

## Inhibitory Effects of *Acanthopanax chiisanensis* Ethanolic Extracts on FcεRI α Chain Expression

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Basophils and mast cells play an important role in FcεRI-mediated allergic reaction as effector cells. We studied the effects of *Acanthopanax chiisanensis* on FcεRI α chain expression in human basophilic KU812F cells. Ethanol extracts from root and stem of *A. chiisanensis* were tested for inhibitory effects of FcεRI α chain expression. The cell surface FcεRI α chain expression was examined by flow cytometric analysis. All of the extracts of *A. chiisanensis* reduced the cell surface FcεRI α chain expression. Furthermore, *A. chiisanensis* extracts caused a decrease in the level of FcεRI α chain mRNA level and FcεRI-mediated histamine release. These results suggest that root and stem extracts of *A. chiisanensis* play an important role in anti-allergic activity via down-regulation of FcεRI α chain expression and decrease in release of inflammatory mediator such as histamine.

**Key words** : *Acanthopanax chiisanensis*, FcεRI, histamine, KU812F

### Introduction

Allergic diseases such as pollen allergy, atopic dermatitis, bronchial asthma, and allergic rhinitis has increased in many industrialized countries with changes of life style, increment of environmental pollution and stress [5]. Human basophils and mast cells express a high affinity IgE receptor, FcεRI on the cell surface. A cross-linking of two adjacent FcεRI molecules attached allergen-specific IgE leads to release of inflammatory mediator including histamine, leukotrienes, and prostaglandins from activated basophils and mast cells [2,4,10,16,25]. FcεRI molecules is tetrameric complex and consist of one α chain, one β chain and two γ chains. The α chain of FcεRI extends out to the extracellular region, bind to the Fc portion of IgE with high affinity, and initiates allergic reaction [6]. It is expected that down-regulation of FcεRI α chain expression on effector cells will be useful to study physiologically functional substances in allergic diseases.

*Acanthopanax* species are widely distributed in Korea,

Japan, China and belong to the family Araliaceae, and has been traditionally used as tonic, sedative as well as in the treatment of paralysis, ischaemic heart disease, hypertension, rheumatoid arthritis and diabetes mellitus [7,11,13]. The genus *Acanthopanax* is approximately fifteen species, and is known to be self-grown plant in the Korean peninsula. *A. chiisanensis* is one of the most abundant indigenous species, and distributed from 200 to 1400 m altitude from Mt. Jiri. *A. chiisanensis* contains lignans as bioactive components with pharmaceutical properties such as anti-inflammatory, and antiestrogenic activities [1,7-9,11-14,24]. Moreover, the root and stem water extracts of *A. senticosus* have anti-allergic activity through inhibition of mast cell-dependent anaphylaxis [26,27]. However, the regulation of FcεRI expression by *Acanthopanax* species has not been studied. Therefore, in the present study, we investigated whether root and stem extract of *A. chiisanensis* have inhibitory effects on FcεRI α chain expression in human basophilic KU812F cells.

### Materials and Methods

#### Reagents

RPMI-1640 medium, fetal bovine serum (FBS), pen-

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icillin-streptomycin, and TRIzol reagent were purchased from GIBCO BRL (Gaithersburg, MD, USA). Anti-human FcεRI antibody (CRA-1) was purchased from Kyokuto (Tokyo, Japan). Mouse IgG was purchased from Biosources (Burlingame, CA, U.S.A.). FITC-conjugated F(ab')<sub>2</sub> goat anti-mouse IgG was purchased from Jackson Immuno-Research Lab. (Baltimore, PO, USA). Celltiter 96<sup>R</sup> Aqueous one solution cell proliferation assay, oligo dT primer, reverse transcriptase and Taq DNA polymerase were purchased from Promega (Madison, WI, USA). Hydroxyethyl piperazinylethanesulfonic acid (HEPES), histamine and o-phthalaldehyde (OPA) were purchased from Sigma Chemicals (St. Louis, MO, USA).

#### Preparation of *A. chiisanensis* extract

The dried root and stem of *A. chiisanensis* were purchased from Jirisan Ogalpi Farm Association Corporation. The dried root and stem of *A. chiisanensis* were grinded, and were added to 10 volumes of ethanol, and extracted at 35°C for 24 hr. The extract was filtered, evaporated under vacuum, and stored at -20°C. The extract was dissolved in DMSO before using.

#### Cell culture and determination of cell viability

The human basophilic KU812F cells were received from Dr. Shirahata in Kyushu University in Japan. The cells were maintained in a RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 10 mM HEPES, cultured at 37°C in a humidified atmosphere with 5% CO<sub>2</sub> and passaged every 3-4 days. Cells were cultured in serum-free RPMI-1640 medium with or without root and stem extracts of *A. chiisanensis* with different concentration for 24 hr. Cell viability was spectrophotometrically evaluated at 490 nm using Celltiter 96<sup>R</sup> Aqueous one solution cell proliferation assay.

#### Flowcytometric analysis

The cell surface FcεRI expression was measured by flowcytometric analysis. KU812F cells (1×10<sup>6</sup> 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')<sub>2</sub> goat anti-mouse IgG (20 μg/ml) for 60 min on ice, followed by washing with ice-cold PBS. As a negative control, a mouse IgG antibody (10 μg/ml), replacing anti-human FcεRI α-chain antibody was used.

#### Reverse-transcriptase polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated using TRIzol reagent according to the manufacturer's instruction. For cDNA synthesis, 1 μg of total RNA was reversely transcribed using a oligo (dT)<sub>15</sub> primer and reverse transcriptase. The resultant cDNA samples were subjected to PCR amplification in the presence of specific sense and antisense primers. Human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as an internal control. Primer sequences used in this study were as follow, for the FcεRI α-chain, sense 5'-CTTAGGATGTGGGTTTCAGAAGT-3' and antisense 5'-GACAGTGGAGAATACAAATGTCA-3'; for the G3PDH, sense 5'-GCTCAGACACCATGGGGAAGGT-3' and antisense 5'-GTGGTGCAGGAGGCATTGCTGA-3'. The PCR was performed as follows: 94°C, 30 sec denaturing; 55°C, 30 sec annealing; and 72°C, 1 min extension, and subjected to 18 cycles for FcεRI α chain gene. The amplified PCR product was analyzed by agarose gel electrophoresis.

#### Histamine measurement

The histamine content was measured by a spectrofluorometric assay [22]. KU812F cells (1×10<sup>6</sup> cells/ml) were treated with different concentration of *A. chiisanensis* extracts for 24 hr. The treated cells were stimulated with Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH<sub>2</sub>PO<sub>4</sub> · 2H<sub>2</sub>O, 1 mM MgCl<sub>2</sub> · 2H<sub>2</sub>O, 12 mM NaHCO<sub>3</sub>, 1.8 mM CaCl<sub>2</sub> · 2H<sub>2</sub>O) containing 10 μg/ml of anti-human FcεRI α chain antibody at 37°C for 30 min. After centrifugation, 1 N NaOH and 0.2% OPA were added, and incubated for 40 min on ice. The reaction was terminated by addition of 3 N HCl. The fluorescence intensity was measured at the excitation at 360 nm and the emission at 460 nm.

## Results and Discussion

#### Cell viability in KU812F cells

Human basophilic KU812F cells express a high affinity IgE receptor, FcεRI on the cell surface. *A. chiisanensis* is the traditional medicinal plant for the treatment of inflammation, rheumatoid arthritis, and diabetic mellitus, and recognized to have ginseng-like effects [5,8,15].

To determine the non-toxic concentration, human basophilic KU812F cells were treated with various concentration of *A. chiisanensis* extracts, and the viability of these cells was determined by MTS assay. As shown in Fig. 1,

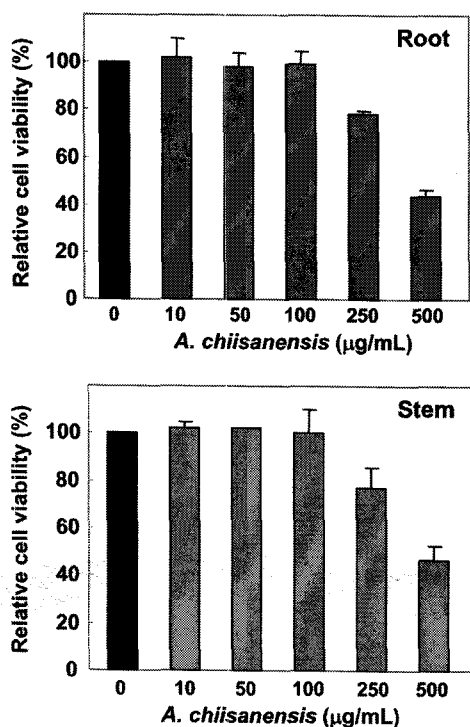


Fig.1. Effects of *A. chiisanensis* extracts on the viability of KU812F cells. KU812F cells ( $5 \times 10^4$  cells) were cultured in serum-free RPMI-1640 medium with or without test compounds for 24 hr and cell viability was measured by MTS assay. The viability of cells is shown as means of  $\pm$  S.D.

Root and stem extracts of *A. chiisanensis* did not show any cytotoxic effects at the concentration range of 1-100  $\mu\text{g/ml}$ .

#### Effects of *A. chiisanensis* extracts on cell surface Fc $\epsilon$ RI $\alpha$ chain expression

Fc $\epsilon$ RI  $\alpha$  chain is the specific component of Fc $\epsilon$ RI, and mostly extends out to the extracellular region and directly binds to IgE. To evaluate the inhibitory effects of root and stem extracts of *A. chiisanensis* on the cell surface Fc $\epsilon$ RI  $\alpha$  chain expression, KU812F cells were treated with different concentration of *A. chiisanensis* extracts for 24 hr under serum-free condition, the cell surface Fc $\epsilon$ RI  $\alpha$  chain expression was assessed by flowcytometric analysis using anti human Fc $\epsilon$ RI  $\alpha$  chain antibody, CRA-1. As shown in Fig. 2, non-treated cells expressed Fc $\epsilon$ RI  $\alpha$  chain expression about 29.8%. However, when the cells were treated with 50 and 100  $\mu\text{g/ml}$ , the Fc $\epsilon$ RI  $\alpha$  chain expression on the cell surface was 21.3 and 18.5% in root extract, and was 20.7 and 17.1% in stem extract. The cell surface Fc $\epsilon$ RI  $\alpha$  chain expression in human basophilic KU812F cells was observed to reduce in root and stem extracts of *A. chiisanensis* - treated cells.

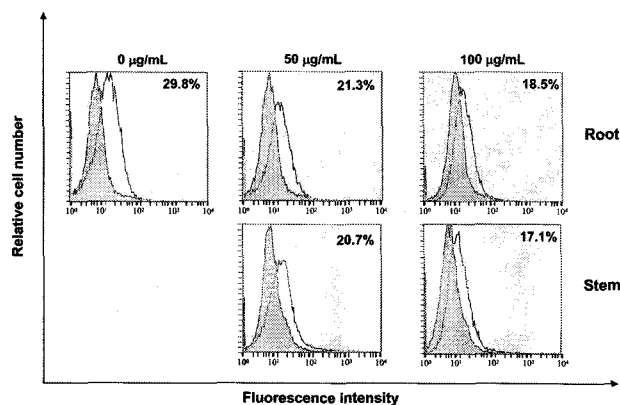


Fig. 2. Effects on the cell surface Fc $\epsilon$ RI  $\alpha$  chain expression. KU812F cells were cultured in the presence of root and stem extracts of *A. chiisanensis* (0, 50, 100  $\mu\text{g/ml}$ ) for 24 hr. The cells were then incubated with anti-human Fc $\epsilon$ RI  $\alpha$ -chain monoclonal antibody (CRA-1) followed by staining with the FITC-conjugated F(ab') $_2$  goat anti-mouse immunoglobulins. Stained cells were then analyzed by flow cytometry. The percentage of Fc $\epsilon$ RI-positive cells were calculated with the arbitrary 2% cutoff position as determined by the negative control. The percentage of cell expressing the cell surface Fc $\epsilon$ RI is shown in each figure, and the value in the figure is the mean fluorescence intensity of CRA-1. The figures are the representatives of three independent experiments.

#### Effects on Fc $\epsilon$ RI $\alpha$ chain mRNA level

To examine the inhibitory effects of Fc $\epsilon$ RI  $\alpha$  chain mRNA levels, KU812F cells were treated with 50  $\mu\text{g/ml}$  extracts for 24 hr under serum-free condition, and Fc $\epsilon$ RI  $\alpha$  chain mRNA level was determined by RT-PCR. As shown in Fig. 3, the Fc $\epsilon$ RI  $\alpha$  chain mRNA level was observed to reduce in root and stem extracts of *A. chiisanensis* - treated cells. The  $\alpha$  chain is expressed in Fc $\epsilon$ RI-positive cells, and is essential for functioning cell surface Fc $\epsilon$ RI [3,10]. We suggest that the inhibitory effect by root and stem extracts of *A. chiisanensis* is related to Fc $\epsilon$ RI  $\alpha$  chain gene expression. Fc $\epsilon$ RI  $\alpha$  chain gene expression is regulated by transcription factors, GATA-1, Elf-1 and PU 1 in rodents and other mammals including human [18,21,23]. To understand the inhibitory mechanism of Fc $\epsilon$ RI expression by root and stem extracts of *A. chiisanensis*, further studies on the regulation of the signal transcription in gene-coding Fc $\epsilon$ RI  $\alpha$  chain need to be performed.

#### Effects on Fc $\epsilon$ RI-mediated histamine release

Cross-linking of Fc $\epsilon$ RI leads to the secretion of inflammatory mediator such as histamine, which is causes

the symptoms of allergic disease [2]. To access whether root and stem extracts of *A. chiisanensis* could inhibit histamine release from anti-FcεRI α chain antibody-sensitized cells, KU812F cells were treated with root and stem extracts of *A. chiisanensis* and histamine content released from the cells was determined by spectrofluorometric assay [22]. As shown in Fig. 4, stem and root extracts of *A. chiisanensis*-treated cells were decreased compared to that of cells treated with DMSO. Root and stem extracts of *A. chiisanensis* inhibited about 22 and 29% at 50 μg/ml, and 36 and 40% at 100 μg/ml, respectively. These results demonstrated that the FcεRI-mediated histamine release by basophils were down-regulated by root and stem extracts of *A. chiisanensis*, and shown that *A. chiisanensis* extracts inhibits degranulation of human basophils. Thus root and stem extracts of *A. chiisanensis* might negatively regulate the activation of

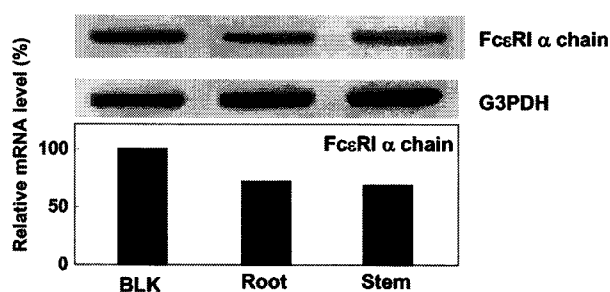


Fig. 3. Effects on FcεRI α chain mRNA level. KU812F cells were cultured in 50 μg/ml of root and stem extracts of *A. chiisanensis* for 24 hr under serum-free conditions, total cellular RNA was isolated from the cells, and FcεRI α and G3PDH mRNA levels were analyzed by RT-PCR.

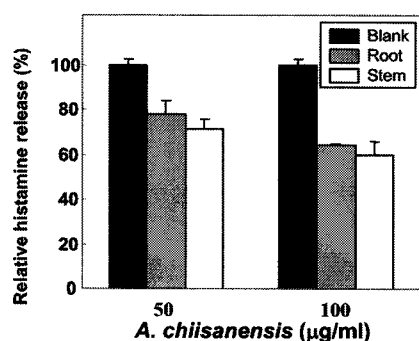


Fig. 4. Effects on FcεRI-mediated histamine release. KU812F cells were cultured in the presence of root and stem extracts of *A. chiisanensis* (0, 50, 100 μg/ml) for 24 hr. The cells were stimulated with CRA-1 antibody for 30 min. Histamine content was determined by a spectrofluorometric method. Each value represents the mean ± S.D of three different experiments.

basophils through inhibition of FcεRI α chain expression and so contribute to the attenuation of allergic reactions. It is reported that *A. chiisanensis* root contains lignans such as with anti-inflammatory and anti-estrogenic activities [1,7-9, 11-14,24]. However, the inhibitory effects of FcεRI α chain expression by root and stem extracts of *A. chiisanensis* in basophils and mast cells have not been studied. Therefore, based on these results, further studies on its biologically active components of *A. chiisanensis* need to be performed for elucidation the inhibitory mechanism of FcεRI expression. In conclusion, our results suggest the suppressive mechanism of FcεRI α chain expression in the presence of *A. chiisanensis* extracts, and these findings may contribute to the increasing number of studies to identify potent antiallergic candidates from medicinal plants.

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**초록 : 지리 오갈피의 FcεRI α chain 발현 저해 효과**

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호염기구와 비만세포는 FcεRI을 매개로 한 알러지 반응에 있어 효과세포로서 중요한 역할을 담당한다. 인간 유래 호염기구성 세포주 KU812F 세포의 FcεRI α chain 발현에 있어 지리 오갈피의 저해 효과에 대해 연구하였다. 지리 오갈피의 뿌리 및 줄기를 에탄올로 추출하여, FcεRI α chain 저해 활성 실험에 이용하였다. 세포 표면의 FcεRI α chain 발현량을 flow cytometry로 분석한 결과, 지리 오갈피 뿌리 및 줄기 추출물에서 세포표면의 FcεRI 발현을 억제하는 효과를 나타냈다. 또한 지리 오갈피 뿌리 및 줄기 추출물은 FcεRI α chain mRNA 발현을 감소시켰으며, FcεRI을 매개로 한 히스타민 유리를 감소시켰다. 이러한 결과는 지리 오갈피의 뿌리 및 줄기 추출물이 FcεRI α chain 발현의 저하 조절 및 히스타민과 같은 염증매개인자의 분비를 저하시킴으로서 항 알러지 활성을 갖는데 중요한 역할을 할 것으로 판단된다.