Research Note

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Inhibitory Effects of Ginsenoside Re Isolated from Ginseng Berry on Histamine and Cytokine Release in Human Mast Cells and Human Alveolar Epithelial Cells

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The berry of *Panax ginseng* significantly inhibited the histamine releases at the concentration of 30 μg/mL (p<0.05) and 10 μg/mL (p<0.01). The ginsenoside Re from ginseng berry was found out to have a potent effect in the experiment of histamin and cytokine release.

Keywords: *Panax ginseng*, Ginseng berry, Ginsenoside Re, Histamine, Cytokine

The root of ginseng (*Panax ginseng* Meyer) has been traditionally used for medicine and food stuff. The primary physiologically active substances of ginseng are ginsenosides, polyacetylenes, ginseng proteins, polysaccharides, and phenolic compounds, etc. [1-4]. Ginsenosides, in particular, have been noticed as the principal effective component of ginseng showing various biochemical and pharmacological efficacies. A number of researchers have studied the components of ginseng since the late 1960s starting with the research of Shibata [5,6], whose research group identified the chemical structures of ginsenoside. The physiological activities of these ginsenosides have been reported to show anti-cancer effects [7], anti-diabetic effects [8], protection of the central nervous system [9], anti-arteriosclerotic and hypertensive effects [10,11], improvement of liver function and clearing of hangovers [12], anti-fatigue and anti-stress effects [13,14], anti-oxidative qualities [15], anti-inflammatory effects [16], promotion of protein synthesis [17], and strengthening the immune system [18]. To examine and identify the efficacies of ginsenosides as mentioned above, a number of biochemical and pharmacological studies have been conducted. Many researchers are still eagerly trying to discover new efficacies of ginsenoside.

Until recently, most of the ginseng research has focused on the components of the ginseng root [19-21]. However, in addition to the ginseng root, the components of the ginseng berry and their pharmacological activities

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have also been reported such as: anti-diabetic [22-24], anti-cancer [25,26], anti-oxidant, anti-aging [27-30], and anti-stress [31], etc. As the interest in the ginseng berry has increased recently, many studies have been carried out. However, the research on the anti-allergic effects of ginseng berries has not been performed. Therefore, the present study investigated the influence on anti-histamine releasing activity of the ginseng berry (except the seeds from the berry), and the active components were purified and identified as ginsenoside Re.

The berry of a 4-year cultivated ginseng plant was collected at Kimjae (cultivated by Gil Kim) in Korea on July 14, 2007. Images of the ginseng berry are shown in Fig. 1. These specimens were stored at the Oriental Medical Food Research Laboratory of Semyung University. Dried ginseng berries (5 kg) were ground to powder and extracted twice with 1 L of 95% ethyl alcohol for 2 h in a water bath (60°C). The extracts were concentrated by a vacuum evaporator (Eyela Co., Tokyo, Japan).

In order to determine the effects of ginseng berries on histamine release, the human leukemic mast cells (HMC-1) were used. The HMC-1 was cultured in Iscove's modified Dulbecco's media containing 10% bovine serum albumin, 2 mM L-glutamine, 100 IU/mL penicillin, and 50 μg/mL streptomycin. The cells were cultured at 37°C in a 5% CO_2 with 95% humidity and were passaged every 2 to 3 d. HMC-1 was subcultured in a 24 well plate and stabilized for 24 h. They were divided into six groups; vehicle-treated blank, control, 30, 10, 3, and 1 μg/mL ginseng berry-treated groups. After 30 min, every well except the blank group was treated with compound 48/80 (1 mg/mL). The same volume of distilled water was treated in the blank wells. After incubation for 15 min, histamine levels of the supernatants were measured. Specific processes of histamine assay were as follows. NaOH (1M) and o-phthaldialdehyde (1 mg/mL in EtOH) were added to all wells. After 4 min, fluorescent measurements were made using a Synergy HT (Bio-Tek Instruments Inc., Winooski, VT, USA).

In order to determine release of inflammatory cytokines on human alveolar basal epithelial cells (A549) by treatment with ginseng berry, cytokines were measured using the RayBio Human Cytokine Antibody Array kit (RayBiotech, Norcross, GA, USA) according to the protocol of the manufacturer. In brief, A549 cells was seeded into a 6 well plate at a density of 2×10^5 cells/well and incubated at 37°C for 24 h. After then, ginseng berry or ginsenoside Re with lipopolysaccharide (LPS) was treated and incubated for 24 h, and the cellular proteins were extracted from the cells using a protein extract buffer. Array membranes were incubated with blocking buffer at room temperature for 30 min to block membranes and blocking buffer was decanted and 1 mL of each protein was added each membrane. After 2 h, each protein was decanted and membranes were washed 5 times with wash buffer I and II. And then, biotin-conjugated anti-cytokines were diluted with blocking buffer and were added and incubated to each membrane for 2 h. Membranes were washed 5 times and incubated with 1,000 fold diluted horseradish peroxidase-conjugated streptavidin for 2 h and then washed 5 times. Enhanced chemiluminescence buffer was added to each membrane and the signals were detected using Fusion chemiluminescence imaging system and analyzed by Bio1D software (Vilber Lourmat, Marne La Vallee, France).

The results were expressed as mean±SD. The data

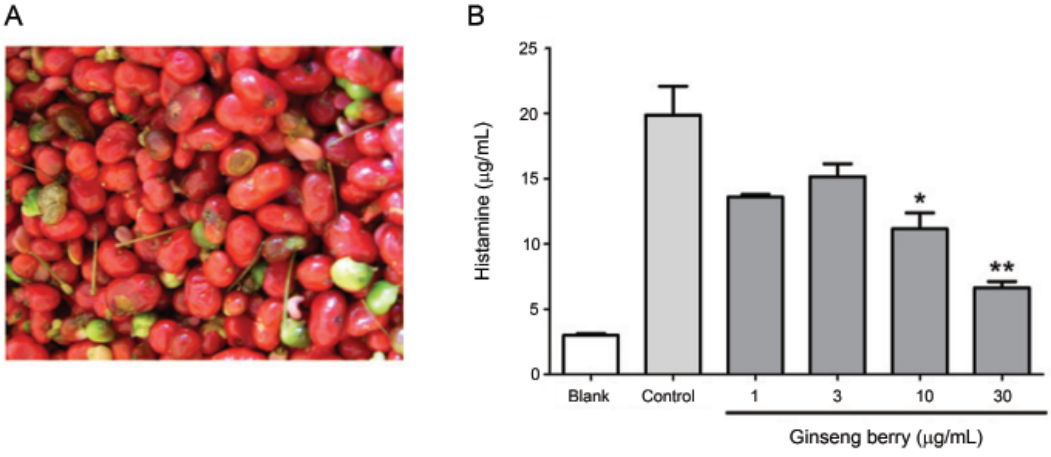

Fig. 1. Photograph of ginseng berry (A) and histamine content of cultured supernatants of human mast cells (B). Blank group was not incubated with compound 48/80, and other experimental groups were incubated with compound 48/80 (*p<0.05, **p<0.01 *vs*. control).

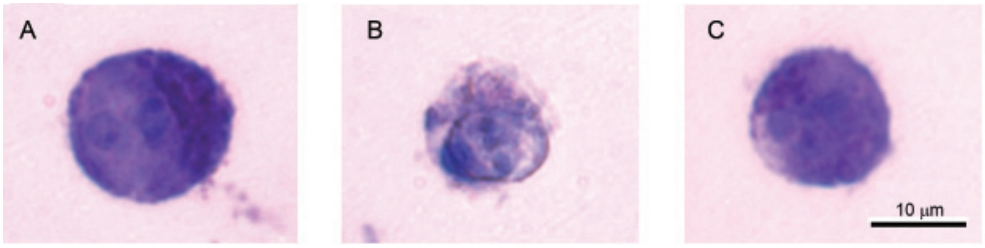

Fig. 2. Microphotographs of degranulation in human mast cells. (A) An unstimulated mast cell. (B) The cell has been activated to secrete its stored histamine by compound 48/80. (C) The extracts of ginseng berry (30 μg/mL) prevented the histamine secretion induced by compound 48/80.

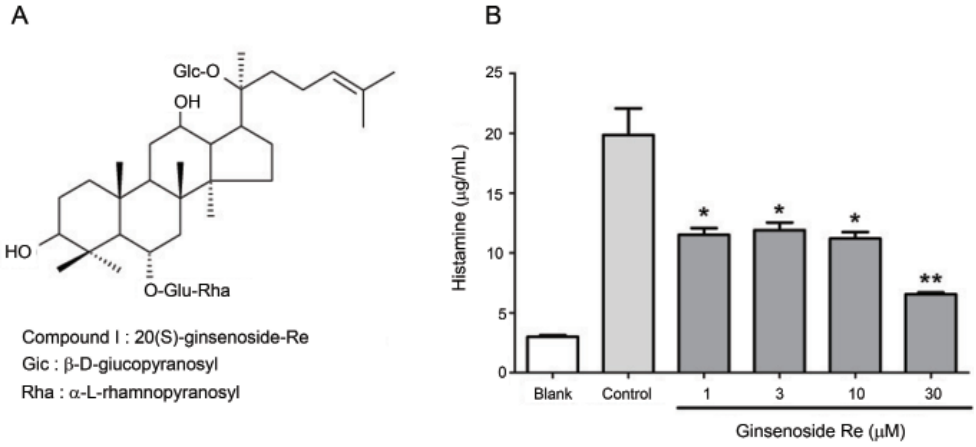

Fig. 3. Chemical structures of compound I (A) and histamine content of cultured supernatants of human mast cells (B). Blank group was not incubated with compound 48/80, however, other experimental groups were incubated with compound 48/80 (*p<0.05, **p<0.01 *vs*. control).

was analyzed by one-way ANOVA, followed by Tukey's *post-hoc* analysis using SPSS (SPSS Inc., Chicago, IL, USA). Differences were considered significant at p<0.05.

Toluidine blue (pH 5.0) staining was performed to observe the cytotoxicity of tested materials, because cell death including apoptosis and necrosis could increase histamine concentration. The degranulations in HMC-1 stimulated with compound 48/80 were observed morphologically after 0.05% toluidine staining (Fig. 2B) whereas not stimulated HMC-1 showed the darker staining of granules in cytoplasm (Fig. 2A). The extract of ginseng berry prevented the degranulation of HMC-1 (Fig. 2C).

As shown in Fig. 1B, the histamine secretion in blank group was 3.0±0.2 μg/mL and it was increased by compound 48/80 treatment to 19.9±3.1 μg/mL in the control group. The compound 48/80-induced histamine secretions were reduced by the ginseng berry treatment in a dose-dependent manner. The histamine secretions at the concentrations of 30, 1, 3, 10, and 30 μg/mL were 13.6±0.3, 15.2±1.4, 11.1±1.7, and 6.6±0.7 μg/mL, respectively (Fig. 1B), with the statistical significances at 30 μg/mL (p<0.01) and 10 μg/mL (p<0.05).

Ninety-five percent EtOH extract of ginseng berry (1,080 g) was dissolved in distilled water and it was partitioned 10 times with diethyl ether resulting in 45 g of diethyl ether fraction and 998 g of water fraction. The water fraction (998 g) was suspended in distilled water and was adsorbed in a Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan) ion exchange resin column. Thirty percent MeOH fraction, 50% MeOH fraction, 70% MeOH fraction, and 100% MeOH fraction were eluted in the order named. The 30% MeOH fraction (10 g) was then subjected to an octadecyl silane (12 nm S-75 um; YMC ODS-A, YMC Co., Kyoto, Japan) gel column by gradient elution with 30% to 100% MeOH and resulted in 4 subfractions (F1-F4). The F3 subfraction (1.2 g) that exerted the highest inhibitory effect on histamine release was rechromatographed on silica gel column with a mixture of the solvents ($CHCl_3$:MeOH:H_2O=70:30:4 v/v) and 0.6 g of compound I was isolated and identified as ginsenoside Re by the spectroscopic methods of ^{1}H-NMR, ^{13}C-NMR, and FAB-MS (Fig. 3A). The isolated ginsenoside Re treatment at concentrations of 1, 3, 10, and 30 μM significantly reduced histamine secretion

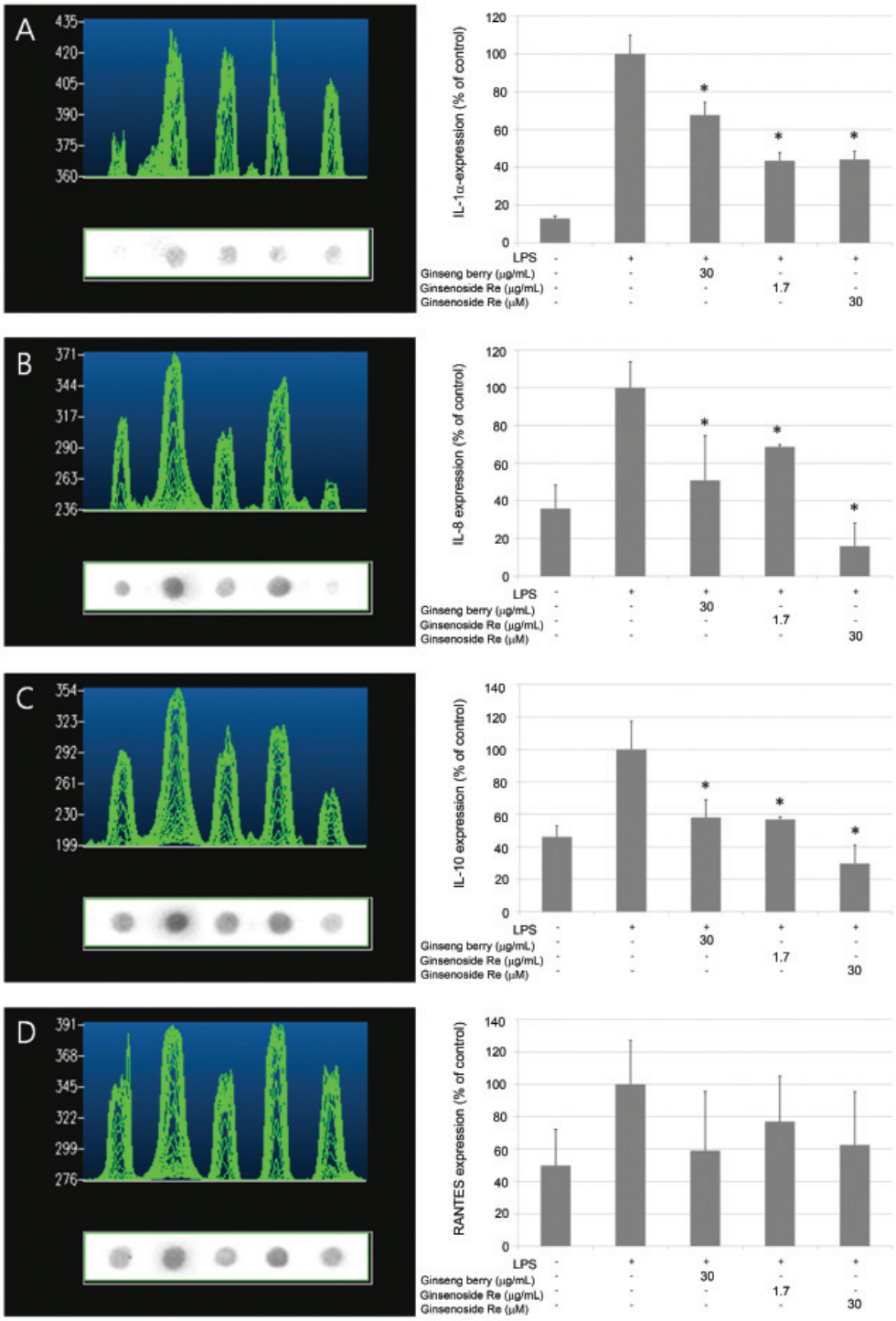

Fig. 4. (A-D) Cytokine array photographs and quantitatively analyzing graphs. A549 cells were incubated lipopolysaccharide (LPS) and/or each sample (*p<0.05 *vs*. LPS treated control). RANTES, regulated and normal T cell expressed and secreted.

exhibiting 49.6±4.8%, 47.2±5.3%, 51.3±4.5%, and 79.0±1.2%, respectively (p<0.05) (Fig. 3B).

The content of ginsenoside Re in crude extract was found out via HPLC analysis (5.9%). The ginsenoside content in 30 μg of crude extract is 1.7 μg, accordingly. As the molecular weight of ginsenoside Re is 947.14, the molar concentration of ginsenoside Re (1.7 μg/mL, the content in 30 μg/mL of crude extract) is 1.8 μM. The effect of 1 and 3 μM of ginsenoside Re could significantly reduce the histamine secretion. Therefore ginsenoside

Re might be thought to be a main effective component of ginseng berry in the present histamine secreting experiment.

In cytokine assay experiments, the most effective dosages of ginseng berry and ginsenoside Re were determined to be 30 μg/mL and 30 μM, respectively. Because ginsenoside Re is a component of ginseng berry extract (5.9%), 1.7 μg/mL ginsenoside Re treated group was also tested to find out the role of ginsenoside Re in total crude extract.

The IL-1α, IL-8, IL-10, and regulated and normal T cell expressed and secreted (RANTES) secretion were increased by LPS treatment (Fig. 4). Ginseng berry extract could reduce the IL-1α, IL-8, IL-10, and RANTES secretion induced by LPS treatment. Similarly, ginsenoside Re (1.7 μg/mL, the amount in 30 μg of ginseng berry extract) could reduce the IL-1α, IL-8, IL-10, and RANTES secretion. In the experiments of IL-8 and IL-10, ginsenoside Re (30 μM) showed the effects in a dose-dependent manner.

As results of the present study, ginsenoside Re was thought to be a main effective component of ginseng berry and the effects of ginsenoside Re were more powerful in higher dosages. The health promoting product having higher content of ginsenoside Re might be a health functional food.

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