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黃芩이 사람 비만세포의 사이토카인 및 케모카인 분비에 미치는 영향

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Effect of Scutellariae Radix on Expression of Cytokines and Chemokines Levels in Human Mast Cells (HMC-1)

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ABSTRACT

Objectives : Scutellariae Radix (Hwanggeum in Korean) is the root of ***Scutellaria baicalensis*** Georgi. Scutellariae Radix is well known to be used as a medicine for common cold, upper respiratory infections, and to strengthen and regulate the immune system and anemia etc. Little is understood about the roles of Scutellariae Radix in the cytokine and chemokine secretion by immune cells. This study was designed to find out the effects of Scutellariae Radix on the cytokine and chemokine secretion in human mast cells (HMC-1).

Methods : We treated hwanggeum according to consistency on HMC-1 and measured cytokines and chemokines levels using flow cytometry CBA system.

Results : In hwanggeum treated group, the expression of interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), chemokine (C-X-C motif) ligand 9 (CXCL9, MIG), interleukin 8 (IL-8), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 10 (IL-10), and interferon ɣ (IFN-ɣ) were decreased significantly.

Conclusion : These results suggest that hwanggeum may support some of immune diseases by means of amiliorating some chemokines or cytokines such as IP-10, MCP-1, MIG, IL-8, IL-2, IL-4, IL-5, IL-10, and IFN-ɣ.

Keywords : Scutellariae Radix, Human Mast Cell, HMC-1, Cytokine, Chemokine

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Introduction

Scutellariae Radix (Hwanggeum in Korean) is the root of Scutellaria baicalensis Georgi. Scutellariae Radix is a famous drug in Korean Traditional Medicine. Scutellariae Radix was traditionally used for common cold and upper respiratory infections, and to strengthen and regulate the immune system, and anemia etc. It clears heat, dries dampness, stops bleeding, calms the fetus, and sedates ascendant liver Yang[1-3]. Recently, Scutellariae Radix has been reported as potent anti-inflammatory properties[4]. Preliminary research suggests baicalin, a famous ingredient of Scutellariae Radix, might decrease inflammation and relieve pain, possibly by inhibiting proinflamm atory cytokines, nitric oxide, and prostaglandin E[5,6]. Some evidence suggests it can inhibit tumor growth and suppress carcinoma cell proliferation[4].

Mast cells are a key role in the inflammatory process, and for their ability to respond to both immunologic and non-immunologic stimulation. The range of both preformed and newly synthesized cytokines and chemokines from mast cells which regulate immune responses and activate T cells includes tumor necrosis factor alpha (TNF-a), interleukins, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory proteins, and chemokine (C-C motif) ligand 5 (RANTES)7,8. Some of the cytokines, such as TNF-a and interleukin 16, release rapidly from preformed stores within the cell, and some of them release very slowly even on several hours[7,9,10].

Flow cytometry is an analysis tool that allows for the discrimination of different particles on the basis of size and color. Multiplexing is the simultaneous assay of many analytes in a single sample. The cytomeric bead array (CBA) employs a series of particles with discrete fluorescence intensities to simultaneously detect multiple soluble analytes. Combined with flow cytometry, CBA is a powerful multiple analyte assay system. CBA multiplex beads simplify panel assays only one sample is required to detect and quantify several parameters, and the independent measurement for each bead population ensures high precision[11-14].

This study was designed to find out the effects of hwanggeum on the cytokine and chemokine secretions in HMC-1.

Material and Methods

1) Cell preparation

Human mast cell line (HMC-1, Korean Cell Line Bank) were cultured in Iscove's modified Dulbecco's medium supplemented with 10% bovine serum albumin, 2 mM L-glutamine, 100 IU/ml penicillin 50 ug/ml streptomycin, and 1.2 mM a-thioglycerol. The cells were passaged every 3-4 days.

2) Drug preparation.

Scutellariae Radix were purchased and identified from Semyung University Oriental Medicine Hospital. 100 g of Scutellariae Radix was extracted with water and filtered. Then evaporated on a rotatory evaporator and finally dried by a freeze drier. The yield of extract was 20.3% (W/W).

3) Cytometric bead array

(1) Human chemokine array

HMC-1 were placed in 6 well plate and hwanggeum 100 and 10 ug/ml were treated on the cells. Phosphate buffered saline (PBS) was treated as control. After 8 hours, each medium were collected and freeze until next step. The assay were used human chemokine cytometric bead array (CBA) kit (BDbioscience, USA). At first, 9 step of standards were prepared. 100 μl of Stock Standard buffer was added to 1,900 μl of assay diluent buffer to make 2,500 pg/ml of standard buffer. And rest of standards were made by serial dilution and assay diluent were used as the negative control.

Five of capture beads (human CXCL8/IL-8 capture beads, human CCL5/RANTES capture beads, human CXCL9/MIG capture beads, human

CCL2/MCP-1 capture beads and human CXCL10/IP-10 capture beads; 10 μl/test) were mixed to make master mix. The master mix was vortexed and divided to the appropriate assay tubes. And 50 μl of the human chemokine standard dilutions and samples were added. 50 μl of the human chemokine I PE detection reagents were added to the appropriate assay tubes, vortexed and incubated for 3 hours at room temperature (RT) and protected from direct exposure to light. 1 ml of wash buffer was added to each assay tube and centrifuged at 200 x g for 5 minutes, then the supernatants were discarded. 300 μl of wash buffer was added to each assay tube and vortexed. Each sample was analyzed on a flow cytometer. BD CBA software was used for analysis. Standard curves were created by using 9 standards and 1 negative control to get the quantity of each chemokine.

(2) Human Th1/Th2 array

HMC-1 were placed in 6 well plate and hwanggeum 100 and 10 ug/ml were treated on the cells. PBS was treated as control. After 8 hours, each medium were collected and freeze until next step. The assay were used human Th1/Th2 (CBA) kit (BDbioscience, USA). 9 step of standards were prepared. 100 μl of Stock standard buffer was added to 1,900 μl of assay diluent buffer to make 5,000 pg/ml of standard buffer. And rest of standards were made by serial dilution and assay diluent were used as the negative control.

Six of capture beads (human IL-2 capture beads, human IL-4 capture beads, human IL-5 capture beads, human IL-10 capture beads, human TNF capture beads and human IFN-γ capture beads; 10 μl/test) were mixed to make the master mix. The master mix was vortexed and centrifuged at 200 x g for 5 minutes. The supernatants were discarded and same volume of serum enhancement buffer were added. The master mix was incubated for 30 minutes at RT and protected from direct exposure to light. After then the master mix was vortexed and divided to the appropriate assay tubes. And 50 μl of the Standard dilutions and Samples were added. 50 μl of the Human Th1/Th2 PE detection reagents were add to the appropriate assay tubes, vortexed and incubated for 3 hours at RT and protected from direct exposure to light. 1 ml of wash buffer was added to each assay tube and centrifuged at 200 x g for 5 minutes, then the supernatants were discarded. 300 μl of wash buffer was added to each assay tube and vortexed. Each sample was analyzed on a flow cytometer. BD CBA software were used for analysis. Standard curves were created by using 9 standards and 1 negative control to get the quantity of each cytokine.

4) Statistical analysis

Values are expressed as means ± standard error (S.E.). The data were analyzed by one-way ANOVA followed by Dunnett's post-hoc analysis using SPSS. Differences were considered significant at $P < 0.05$.

Results

1) Human chemokine array

Standard curves of five chemokines were created using BD CBA software (Figure 1) and five chemokines of each samples were calculated by standard curves. The IP-10 expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 4.5%, 82.5 ± 1.5% ($P < 0.05$) and 94.0 ± 1.6%, respectively. The MCP-1 expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 3.3%, 74.6 ± 0.4% ($P < 0.05$) and 95.9 ± 3.0%, respectively. The MIG expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 5.1%, 76.6 ± 0.0% ($P < 0.05$) and 80.5 ± 0.8% ($P < 0.05$), respectively. The RANTES expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 2.4%, 96.8 ± 1.8% and 103.0 ± 0.9%, respectively. The IL-8 expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 6.3%, 75.5 ± 1.8% ($P < 0.05$) and 92.4 ± 0.0%,

respectively (Figure 2).

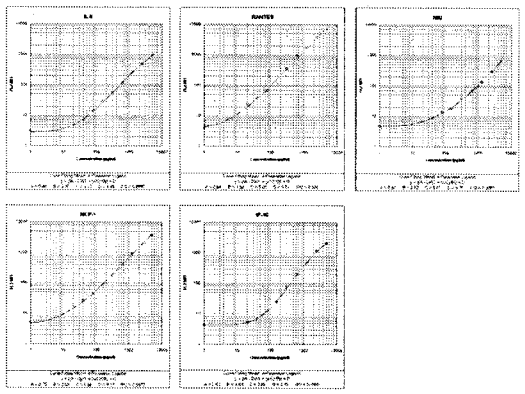

Figure 1. Standard curves of five chemokines calculated by CBA program.

IP-10 : interferon-inducible protein 10 ; MCP-1 : monocyte chemoattractant protein-1 ; MIG : chemokine (C-X-C motif) ligand 9 (CXCL9) ; RANTES : chemokine (C-C motif) ligand 5 (Ccl5) ; IL-8 : interleukin 8.

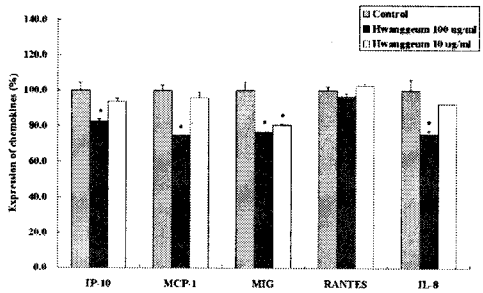

Figure 2. Expression of five chemokines in HMC-1.

100 and 10 ug/ml of hwanggeum were treated on the HMC-1 cells and assay the cytometric bead array after 8 hours. Expressions of some chemokines (such as IP-10, MCP-1, MIG and IL-8) were decreased by hwanggeum treatment in HMC-1 cells.

IP-10 : interferon-inducible protein 10 ; MCP-1 : monocyte chemoattractant protein-1 ; MIG : chemokine (C-X-C motif) ligand 9 (CXCL9) ; RANTES : chemokine (C-C motif) ligand 5 (Ccl5) ; IL-8 : interleukin 8 ; Control : PBS treated group ; hwanggeum 100 ug/ml : Scutellariae Radix 100 ug/ml treated group ; hwanggeum 10 ug/ml : Scutellariae Radix 10 ug/ml treated group. *$P < 0.05$.

2) Human Th1/Th2 cytokine array

Standard curves of six cytokines were created using BD CBA software (Figure 3) and six cytokines of each samples were calculated by standard curves. The IFN-γ expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 8.4%, 106.7 ± 8.4% and 61.7 ± 2.8% ($P < 0.05$), respectively. The TNF-a expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 6.6%, 77.5 ± 0.6% ($P < 0.05$) and 103.7 ± 0.0%, respectively. The IL-10 expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 5.8%, 82.7 ± 1.2% ($P < 0.05$) and 63.7 ± 0.0% ($P < 0.05$), respectively. The IL-5 expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 2.6%, 102.4 ± 0.3% and 82.5 ± 1.8% ($P < 0.05$), respectively. The IL-4 expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 4.8%, 104.7 ± 8.6% and 91.3 ± 6.8%, respectively. The IL-2 expression of control and hwanggeum treated groups (100 and 10 ug/ml) were 100.0 ± 8.0%, 82.2 ± 22.0% and 82.9 ± 0.7% ($P < 0.05$), respectively (Figure 4).

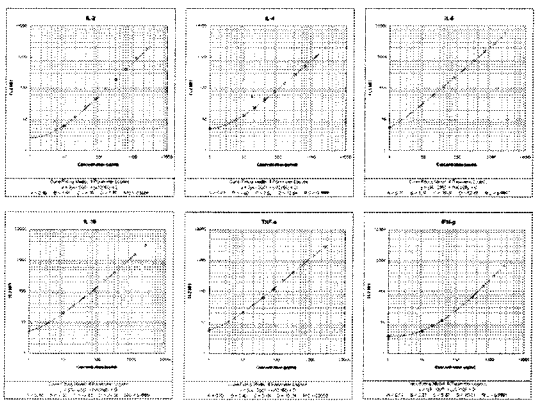

Figure 3. Standard curves of six cytokines calculated by CBA program.

IL-2 : interleukin 2 ; IL-4 : interleukin 4 ; IL-5 : interleukin 5 ; IL-10 : interleukin 10 ; IFN-γ : interferon γ ; TNF-a : tumor necrosis factor alpha.

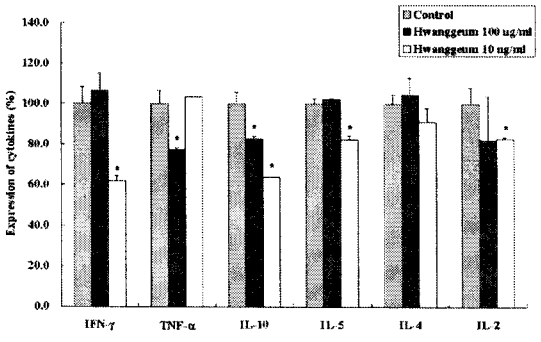

Figure 4. Expression of six cytokines in HMC-1.

100 and 10 ug/ml of hwanggeum were treated on the HMC-1 cells and assay the cytometric bead array after 8 hours. Expressions of some cytokines (such as IFN-γ, TNF-a, IL-10, IL 5 and IL-2) were decreased by hwanggeum treatment in HMC-1 cells.

IL-2 : interleukin 2 ; IL-4 : interleukin 4 ; IL-5 : interleukin 5 ; IL-10 : interleukin 10 ; IFN-γ : interferon γ ; TNF-a : tumor necrosis factor alpha ; Control : PBS treated group ; hwanggeum 100 ug/ml : Scutellariae Radix 100 ug/ml treated group ; hwanggeum 10 ug/ml : Scutellariae Radix 10 ug/ml treated group. *$P < 0.05$.

Discussions

Cytokines are small secreted proteins which mediate and regulate immunity, inflammation, and hematopoiesis. They must be produced de novo in response to an immune stimulus. They generally act over short distances and short time spans and at very low concentration. They act by binding to specific membrane receptors, which then signal the cell via second messengers, often tyrosine kinases, to alter its gene expression. Responses to cytokines include increasing or decreasing expression of membrane proteins, proliferation, and secretion of effector molecules.

Cytokine is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action)[11-13].

In this study we treated hwanggeum according to consistency on HMC-1 and measured cytokine and chemokine levels. In hwanggeum treated group, the expression of interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), chemokine (C-X-C motif) ligand 9 (CXCL9, MIG), interleukin 8 (IL-8), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 10 (IL-10), and interferon γ (IFN-γ) were decreased significantly. IL-10 has pleiotropic effects on immunoregulation and inflammation. It down-regulates the expression of Th1 cytokines, MHC class II Ags, and costimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production18-20). IL-8 is the most extensively studied member of the chemokine superfamily, with its major actions being as a neutrophil chemoattractant and activator. It was reported to play a major role in triggering and sustaining the allergic inflammatory response21). These results suggest that hwanggeum may support some of immune diseases by means of ameliorating some chemokines or cytokines such as IP-10, MCP-1, MIG, IL-8, IL-2, IL-4, IL-5, IL-10, and IFN-γ.

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