Polygoni Cuspidati Radix Inhibits the Activation of Syk Kinase in Mast Cells for Antiallergic Activity

BEONG OU LIM,* JUN HO LEE,† NA YOUNG KO,† SE HWAN MUN,† JIE WAN KIM,† DO KYUN KIM,† JU DONG KIM,† BO KYUNG KIM,† HYUNG SIK KIM,‡ ERK HER,† HOI YOUNG LEE,§ AND WAHN SOO CHOI†,[1]

*College of Biomedical & Health Science, Konkuk University, Chungju 380-701, Korea; †Department of Immunology and Physiology, College of Medicine, Konkuk University, Chungju 380-701, Korea; ‡College of Pharmacy, Pusan National University, Busan 609-735, Korea; and §College of Medicine, Konyang University, Nonsan 320-711, Korea

The antiallergic activity of *Polygoni cuspidati* radix (PR) and the mechanism of action by which it functions were investigated in this study. The extract of PR exhibited potent inhibitory activity in mast cells; its IC_{50} values were 62 ± 2.1 μg/ml for RBL-2H3 mast cells and 46 ± 3.2 μg/m for bone marrow–derived mast cells by antigen stimulation, and it also suppressed the expression of tumor necrosis factor-α and interleukin-4 in RBL-2H3 cells. According to the *in vivo* animal allergy model, it inhibited a local allergic reaction, passive cutaneous anaphylaxis, in a dose-dependent manner. With regard to its mechanism of action, PR inhibited the activating phosphorylation of Syk, a key signaling protein for the activation of mast cells. It also suppressed Akt and the mitogen-activated protein kinases ERK1/2, p38, and JNK, which are critical for the production of various inflammatory cytokines in mast cells. The results of the study indicate that the antiallergic activity of PR is mediated through the inhibition of histamine release and allergic cytokine production by the inhibition of Syk activating phosphorylation in mast cells. Exp Biol Med 232:1425–1431, 2007

Key words: *Polygoni cuspidati* radix; anti-allergic activity; mast cells; Syk kinase

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[1] To whom correspondence should be addressed at 322 Danwol-dong, Chungju, Chungbuk-do 380-701, Republic of Korea. E-mail: wahnchoi@kku.ac.kr

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Introduction

It is well established that IgE-dependent mast cell activation is responsible for several allergic diseases, such as allergic rhinitis, asthma, atopic dermatitis, and atopic eczema (1, 2). The stimulation of mast cells through the cross-linking of FcεRI with an antigen activates Src family kinases (SFKs), such as Lyn and Fyn, and subsequently other signaling molecules, such as Syk, LAT, SLP-76, and Gab2. Accumulated evidence suggests that Syk plays a pivotal role in the activation of mast cells and is an upstream molecule for the activation of LAT, SLP-76, and Gab2 (3). Subsequently, mast cells rapidly release various allergic mediators, including histamine, cytokines, and arachidonic derivatives, and various acute and chronic allergic reactions are induced by the mediators (4, 5). There are, however, many approaches to the treatment of these diseases, such as allergen-specific immunotherapy, DNA vaccination, humanized anti-IgE antibody administration, soluble interleukin (IL)-4 receptor treatment, and treatment with antagonists to leukotriene and histamine receptors. However, certain difficulties and side effects are associated with these therapies (6, 7). Consequently, novel approaches are being explored to develop new therapies. Complementary and alternative medicines are considered promising avenues for the development of such new therapies.

In Asian countries, many herbal extracts have been used as traditional folk remedies for treating diseases. However, the pharmacological activities of most herbal extracts have not been investigated scientifically. Among these remedies, *Polygoni cuspidati* radix (PR), also known as *Ho-Jang Geun* in Korea, of *Polygonum cuspidatum* Sieb. et Zucc. (Polygonaceae), is a well-known traditional Chinese medicine (8). It is traditionally used in Korea, China, and Japan as a folk remedy for menoxenia, skin burn, gallstone, hepatitis, inflammation, and osteomyelitis (8).

Recently, the extract of *Polygonum cuspidatum* has been reported to have anticancer activity (9), antibacterial activity (10), anti–bone loss activity (11), and antiviral activity (12). No studies documenting the antiallergic activity of PR, however, have been performed to date. In this study, it is reported for the first time that PR exhibits potent antiallergic activity through the inhibition of mast cells by suppressing the activating phosphorylation of Syk and mitogen-activated protein (MAP) kinases, suggesting that PR has potential in the treatment of allergic diseases.

Materials and Methods

Reagents. 2,4-Dinitrophenol (DNP)-specific monoclonal IgE, 2,4-dinitrophenylated bovine serum albumin (DNP-BSA), formamide, Arabic gum, and diphenylhydramine were obtained from Sigma Chemical Co. (St. Louis, MO), and PP2 was obtained from Calbiochem (La Jolla, CA). Monoclonal or polyclonal antibodies were obtained from the following sources: antibodies against the phosphorylated forms of ERK1/2 (monoclonal), p38 (polyclonal), JNK (polyclonal), Akt (polyclonal), and the phosphorylated form of Y317 Syk (monoclonal) from Cell Signaling Technology, Inc. (Danvers, MA); antibodies against Syk (polyclonal) and Actin (monoclonal) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); and polyclonal antibodies against LAT, SLP-76, and Gab2 from Upstate Biotechnology (Lake Placid, NY). The monoclonal antibody against the FcεRIβ subunit was kindly supplied by Dr. J. Rivera (National Institutes of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD). The horseradish peroxidase (HRP)–linked antibody against mouse or rabbit IgG was obtained from Cell Signaling Technology, Inc. (Danvers, MA). The minimal essential medium (MEM) and other cell culture reagents were purchased from GIBCO/Life Technologies, Inc. (Rockville, MD).

Animals. Male ICR mice (aged 4 weeks) were purchased from the Dae Han Experimental Animal Center (Eumsung, Korea) and were housed in the animal facilities at the College of Medicine in Konkuk University. Ten mice were placed in each cage, all of which had been fitted with a laminar airflow cabinet. The mice were kept at a temperature of 22° ± 1°C and at a relative humidity of 55% ± 10% throughout the study. This study was done in accordance with the institutional guidelines.

Preparation of Ethanol Extract of PR. The PR was imported from China and was authenticated by Dr. Hyung Kyu Lee at the Korea Research Institute of Bioscience and Biotechnology in Korea. It was prepared as follows: Dried root (100 g) from *Polygonum cuspidatum* Sieb. et Zucc. (Polygonaceae) was extracted with 1000 ml of ethanol at 50°C using an ultrasonic cleaner (Branson Ultrasonics Corporation). The extracted materials were concentrated with a speed bag (Biotron Corporation) at 40°C for 24 hrs, and the yield of extraction was about 15% (w/w). A voucher specimen (CA03–097) was deposited at the College of Medicine, Konkuk University.

Preparation and Stimulation of Bone Marrow–Derived Mast Cells (BMMCs) and RBL-2H3 Cells. BMMCs from male Balb/cJ mice were cultured for up to 10 weeks in a 50% enriched medium (RPMI 1640, containing 2 m*M* L–glutamine, 0.1 m*M* nonessential amino acids, antibiotics, and 10% fetal calf serum) containing 10 ng/ml IL-3. After 3 weeks, >98% of the cells were verified as being BMMCs through the previously described procedure (13), and the FcεRIβ subunit was subjected to immunoblot analysis. For cell stimulation, BMMCs were primed overnight with 50 ng/ml of DNP-specific IgE. The BMMCs were washed and resuspended in a Tyrode-BSA buffer (20 m*M* HEPES [pH 7.4], 135 m*M* NaCl, 5 m*M* KCl, 1.8 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 5.6 m*M* glucose, and 0.05% BSA) before stimulation with 20 ng/ml of the antigen DNP-BSA for 10 mins. RBL-2H3 cells were grown as monolayers in MEM with Earle's salts, supplemented with glutamine, antibiotics, and 15% fetal bovine serum.

Measurement of Degranulation in BMMCs and RBL-2H3 Mast Cells. In each experiment, the cells were transferred to 24-well (2×10^5 cells/0.4 ml/well) cluster plates (14) and incubated overnight in a complete growth medium with 50 ng/ml DNP-specific IgE. The cultures were washed, and the required buffered solution was added (0.2 ml/well). Experiments on intact cells were performed in a PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid))-buffered medium for RBL-2H3 cells and in a Tyrode buffer for BMMCs. Unless otherwise stated, the cultures were incubated for 30 mins, with or without PR, before adding 25 ng/ml of the antigen DNP-BSA for 10 mins. The secretion of granules containing various allergic mediators was determined through the measurement of the release of the granule marker β-hexosaminidase using a colorimetric assay, through which the release of p-nitrophenol from p-nitrophenyl-N-acetyl-β-D-glucosaminide was measured. The values were expressed as percentages of intracellular β-hexosaminidase released into the medium. The IC_{50} values, the concentration necessary to obtain a 50% inhibition of the response, were calculated through nonlinear regression analysis using GraphPad software (GraphPad, San Diego, CA).

Immunoblotting Analysis. BMMCs were washed, and the medium was replaced with the Tyrode buffer. The cells were then stimulated with a 25 ng/ml antigen (DNP-BSA) for 10 mins, with or without PR, and then chilled with ice to terminate the stimulation. Following this step, the cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed in 0.5 ml of ice-cold lysis buffer (20 m*M* HEPES [pH 7.5], 150 m*M* NaCl, 1% Nonidet p-40, 10% glycerol, 60 m*M* octyl β-glucoside, 10 m*M* NaF, 1 m*M* Na_3VO_4, 1 m*M* phenylmethylsulfonyl fluoride, 2.5 m*M* nitrophenylphosphate, 0.7 μg/ml pepstatin, and a protease-inhibitor cocktail tablet). The lysates were kept on ice for 30 mins, followed by centrifugation at 15,000 *g* for 15 mins at

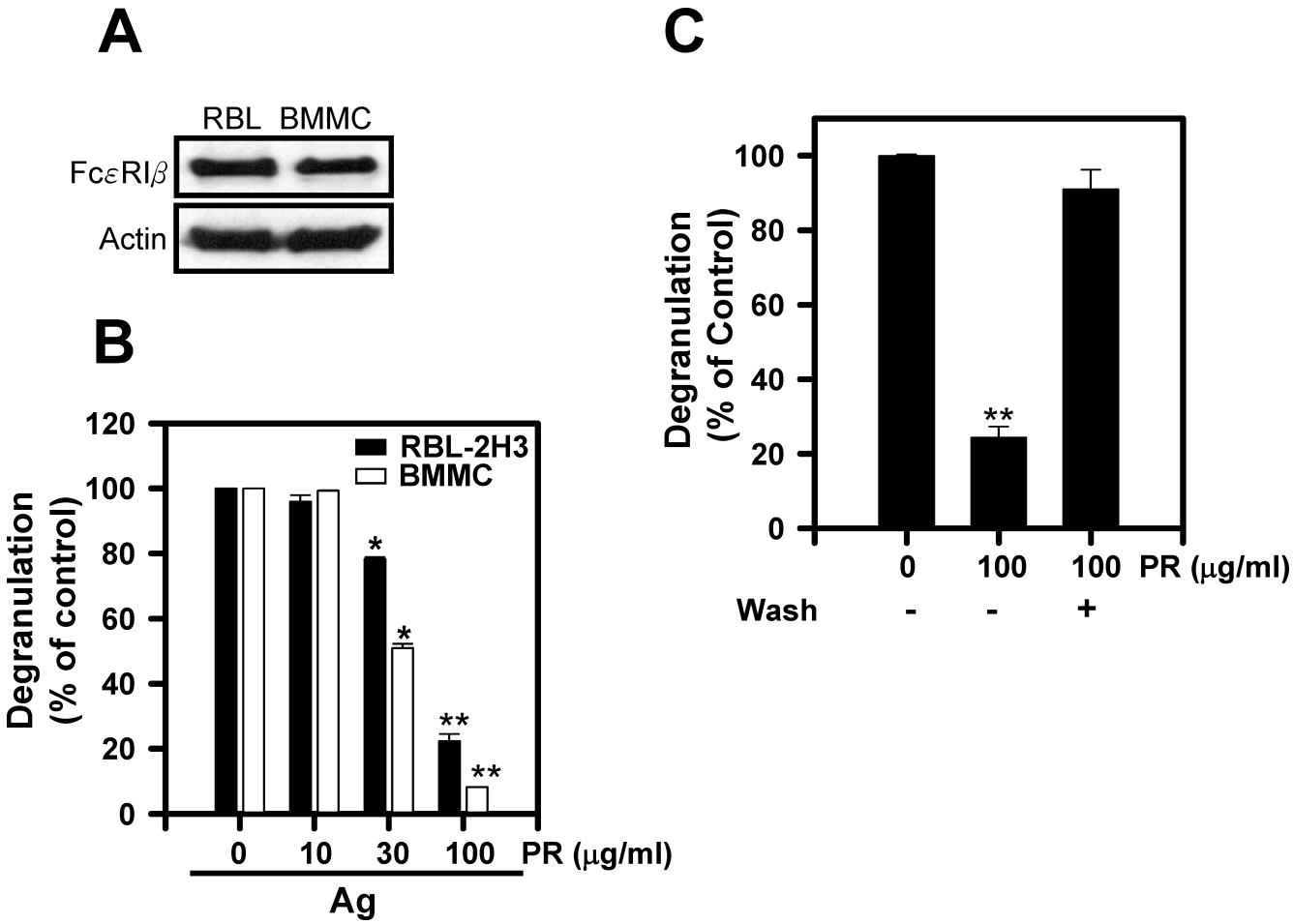

Figure 1. Effect of PR on antigen-induced degranulation in mast cells, and reversibility of the activity of PR. (A) The isolated BMMCs were verified, through immunoblot analysis, for the FcεRIβ subunit. (B) BMMCs or RBL-2H3 cells were incubated overnight in 24-well cluster plates, with 50 ng/ml DNP-specific IgE, in a complete growth medium. The medium was replaced with a Tyrode or PIPES buffer that contained the indicated concentration of PR, before stimulation with 25 ng/ml DNP-BSA, to measure the release of β-hexosaminidase. (C) RBL-2H3 cells were preincubated for 1 hr with DMSO or 100 μg/ml PR. One of the PR-treated cells was washed twice with a PIPES buffer before the treatment with antigen. The values are expressed as mean ± SEM from the three independent experiments.

4°C. The cell lysates were denatured by boiling at 95°C for 5 mins in a 2× Laemmli buffer (15). The proteins were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis and were then transferred to nitrocellulose membranes (Schleicher and Schuell, BA85). Subsequent to blocking in Tris-buffered saline + Tween 20 (TBS-T buffer; 10 m*M* Tris-HCl [pH 7.5], 150 m*M* NaCl, and 0.05% Tween 20) containing 5% skimmed milk powder, the membrane was incubated with individual antibodies. The primary antibodies were diluted 1:2000-fold, unless otherwise noted, and were incubated at 4°C overnight. The membranes were washed three times for 5 mins each with TBS-T buffer. The immunoreactive proteins were incubated with the use of HRP-coupled secondary antibodies diluted 1:2000-fold for 1 hr at room temperature, subsequently washed five times (for 5 mins each) with TBS-T buffer and enhanced chemoluminescence, according to the manufacturer's protocols (Amersham Biosciences, Piscataway, NJ).

Reverse Transcription Polymerase Chain Reaction (RT-PCR) for Tumor Necrosis Factor-α (TNF-α) and IL-4 mRNA. The RBL-2H3 cells were harvested by trypsinization and were transferred to 6-well (1×10^6 cells/3 ml/well) cluster plates, which were incubated overnight in a complete growth medium containing 25 ng/ml DNP-specific IgE. The cells were washed, followed by medium replacement with a PIPES-buffered medium (25 m*M* PIPES, pH 7.2, 159 m*M* NaCl, 5 m*M* KCl, 0.4 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 5.6 m*M* glucose, and 0.1% fatty-acid-free fraction V from a bovine serum), stimulated with 25 ng/ml DNP-BSA for 15 mins, with or without PR, and finally washed twice with ice-cold PBS. The total RNA was isolated using Trizol Reagent (Invitrogen, Carlsbad, CA) and was reverse-transcribed using the Superscript first-strand synthesis system (Invitrogen), according to the manufacturer's protocol. Polymerase chain reaction was performed at 94°C for 45 secs, at 55°C for 45 secs, and at 72°C for 60 secs, for 30 cycles. The following

primers were used: rat TNF-α forward 5′-CACCACG-CTCTTCTGTCTACTGAAC-3′, reverse 5′-CCGGA-CTCCGTGATGTCTAAGTACT-3′; rat IL-4 forward 5′-ACCTTGCTGTCACCCTGTTC-3′, reverse 5′-TTGT GA-GCGTGGACTCATTC-3′; and rat GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) forward 5′-GTGG-AGTCTACTGGCGTCTTC-3′, reverse 5′-CCAAGGCT-GTGGGCAAGGTCA-3′.

Induction of Mast Cells–Mediated Passive Cutaneous Anaphylaxis (PCA) in Mice. An anti-DNP-BSA–specific IgE (0.5 μg) was intradermally injected into a mouse ear, followed by PR dose treatments ranging from 100 to 1000 mg/kg or 50 mg/kg diphenylhydramine (DPH) as a control drug 24 h later. One hour after the oral treatment of either vehicle or PR, the mice were then challenged with an intravenous injection of a 250-μg antigen (DNP-BSA) in 250 μl PBS containing 4% Evans blue (Sigma-Aldrich). The mice were then euthanized an hour after the challenge of antigen, followed by the removal of an ear for the measurement of the amount of dye extravasated by the antigen. The dye was extracted overnight from the ear in 700 μl formamide at 63°C as described by a previous report (16). The intensity of the absorbance was measured at 620 nm.

High-Performance Liquid Chromatography (HPLC) Analysis of PR. To analyze the composition of PR, the ethanol extract was dissolved in 50% methanol. Before injection onto HPLC, all the samples were filtrated through a 0.2-μm microspin polyvinylidene fluoride filter (Millipore Corp., Billerica, MA). The components of PR were determined through an HPLC system (Gilson Unipoint Chromatography Data System; Gilson, Inc., Middleton, WI) equipped with 321 pumps and a UV/Vis-151 detector. The constituents of the extract were separated using Shiseido CAPCELL PAK C18 MG 5 μm (4.6 × 150 mm) at the flow rate of 1.0 ml/min. The mobile phase was acetonitrile, with the following gradient programs: isocratic at 15% acetonitrile for 5 mins; linear gradient to 30% acetonitrile for 15 mins; linear gradient to 80% acetonitrile for 20 mins; and isoclatic at 80% acetonitrile for 5 mins. The analytes were detected through UV absorption at 346 nm.

Statistical Analysis. The data were presented as the mean ± SEM from three or more separate experiments. Statistical analysis was performed using one-way analysis of variance and the Dunnett's test. All statistical calculations (*, $P < 0.05$ and **, $P < 0.01$) were performed using the SigmaStat software (Systat Software, Inc., Point Richmond, CA).

Results

Effects of PR on Antigen-Induced Degranulation in Mast Cells and Reversibility of the Inhibition. Two kinds of mast cells were used to measure the *in vitro* antiallergic activity of PR. The RBL-2H3 cells are a type of mast cell isolated from rats; they have the properties of mast cells (17). The primary BMMCs were isolated from the bone marrow of mice, as described in the "Methods" section, and the expression of FcεRI in the cells was analyzed through immunoblot analysis. During the continuous search for novel antiallergic agents from natural products (18, 19), assay systems were employed for the screening of potential suppressors of degranulation in cultured mast cells and primary BMMCs. As shown in Figure 1A, the FcεRIβ subunit was expressed in the isolated BMMCs, which is comparable to the expression level of RBL-2H3 mast cells. The cells were stimulated well by the DNP-BSA antigen after they were primed with DNP-specific IgE (Fig. 2B). Therefore, the isolated cells were verified as the bone marrow–derived mast cells (BMMCs) by the above results (Fig. 1A and B). PR significantly suppressed antigen-induced degranulation in a dose-dependent manner in both the RBL-2H3 cells and the BMMCs (Fig. 1B). The IC_{50} values were approximately 62.0 ± 2.1 μg/ml and 46.0 ± 3.2 μg/ml, respectively. Next, it was determined whether the inhibitory activity of PR was reversible. The RBL-2H3 cells were washed out twice with a PIPES buffer after incubating with PR for 1 hr, and the remaining activity was measured. As shown in Figure 1C, the degranulation of the mast cells was recovered by washing out with a PIPES buffer. These results indicate that the inhibitory activity of PR is reversible.

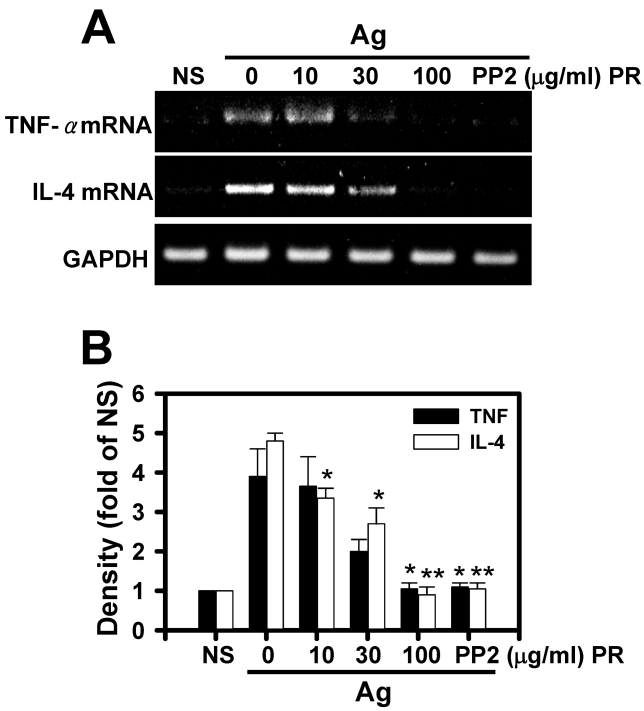

Figure 2. Effects of PR on the expression of TNF-α and IL-4 mRNA in mast cells. (A) The indicated amounts of PR were added to the RBL-2H3 cultures 30 mins before the addition of 25 ng/ml DNP-BSA, or were left unstimulated (NS) after incubating overnight with 20 ng/ml DNP-BSA-specific IgE. The cells were stimulated for 15 mins for the assay of TNF-α mRNA by RT-PCR. The results were representative gel pictures from the three independent experiments. (B) Band densities are expressed as mean ± SEM for the three independent experiments. PP2 is a general SFK inhibitor.

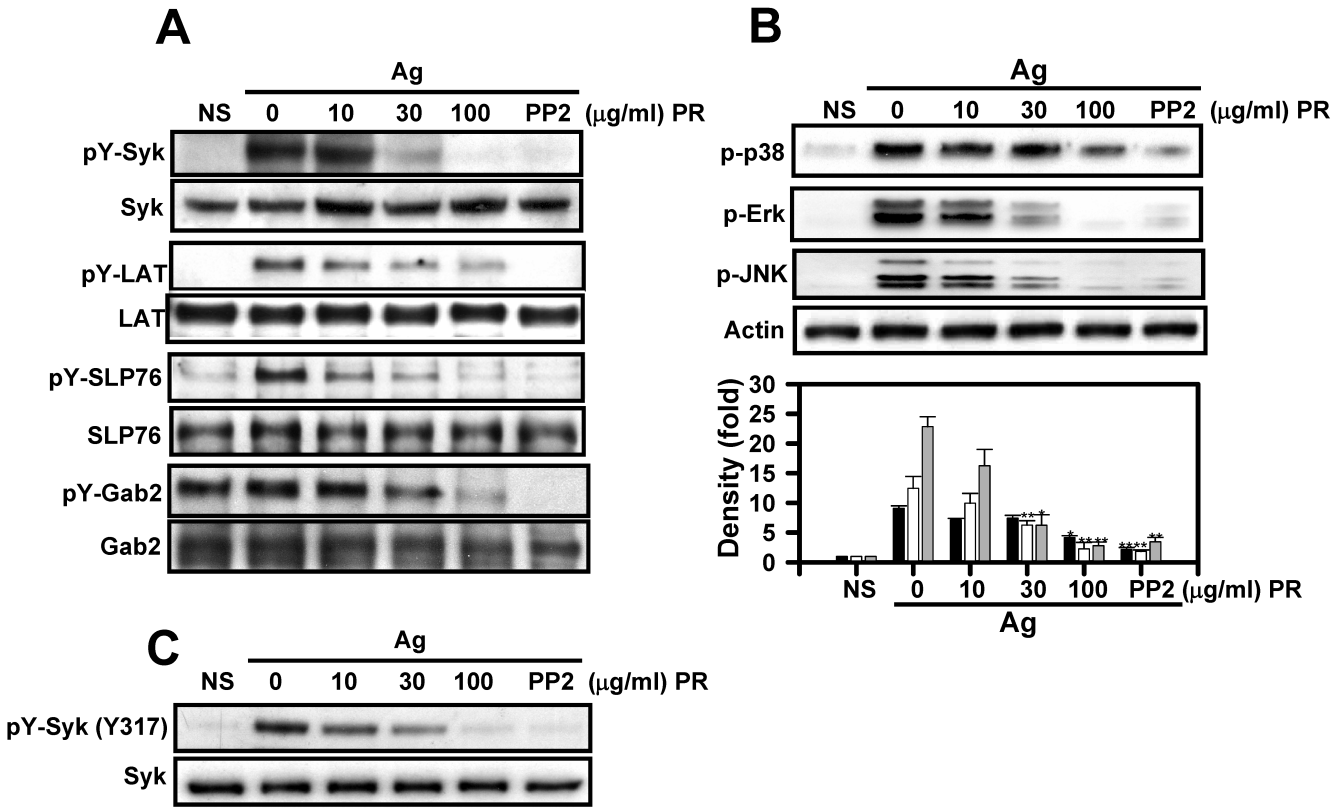

Figure 3. Effect of PR on the activating phosphorylation of Syk and MAP kinases. (A) The RBL-2H3 cells were incubated overnight in 6-well plates, with 50 ng/ml DNP-specific IgE, in a complete growth medium. The cells were stimulated with 25 ng/ml DNP-BSA, with or without PR, for 7 mins. Syk, LAT, SLP-76, and Gab2 were immunoprecipitated with the specific antibodies, and the immunoprecipitated proteins were subjected to immunoblot analysis to detect phosphorylated or total proteins. (B) The proteins derived from the RBL-2H3 cell lysates after antigen stimulation for 7 mins were subjected to immunoblot analysis to detect phosphorylated forms of MAP kinases. Band densities are expressed as mean ± SEM for the three independent experiments. (C) The BMMCs were incubated with 0.5 μg/ml DNP-specific IgE in a complete growth medium overnight. The cells were stimulated with 25 ng/ml DNP-BSA, with or without PR, for 7 mins. The proteins derived from cell lysates were subjected to immunoblot analysis to detect the phosphorylated forms (Y317) of Syk. Representative blots are shown. PP2 is a general SFK inhibitor.

Effect of PR on the Expression of TNF-α and IL-4 mRNA. Accumulated evidences suggest that various cytokines, including TNF-α and IL-4, are critical for allergic inflammation (20). Whether or not PR suppressed the expression of TNF-α and IL-4, which are produced by antigen stimulation in mast cell–dependent inflammatory events, was investigated. PR was found to have significantly inhibited the antigen-stimulated expression of TNF-α and IL-4 mRNA in the cells in a dose-dependent manner (Fig. 2). The inhibitory potency of 100-μg/ml PR was similar to that of 20-μ*M* PP2, a typical SFK inhibitor.

Effects of PR on Cellular Signaling Molecules in an IgE-Mediated Signaling Pathway. To identify the mechanism of the action of PR, the effects of PR on the activating phosphorylation of Syk kinase and the downstream signaling molecules in the cells, including LAT, SLP-76, and Gab2, were tested. As shown in Figure 3A, the activating phosphorylation of Syk kinase, LAT, SLP-76, and Gab2 were significantly suppressed by PR (Fig. 3A). MAP kinases are also important for the production of cytokines, including TNF-α and IL-4. The activating phosphorylation of the major MAP kinases p38, Erk1/2, and JNK was also significantly suppressed by PR in a dose-dependent manner (Fig. 3B). Next, whether the effect of PR on the inhibition of Syk phosphorylation was mediated by the inhibition of Lyn activity was determined using the specific antibody against phosphorylation on tyrosine 317 of Syk. The phosphorylation of Syk on tyrosine 317 was found to be dependent on SFKs, mostly Lyn, the first kinase to be activated (21, 22). As shown in Figure 3C, PR and the control compound, PP2, a general SFK inhibitor, potently inhibited the phophoryation of Syk (Y317). This result strongly suggests that the effect of PR on the activating phosphorylation of Syk is mediated through the inhibition of SFK in mast cells.

Effect of PR on Mast Cell–Mediated Allergic Reaction in Mice. The above *in vitro* results led these researchers to measure the *in vivo* antiallergic activity of PR by using a local allergic reaction that is mediated by mast cells in mice. As seen in the previous report (16), the PCA was successfully induced by IgE and antigen injection in mice, as described in the "Methods" section. Consistent with the above *in vitro* results, PR inhibited the PCA in a dose-dependent manner (Fig. 4). The potency of the activity

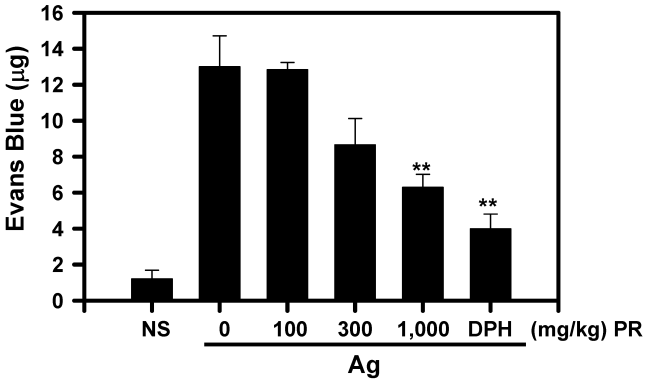

Figure 4. Effect of PR on a mast cell–mediated allergic reaction. An anti-DNP-BSA–specific IgE (0.5 μg) was intradermally injected into a mouse ear. An injection of antigen, 250 μg DNP-BSA (1 μg/ml in a PBS containing 4% Evans blue), was administered 24 hrs later into the mouse tail vein. The PR dose ranged from 100 to 1000 mg/kg, and was administered 1 hr before the treatment of antigen. The mouse died 1 hr after the challenge of antigen was introduced, and the ear was removed for the measurement of the amount of dye extravasated by antigen treatment. The values are expressed as mean ± SEM from the three independent experiments.

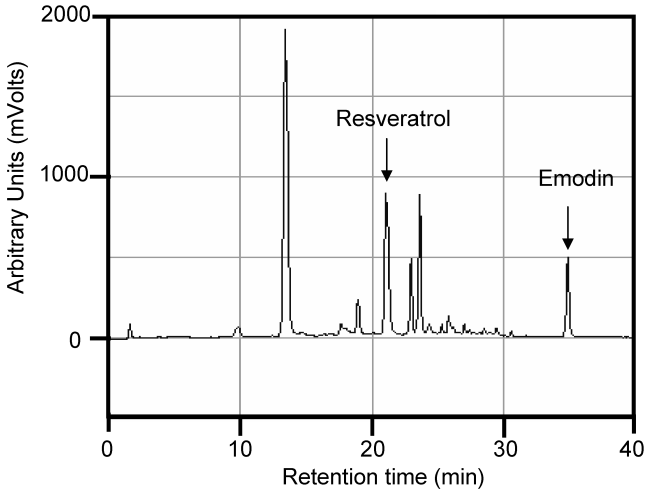

Figure 5. HPLC chromatogram of PR. The components of PR were determined through an HPLC system equipped with 321 pumps and a UV/Vis-151 detector. The constituents of the extract were separated, using Shiseido CAPCELL PAK C18 MG 5 μm (4.6 × 150 mm), at a flow rate of 1.0 ml/min. The mobile phase was acetonitrile, with the following gradient programs: isocratic at 15% acetonitrile for 5 mins; linear gradient to 30% acetonitrile for 15 mins; linear gradient to 80% acetonitrile for 20 mins; and isoclatic at 80% acetoitrile for 5 mins. The analytes were detected through UV absorption at 346 nm. Authentic resveratrol and emodin were used to identify the peaks.

of PR is similar to that of DPH, a typical anti-histamine drug (Fig. 4).

HPLC Analysis of PR. The stem and root of crude drugs contain polydatin, emodin, resveratrol, anthraglycosides, physcion, rhein, and others (23, 24). Among these, resveratrol, which can also be found in grapes (25), has been reported to have activities lowering the cholesterol level, as well as an anti-inflammatory property (26). Emodin can act as a strong inhibitor of protein tyrosine kinase in cancer cells (27). PR was analyzed to identify resveratrol and emodin, as previously reported (23). Consistent with the previous report, resveratrol and emodin were identified, and the retention times of both were found to be 21.1 and 34.8 mins, respectively (Fig. 5).

Discussion

Type I hypersensitivity allergic reaction is induced by the rapid local and systemic release of allergic inflammatory mediators such as histamine, serotonin, heparin, and various pro-inflammatory cytokines from mast cells located throughout the human body (28). In this study, PR inhibited degranulation, the release of several allergic mediators in mast cells, RBL-2H3 cells, and BMMCs as a function of PR dose (Fig. 1), indicating that PR inhibited several signaling molecules in the cells, including Syk, LAT, and SLP-76 (Fig. 3A) and MAP kinases (Fig. 3B). Mast cells are stimulated by IgE-mediated antigen *via* the high-affinity receptor for IgE, FcεRI. The cross-linking of the IgE/FcεRI complex with antigen activates the SFKs, such as Lyn and Fyn, and subsequently other signaling proteins, including Syk, LAT, SLP-76, Gab2 (3), and phospholipase (PL) D2 (29). It is well known that Syk plays a key role in the activation of mast cells and is an upstream kinase for the activation of LAT, SLP-76, and Gab2 (3). Mast cells are primarily known for their critical role in the induction of allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, and anaphylaxis (1, 2). As such, many studies have been conducted to search for therapeutic targets for the cure of allergic diseases using tyrosine kinase inhibitors to suppress the antigen-induced FcεRI signaling (30). In this study, PR was identified as an inhibitor in the activating phosphorylation of Syk in the cells, most probably through the inhibition of SFKs, especially Lyn (Fig. 3A and C). SFK is the upstream kinase for the phosphorylation of Syk in the cells. The results of this study suggest that PR is an inhibitor of SFK, preventing the activation of Syk. The possibility, however, that PR inhibited the autophosphorylation of Syk in the cells could not be discounted (31). More interestingly, it has been reported that PLD2 has the function of an adaptor molecule for the full phosphorylation and activation of Syk in mast cells (32). Therefore, the possibility that PR inhibits the role of PLD2 for Syk activation remains wide open.

The activated mast cells in this study also produced many cytokines, including IL-1, –3, –4, –5, and –6; TNF-α; and the granulocyte-macrophage colony-stimulating factor. Such cytokines are said to mediate pathogenic inflammatory symptoms at the latter stages of an allergic reaction (20, 33). ERK1/2 was reported to be an essential signal in the production of IL-5, TNF-α, IL-3, and IL-13 in mast cells (34), and in the production of TNF-α in the RBL-2H3 mast cells, depending on the activation of the ERK2 cascade (35). In addition, the production of IL-4 is positively regulated by p38 MAP kinase in BMMCs (36). Based on the findings that TNF-α and IL-4 expression, and the activation of three

typical MAP kinases, were suppressed by PR (Fig. 3B), it was concluded that the inhibition of TNF-α and IL-4 expression by PR is highly correlated with the inhibition of the MAP kinase cascade.

In summary, it was reported for the first time in this study that PR inhibited degranulation in BMMCs and RBL-2H3 mast cells, as well as a local allergic anaphylaxis, as a function of PR dose. In addition, PR inhibited the expression of TNF-α and IL-4 expression, possibly through the inhibition of MAP kinase. The results indicate that PR exhibits antiallergic activity through the inhibition of Syk activation in mast cells, most possibly through the inhibition of SFK and/or PLD2, and that it can be used to treat acute and chronic IgE-mediated allergic diseases.

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