PMA + A23187        | –                     | 1.546 $\pm$ 0.903              |
| PMA + A23187 + PUAE | 0.01                  | 1.244 $\pm$ 0.105              |
|                     | 0.1                   | 1.179 $\pm$ 0.235              |
|                     | 1                     | 0.795 $\pm$ 0.149 <sup>a</sup> |

PMA plus A23187-stimulated HMC-1 cells ( $3 \times 10^5$  cells/mL) were incubated for 10 h in the absence or presence of PUAE. IL-1 $\beta$  secreted into the medium is presented as the mean  $\pm$  SEM of three independent experiments.

<sup>a</sup>  $p < 0.05$ ; significantly different from the saline value.

### PUAE inhibition of IL-1 $\beta$ secretion from HMC-1 cells

The next test examined whether PUAE could regulate IL-1 $\beta$  secretion by HMC-1 cells. PUAE inhibited the secretion of IL-1 $\beta$  in PMA plus A23187-stimulated HMC-1 cells (Table 5). No significant cytotoxicity of PUAE on the culture was observed in the concentrations used in the experiments, as assessed by trypan blue exclusion.

### PUAE inhibition of the gene expression and production of TNF- $\alpha$ in HMC-1 cells

The inhibitory effect of PUAE on TNF- $\alpha$  mRNA and production in HMC-1 cells stimulated with PMA plus A23187 is shown in Fig. 3. RT-PCR products were generated from total RNA obtained from HMC-1 cells. PMA plus A23187-stimulated HMC-1 cells increased TNF- $\alpha$  mRNA expression while unstimulated HMC-1 cells expressed very little TNF- $\alpha$  mRNA. PUAE (1 mg/mL) inhibited the TNF- $\alpha$  mRNA expression increased by PMA plus A23187.  $\beta$ -actin was used as a control for RNA integrity and equal loading (Fig. 3A). The TNF- $\alpha$  protein level in intracellular of HMC-1 cells was determined by Western blotting analysis (Fig. 3B). PMA plus A23187-stimulation of HMC-1 cells led to an

increase in TNF- $\alpha$  production. However, pretreatment of cells with PUAE (1 mg/mL) decreased TNF- $\alpha$  production. The results indicate that PUAE inhibited TNF- $\alpha$  in HMC-1 cells.

## DISCUSSION

The present study showed that PUAE pretreatment profoundly affected compound 48/80-induced systemic allergic reaction and anti-DNP IgE-induced PCA reaction. These results indicated that nonspecific and specific mast cell-dependent allergic reactions were significantly inhibited by PUAE. It is believed that stimulation of mast cells with compound 48/80 or anti-DNP IgE initiates the activation of a signal-transduction pathway, which leads to histamine release. There have been some reports that compound 48/80 is able to activate G-proteins (Mousli *et al.*, 1990a, 1990b). Recently, Chadi *et al.* (2000) announced that compound 48/80 activated mast cell phospholipase D via heterotrimeric GPT-binding proteins. They showed that recombinant G ( $\beta 2\gamma 2$ ) subunit markedly synergized phospholipase D activation by compound 48/80 in permeabilized RBL-2H3 cells. Murine mast cells are a good experimental model for the study of compound 48/80-induced histamine release (Alfonos *et al.*, 2000). The report that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane indicates that the permeability increase may be an essential trigger for the release of mediators from mast cells (Tasaka *et al.*, 1986). In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable. PUAE might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80. The PUAE-administered mice were protected from IgE-mediated local allergic reaction. It is conceivable that PUAE inhibited the initial phase of immediate-type allergic reactions, probably through interference with the mast cell/histamine system. In this study, the compound 48/80-induced or anti-DNP IgE-mediated histamine release from RPMC was significantly inhibited by PUAE at concentrations of 0.1 and 1 mg/mL. The HMC-1 cell line was a useful cell for studying cytokine activation pathways (Sillaber *et al.*, 1993). PUAE inhibited the secretion of IL-1 $\beta$  in PMA plus A23187-stimulated HMC-1 cells. PUAE inhibited the main inflammatory cytokine TNF- $\alpha$  production mRNA expression in HMC-1 cells. The effect of PUAE on mast cell cytokine secretion *in vivo* and the relative importance of mast cells as a source of TNF- $\alpha$ , IL-6 and IL-1 $\beta$  during inflammatory and immune responses are important areas for future studies. In conclusion, the results obtained in the present study provide evidence that PUAE importantly contributes to the prevention or treatment of mast cell-dependent allergic diseases.

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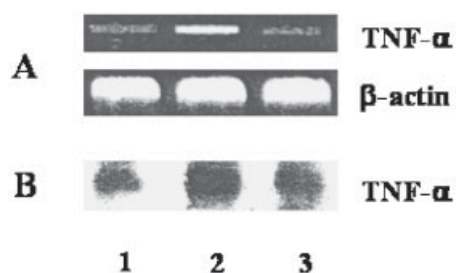


**Figure 3.** Inhibitory effect of PUAE on the TNF- $\alpha$  mRNA and production in HMC-1 cells by RT-PCR (A) and western blot (B). HMC-1 cells were pretreated with PUAE (1 mg/mL) for 30 min prior to PMA plus A23187 stimulation. Lane 1, Saline; Lane 2, Control (PMA plus A23187); Lane 3, PMA plus A23187 plus PUAE (1 mg/mL).

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