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Suppressive Effects of *Scutellaria radix* Water Extract on FcεRI Expression

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Abstract The initiation of immunoglobulin E (IgE)-mediated allergic reactions requires binding of IgE antibody to its high-affinity receptor, FcεRI. Human basophilic KU812F cells express FcεRI on the cell surface and act as effector cells in the allergic response. In this study, we investigated the effects of *Scutellaria radix* extract on the expression of the FcεR1 in human KU812F cells. Flow cytometric analysis showed that *S. radix* extract treatment caused a concentration-dependent decrease in FcεRI expression on the cell surface. Furthermore, the level of FcεRI α, β, and γ chain mRNA in KU812F cells was examined by RT-PCR. *S. radix* extract reduced total cellular FcεRI α and γ chain mRNA expression in a concentration-dependent manner. FcεRI-mediated histamine release was reduced from 21.75±1.34 ng/10^6 cells in non-treated cells to 16.46 ±1.98 ng/10^6 cells in *S. radix* extract treated cells. These results suggested that *S. radix* extract has the potential to down-regulate of FcRI expression and to inactivate basophils.

Keywords: *Scutellaria radix*, IgE, FcεRI, histamine

Introduction

Epidemiological studies have shown that allergic disorders including food allergies are increasing worldwide with changes in life style and increased environmental pollution and stress (1, 2). The high-affinity IgE receptor FcεRI is found on the surface of basophils and mast cells of the immune systems that initiate cellular reactions associated with the allergic response such as atopic dermatitis, bronchial asthma, allergic rhinitis, and food allergy. The cross-linking of the cell bound IgE-FcεRI complex with specific antigens leads to the release of inflammatory mediators such as histamine, prostaglandins, and leukotrienes from activated mast cells and basophils. The FcεRI molecule on effector cells is a tetrameric complex of a single IgE-binding α subunit, a single β subunit, and two disulfide-linked γ subunits. The α chain of FcεRI extends out to the extracellular region, binds to the Fc portion of IgE with high affinity, and initiates allergic responses (3, 4). Thus it is expected that the suppression of FcεRI in basophils and mast cells may lead to a decrease in IgE-mediated allergic disease.

Scutellaria radix, a root of *Scutellaria baicalensis* Georgi (Labiatac), is known to be a medicinal plant with antimicrobial (5), antibacterial (6, 7), anticancer (8), anti-inflammatory (9, 10), and antioxidative (11) properties. The active components, which have various physiological and pharmaceutical activities, are known to be baicalein, baicalin, neobaicalin, oroxylin A, wogonin (5,7-dihydroxy-8-methoxyflavone), and wogonosides (12-14). Historically, *S. radix* has been used for the treatment of respiratory tract infection, diarrhea, jaundice, hepatitis, fever, and cancer. Koda *et al.* (15) reported an anti-allergic effect of *S. radix* against type I and IV hypersensitivity reactions. However, an investigation into the regulation of FcεRI, which is expressed on the cell surface of basophils and mast cells (the effector cells in the immune system), has not yet been carried out. Therefore, in this study, we examined whether *S. radix* extract has any suppressive effect on FcεRI expression in human basophilic KU812F cells.

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Materials and Methods

Reagents RPMI-1640 medium was purchased from Nissui Pharmaceutical Co. (Tokyo, Japan). Fetal bovine serum (FBS) and antibiotics and antimycotics were purchased from Gibco BRL (Gaithersburg, MD, USA). Anti-human FcεRI antibody (CRA-1) was purchased from Kyokuto (Tokyo, Japan). Fluorescence isothiocyanate (FITC)-conjugated $F(ab')_2$ goat anti-mouse IgG was purchased from Jackson ImmunoResearch Lab (West Grove, PA, USA). TRI_{ZOL} reagent and Superscript II reverse transcriptase were purchased from Invitrogen (Carlsbad, CA, USA). Taq DNA polymerase was purchased from Roche (Mannheim, Germany). All other reagents, including hydroxyethy-l piperazinylethanesulfonic acid (HEPES), L-glutamine, histamine, and O-phthalaldehyde (OPA) were purchased from Sigma Chemical Co., (St. Louis, MO, USA).

Preparation of hot-water extract from *S. radix* *S. radix* was obtained from the Hapcheon, Gyeongnam, Korea. The dried and powdered roots of *S. radix* were added to 10 volumes of distilled water and boiled under reflux for 24 hr. After centrifugation at 900×g for 10 min, the supernatant was filtered, concentrated under vacuum, lyophilized, and stored at -20°C. The lyophilized extract was dissolved in PBS and filtered through a 0.45 μm

membrane filter before use.

Cell culture and treatment Human basophilic KU812F cells were obtained from the Japanese Collection of Research Biosources (JCRB; Osaka, Japan). The cells were maintained in an RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 10 mM HEPES, antibiotics and antimycotics, cultured at 37°C in a humidified atmosphere with 5% CO_2 and passaged every 3-4 days. KU812F cells were cultured in serum-free RPMI-1640 medium with or without hot-water extract of *S. radix*.

Flow cytometric analysis Expression of the cell surface FcεRI was assessed by indirect immunofluorescence and flow cytometry. In brief, KU812F cells (1×10^6 cells) were incubated with anti-human FcεRI α chain antibody (10 μg/mL) for 60 min on ice. The cells were then stained with FITC-conjugated $F(ab')_2$ goat anti-mouse IgG (20 μg/mL) for 60 min on ice. After washing with ice-cold PBS, the cells were subjected to flow cytometry. As a negative control, a mouse IgG antibody (10 μg/mL) was used instead of anti-human FcεRI α chain antibody.

Reverse transcription and polymerase chain reaction (RT-PCR) Total cellular RNA was isolated using TRI_{ZOL} reagent according to the manufacturer's instructions. For cDNA synthesis, total RNA was reverse transcribed using an oligo $(dT)_{20}$ primer and SuperScript II reverse transcriptase. The resulting cDNA sample was amplified in the presence of specific sense and anti-sense primers. Human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as an internal control. Primer sequences used in this study were as follows: for the FcεRI α chain, sense 5'-CTTAGGATGTGGGTTCAGAA GT-3' and antisense 5'-GACAGTGGAGAATACAAATGTCA-3'; for the FcεRI β chain, sense 5'-TAATTCTTCATAAAGACGATCATCNGG-3' and antisense 5'-ATATGCCTTTGTTTTGGAACAATG GTGTG-3'; for the FcεRI γ chain, sense 5'-TAGGGCCAG CTGGTGTTAATGGCA-3' and antisense 5'-GATGATTC CAGCAGTGGTCTTGCT-3'; for the G3PDH, sense 5'-GC TCAGACACCATGGGGAAGGT-3' and antisense 5'-GT GGTGCAGGAGGCATTGCTGA-3'. The PCR was performed as follows; 94°C, 30 sec denaturing; 55°C, 30 sec annealing; and 72°C, 1 min extension. The amplified PCR products were analyzed by agarose gel electrophoresis.

Histamine measurement Histamine content was measured by fluorometric assay (16). KU812F cells (1×10^6 cells/mL) were treated with 100 μg/mL of hot-water extract of *S. radix* for 24 hr. The treated cells were washed, suspended in 200 μL of PBS containing 0.1% BSA, and stimulated with 20 μg/mL of CRA-1 at 37°C for 30 min. After centrifugation at 900×g for 5 min, the supernatant was incubated with 1 N NaOH and 0.2% OPA solution for 40 min on ice, and the reaction was terminated by addition of 3 N HCl. The intensity of the fluorescence was measured at an excitation wavelength of 360 nm and an emission wavelength of 450 nm. Positive and negative controls were used to indicate histamine content obtained with or without CRA-1.

Statistical analysis The Student *t* test was used for the statistical analysis.

Results and Discussion

Cell surface expression of the high-affinity IgE receptor, FcεRI Mast cells and basophils express FcεRI on their cell surface and play an important role in IgE-mediated allergic reactions. KU812F cells are a human basophilic cell line originally isolated from chronic myelocytic leukemia that expresses a high affinity receptor of IgE antibody (17).

S. radix is one of the traditional medicinal herbs for the treatment of allergic disease, inflammatory disease, hyperlipemia, and hepatitis (15, 18-20). In the present study, we investigated whether hot-water extract of *S. radix* had an inhibitory effect on the cell surface FcεRI in human basophilic KU812F cells. The cell surface FcεRI expression in KU812F cells treated with various concentrations of hot-water extract from *S. radix* for 24 hr under

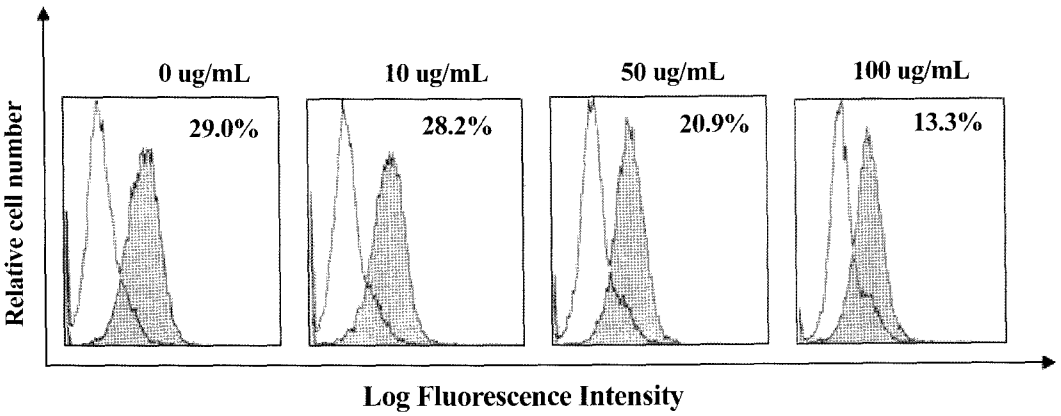

Fig. 1. Effects of hot-water extract of *S. radix* on the cell surface expression of FcεRI. KU812F cells were cultured in the presence of various concentrations of hot-water extract of *S. radix* (0, 10, 50, 100 μg/mL) for 24 hr. Then cells were incubated with CRA-1 followed by staining with the FITC-conjugated $F(ab')_2$ goat anti-mouse immunoglobulin (gray). Mouse IgG was used as an isotype-matched control antibody (white). Stained cells were then analyzed by flow cytometry. The percentage of cells expressing the cell surface FcεRI is shown in each figure.

serum-free conditions was assessed by flow cytometric analysis. As shown in Fig. 1, the FcεRI expression on the cell surface was 29.0, 28.2, 20.9, and 13.3% when the cells were treated with 0, 10, 50, and 100 μg/mL of extract, respectively.

FcεRI α, β, and γ chain mRNA expression The suppression of FcεRI cell surface expression by *S. radix* could be due to a decrease in the amount of total cellular FcεRI α, β, and γ chain gene expression. We used RT-PCR to examine this possibility. After treatment with various concentrations of hot-water extract of *S. radix*, total cellular RNA was isolated from the cells, and the mRNA levels of FcεRI α, β, and γ chains were analyzed by RT-PCR using specific primers. As shown in Fig. 2, the FcεRI α and γ chain mRNA expression of non-treated cells was clearly detected, and the corresponding mRNA expression in *S. radix*-treated cells was reduced. On the other hand, FcεRI β chain mRNA was not detected under any of the conditions. Thus, these results suggested that the suppressive effects of hot-water extract from *S. radix* on the cell surface expression of FcεRI was associated with the down regulation of the expression of the FcεRI α and γ mRNA levels. FcεRI found on mast cells and basophils is a tetrameric complex of a single α, a single β, and two γ subunits. The α chain is necessary for the functioning cell surface FcεRI, and is expressed in FcεRI-positive cells (3, 4). We suggest that the suppressive effect of hot-water extract of *S. radix* is related to FcεR1 α and γ chain gene expression. Nishiyama *et al.* (21, 22) reported that FcεRI α chain gene expression in rodents and other mammals including humans is regulated by at least two transcription factors, GATA-1 and Elf-1. Therefore, to better understand the suppressive mechanism of FcεRI expression by hot-water extract of *S. radix* extract, further studies on the regulation of the transcription of the gene-encoding human FcεRI α chain are necessary.

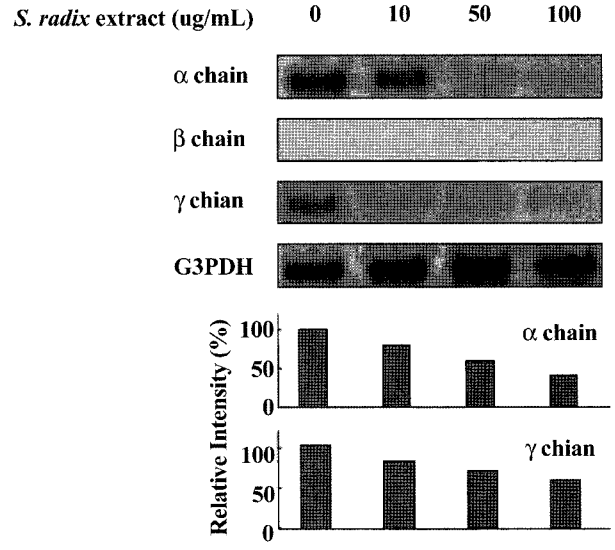

Fig. 2. Effects of hot-water extract of *S. radix* on FcεRI α, β, and γ chain mRNA expression. KU812F cells were cultured in 0, 10, 50, 100 μg/mL of hot-water extract of *S. radix* for 24 hr under serum-free conditions. Total cellular RNA was isolated from the cells, and FcεRI α, β, γ, and G3PDH mRNA levels were analyzed by RT-PCR. G3PDH was used as an internal control.

Histamine release It is well known that the activation of basophils and mast cells through the FcεRI is triggered by aggregation of the FcεRI, which is bound to allergen specific IgE on the cell surface, and degranulation, resulting in a release of mediators such as histamine (23-25). Recently, it is reported that natural products have anti-inflammatory activity (26, 27). KU812F cells released histamine when the cells were stimulated with human IgE and anti-human IgE antibody (28). We assessed whether histamine release from KU812F cells due to stimulation with the FcεRI specific antibody, CRA-1, can be suppressed by hot-water extract of *S. radix*. KU812F cells were cultured with 100 μg/mL of hot-water extract of *S. radix* and stimulated with 10 μg/mL of anti-FcεRI antibody. The amount of histamine released from the cells was determined by a fluorometric assay (Fig. 3). The value of FcεRI-mediated histamine release was 21.75±1.34 ng/10^6 cells in non-treated cells and 16.46±1.98 ng/10^6 cells in hot-water extract of *S. radix*-treated cells. This result demonstrated that the FcεRI-mediated histamine release by basophils was down-regulated by hot-water extract of *S. radix* and indicated that hot-water extract of *S. radix* inhibits degranulation of basophils. Thus, hot-water extract from *S. radix* might negatively regulate the activation of basophils through the suppression of FcεRI expression and so contribute to the attenuation of allergic reactions.

S. radix is a traditional medicinal plant widely used for treatment of allergic disease, inflammatory disease, cancer, bacterial and viral infections, hepatitis, and hyperlipemia, (5-11, 18-20). It has been reported that *S. radix* contains bioactive components such as baicalein, wogonin, oroxylin A, and baicalein with various pharmaceutical properties (12-14). Therefore, in order to further understand the suppressive mechanism of FcεRI expression by the *S. radix*, studies of its bioactive components need to be performed.

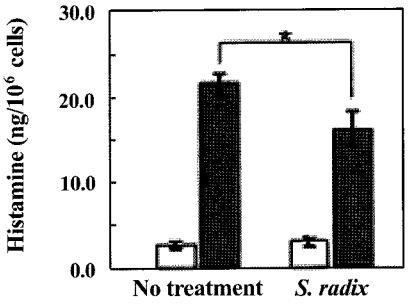

Fig. 3. Effects of hot-water extract from *S. radix* on histamine release from KU812F cells. KU812F cells were cultured in the presence of hot-water extract of *S. radix* (100 μg/mL) for 24 hr under serum-free conditions. KU812F cells were stimulated with anti-FcεRI monoclonal antibody (CRA-1) for 30 min (gray). The negative control used was as an isotype-matched control antibody (white). The amount of histamine was measured by fluorometric assay. Values are expressed as the mean±SD (n=3). *$p<0.05$.

Acknowledgments

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