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Inhibitory effects of Polygoni cuspidati rhizoma on Mast Cell-Mediated Anaphylactic Reaction

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ABSTRACT

Polygoni cuspidati Rhizoma (PCRH) has been used in treatment of menoxenia, skin burn, gallstone, hepatitis, hyperlipidaemia, favus athlete's foot, supperative dermatitis and inflammation. However, its effect in experimental models remains unknown. In this present study, the effect of PCRH for stability on mast cell was analyzed. Two g/kg PCRH inhibited the compound 48/80-induced anaphylaxis by 75%.

In addition, PCRH inhibited the tumor necrosis factor-α and interleukin-8 secretion as compared with the phorbol 12-myristate 13-acetate plus calcium ionophore A23187 stimulated human mast cell line, HMC-1 cells.

These results suggested PCRH may inhibit mast cell-mediated anaphylactic reaction.

Key words : Polygoni cuspidati Rhizoma; Mast cell; Anaphylaxis; TNF-α; IL-8

INTRODUCTION

Polygoni cuspidati Rhizoma (PCRH), the root of the plant, has been used in treatment of menoxenia, skin burn, gallstone, hepatitis, hyperlipidaemia, favus athlete's foot, supperative dermatitis and inflammation (Chi et al., 1982;. Chi, 1975). In addition, it has been used in Korea to maintain oral hygiene (Hur, 1994). However, its effect in experimental models remains unknown. In this present study, we performed to evaluate whether PCRH could modulate the mast cell stability in compound 48/80-induced anaphylaxis or the stimulated human mast cell line, HMC-1.

Mast cells are widely distributed throughout the body in both connective tissue and at mucosal surfaces (Metcalfe et al., 1999). The role of mast

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cells as effector cells of immunoglobulin E (IgE)-dependent immediate-type hypersensitivity reactions and anaphylaxis is well understood (Galli et al.., 2000; Hamelmann et al., 1999) This activation is elicited through the cross-linking of allergen-specific IgE bound to the high-affinity receptor for IgE, FcεR I , on the cell membrane, which results in the degranulation of mast cells and the release of mediators that further aggravate the ongoing allergic process, and the mediators include histamine, proteases, leukotrienes, prostaglandins such as prostaglandin (PG)D_2, Leukotriene (LT) B_4, platelet-activating factor, and the cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 (Joshua A. 2003), and different cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-4, IL-6, and IL-8 (Royer et al., 2001; Artuc et al., 1999). The diverse profile of chemical mediators generated and released by activated mast cells leads to plasma extravasation, tissue edema, bronchoconstriction, leukocyte recruitment, and mucosal inflammation, which result from allergen exposure in sensitized hosts. we used compound 48/80 to activate mast cells, which is known as a potent inducer of degranulation and of the release of histamine and other chemical mediators that are responsible for anaphylactic symptoms (Bronner et al., 1987).

In this study, we tested that PCRH inhibited the production of TNF-α and IL-8 from stimulated human mast cell line, HMC-1 cells.

MATERIALS and METHODS

Preparation of PCRH

100 g of PCRH was decocted with water. The extract was filtered and dried using a freeze-dryer. The PCRH water extract powder was dissolved in phosphate-buffered saline (PBS) and filtered with 0.22 μm syringe filter.

Reagents

Materials Fetal bovine serum (FBS), Iscove's Modified Dulbecco's Medium (IMDM) were purchased from Gibco BRL (Grand Island, NY, USA). Compound 48/80, PMA, A23187, avidin-peroxidase, 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), 3- (4,5-dimethylthiazol-2-yl)-diphenyl -tetrazolium bromide (MTT) and other reagents were obtained from Sigma (St. Louis, MO, USA). Anti-human IL-8/TNF-α antibody (Ab), biotinylated anti-human IL-8/TNF-α Ab, and recombinant human (rh) IL-8/TNF-α were purchased from R&D Systems (Minneapolis, MN, USA).

Animals

The original stock of ICR mice were purchased from the Dae-Han Experimental Animal Center (Eumsung, South Korea), and the animals were maintained in the College of Pharmacy, Wonkwang University. The mice were housed ten per cage in a laminar air-flow room maintained at a temperature of 22 ± 1℃ and relative humidity of 55 ± 10% throughout the study. No animal was used more than once. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

Compound 48/80-induced systemic anaphylactic reaction

Mice were given an intraperitoneal injection of the

mast cell degranulator compound 48/80 (8 mg/kg). PCRH was dissolved in saline and administered orally 1 h before the injection of compound 48/80. Mortality was monitored for 23 min after induction of anaphylactic reaction.

Culture of HMC-1 cells

Human mast cell line, HMC-1 cells were grown in IMDM medium supplemented with 100 U/ml penicillin, 100 g/ml streptomycin, and 10 heat-inactivated FBS at 37℃ in 5% CO_2.

MTT Assay

To test the viability of cells, MTT colorimetric assay was performed as described previously (Kim et al., 2001). Briefly, HMC-1 cells (1 × 10^6 cells/ml) were incubated for 8 h after stimulation in the absence or presence of PCRH (1 mg/ml). After addition of MTT solution, the cells were incubated at 37℃ for 4 h. The crystallized MTT was dissolved in DMSO and measured the absorbance at 540 nm.

ELISA Assay

IL-8/TNF-α secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously (Na et al., 2002). HMC-1 cells were cultured with IMDM plus 10% FBS. The cells were sensitized with PMA (50 nM) plus A23187 (1 μM) for 8 h in the absence or presence of PCRH. The ELISA was performed by coating 96-well plates (Nunc, Denmark) with 1 μg /well of murine monoclonal Ab with specificity for IL-8/TNF-α. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20. All reagents used in this assay and the coated wells were incubated for 1 h at room temperature. For the standard curve, recombinant human IL-8/TNF-α Abs was added to the wells. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human IL-8/TNF-α. After 1 hour, the assay plates were exposed to enzyme, avidin-peroxidase for 30 min.

And then substrates was added to the wells. Optical density readings were made within 10 min of the addition of the ABTS substrate, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) tablets, on a ELISA reader (VersaMax, Molecular Devices) with a 405 nm filter.

Statistical analysis

The results were expressed as mean SEM for a number of experiments. Statistical significance was compared between each treated group and control by analysis of variance (ANOVA), with post hoc test of the means according to Tukey's method. For all tests, P value less than 0.05 was considered significant.

RESULTS

Effect of PCRH oncompound 48/80-induced systemic anaphylaxis

To assess the contribution of PCRH in anaphylactic reactions, we first used the in vivo model of systemic anaphylaxis. As a nonimmunologic stimulator, compound 48/80 (8 g/kg) was used. After the injection of compound 48/80, the mice were monitored for 23 min, after which the mortality rate was determined. As shown in Table 1, an oral administration of saline as a control induced a fatal reaction in 100% of each group. When the PCRH

was orally administered at concentrations of 1 mg/kg or 2 mg/kg 1 h before compound 48/80 injections, the mortality was reduced (Table 1).

Table. 1 Effect of PCRH pretreatment on compound 48/80-induced systemic anaphylactic shock

PCRH concentration (mg/kg)[a]	Compound 48/80 (8 mg/kg)[b]	Mortality (%)[c]
None(saline)	+	100.0
1	+	75.0
2	+	25.0
0	-	0.0

[a]The groups of mice were orally pre-treated with 200 μl of saline or PCRH was given at various doses 1 h before the compound 48/80 injection.
[b]The compound 48/80 solution was intra-peritoneally given to the groups of mice.
[c]Mortality (%) is presented as the No. of dead mice of experimental mice.

Effect of PCRH on HMC-1 Viability

To test cytotoxic effect of PCRH, we has performed MTT assay in HMC-1 cells. Fig. 1 shows the viability of cells 8 h incubation after stimulation in the absence or presence PCRH (1 mg/ml).

PCRH did not significantly affect cell viability and had no toxicity on HMC-1 cells (Fig. 1).

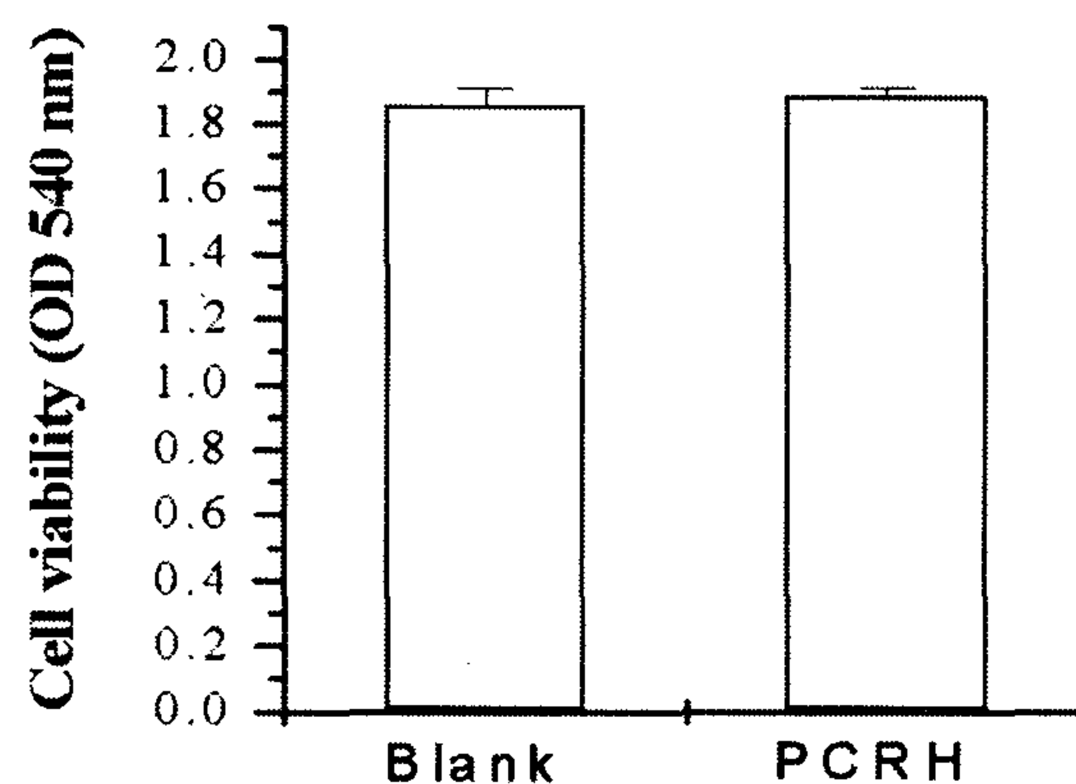

Fig. 1. Effect of PCRH on the cell viability in HMC-1 cells. Cell viability was evaluated by MTT assay 8 h after PCRH treatment (1 mg/ml) in HMC-1 cells. Data represent the mean ± SEM. of three independent experiments.

Effect of PCRH on IL-8 secretion from HMC-1 cells

We examined the inhibitory effect of PCRH on the PMA plus A23187-induced secretion of IL-8 from HMC-1 cells. Culture supernatant was assayed for each cytokine levels by ELISA method. PCRH alon did not affect the secretion of IL-8. However, PMA plus A23187 result in the activation of HMC-1 cells and induction of IL-8 from HMC-1 cells. One mg/ml PCRH inhibited the secretion of IL-8 from PMA plus A23187-stimulated HMC-1 cells compared with PMA plus A23187 group (Fig. 2). However, there was not significant.

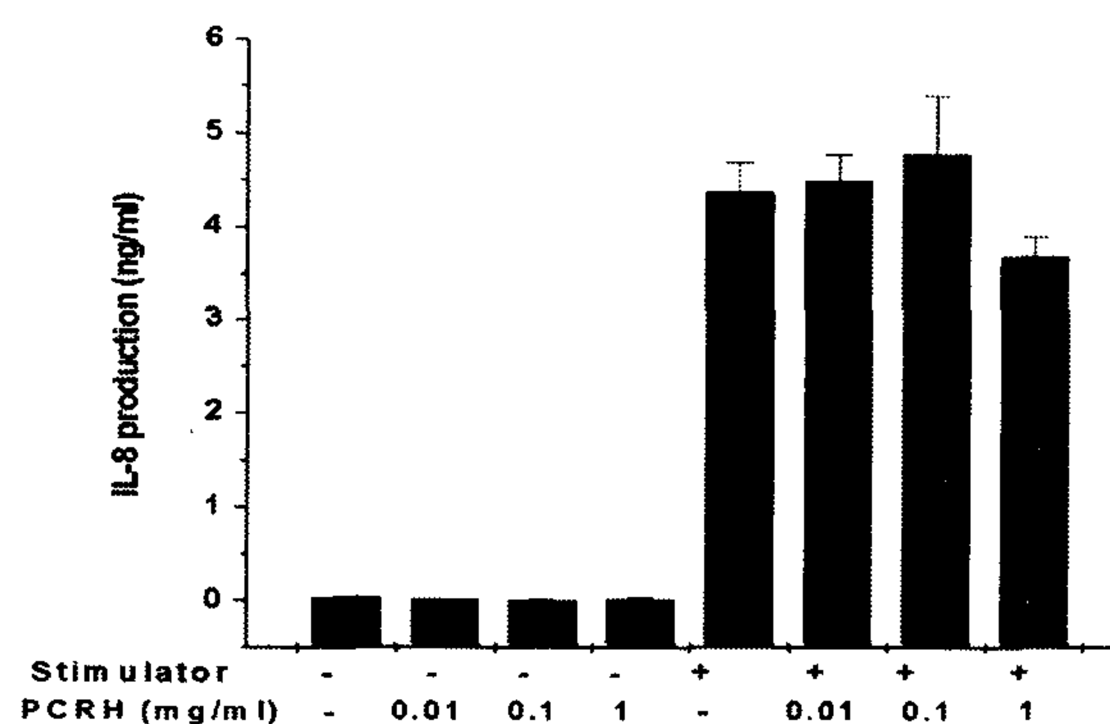

Fig. 3. Inhibition of IL-8 secretion by PCRH in PMA plus A23187-stimulated HMC-1 cells. Cells were pretreated with PCRH for 30 min and then challenged with PMA plus A23187 for 8 h. IL-8 concentrations were measured from cell supernatant using ELISA method. Values are mean ± SEM. of duplicate determinations from three separate experiments.

Effect of PC on TNF-α secretion from HMC-1 cells

We examined the inhibitory effect of PC on the PMA plus A23187-induced secretion of TNF-α from HMC-1 cells. Culture supernatant was assayed for each cytokine levels by ELISA method. PCRH alon did not affect the secretion of TNF-α. However, PMA plus A23187 result in the activation of HMC-1 cells and induction of TNF-α from HMC-1 cells. One mg/ml PCRH inhibited significantly the secretion of TNF-α from PMA plus A23187-stimulated HMC-1 cells compared with PMA plus A23187 group (Fig. 3, $^{*}P<0.001$).

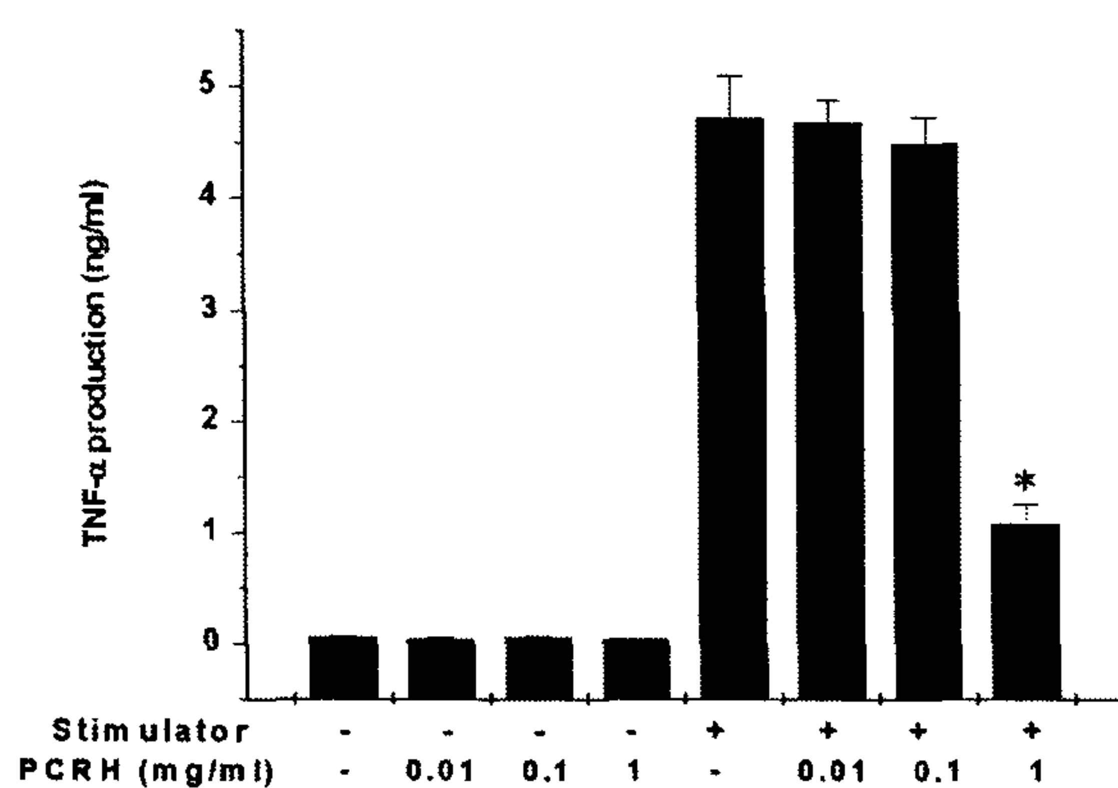

Fig. 3. Inhibition of TNF-α secretion by PCRH in PMA plus A23187-stimulated HMC-1 cells. Cells were pretreated with PCRH for 30 min and then challenged with PMA plus A23187 for 8 h. TNF-α concentrations were measured from cell supernatant using ELISA method. Values are mean ± SEM of duplicate determinations from three separate experiments. $*P < 0.001$; significantly different from the PMA plus A23187 group.

DISCUSSION

The present study showed that PCRH pretreatment profoundly affected compound 48/80-induced systemic anaphylaxis. In addition, the secretion of IL-8 and TNF-α in PMA plus A23187-stimulated mast cells was also inhibited.

Mast cells have been viewed primarily as harmful because of their key role as effector cells of allergic and potentially lethal anaphylactic reactions. Also, the number of mast cells is well known to increase in the inflamed tissue. Stimulation of mast cells with compound 48/80 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., 1990; Shin et al., 2003). Recently, Chadi et al (Chadi et al., 2000) announced that compound 48/80 activates mast cell phospholipase D (PLD) via heterotrimeric GTP-binding proteins. They identified recombinant G22 subunit markedly synergized PLD activation by compound 48/80 in permeabilized RBL-2H3 cells. (Alfonso et al., 2000).

HMC-1 cells activated by PMA plus A23187 are useful in vitro model system for studying of multifunctional effects of the immune and inflammatory reactions (Hosoda et al., 2002 Shin et al., 2003).

IL-8 is the most extensively studied member of the entire chemokine superfamily, with its major actions being as a neutrophil chemoattractant and activator. Free IL-8 has been detected in the sera, and bronchial tissue of subjects with severe atopic asthma but not in samples from normal subjects (Shute et al., 1997). TNF-α is an essential cytokine in many pathological conditions such as allergic diseases, rheumatoid arthritis and pulmonary fibrosis (Camusi et al., 1999).

In the present study, we showed that PCRH inhibited compound 48/80-induced systemic anaphylaxis and IL-8/TNF-α secretion as compared with the

stimulator group. These results suggested PCRH may inhibit mast cell-mediated anaphylactic reaction.

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