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백지의 사람비만세포 사이토카인 및 케모카인 발현 양상

임강현[2#*], 김명규[1], 이세나[2], 임종필[1]

1: 우석대학교 약학대학
2: 세명대학교 한의과대학

Effect of Angelicae Dahuricae Radix on Expression of Cytokines and Chemokines Levels in Human Mast Cells (HMC)

Kanghyun Leem[2#*], Myung-Gyou Kim[1], Se-Na Lee[2], Jong-Pil Lim[1]

1:College of Pharmacy, Woosuk University, Wanju 565-701, South Korea
2:College of Korean Medicine, Semyung University, Jechon 390-711, South Korea

ABSTRACT

Objectives : Angelicae Dahuricae Radix (Baek-Ji in Korean, BJ) is well known to be used as a medicine for cold, headache, supraorbital pain, nasal congestion, and toothache. Little is understood about the roles of BJ in the cytokine and chemokine secretion by immune cells. This study was designed to find out the effects of BJ on the cytokine and chemokine secretion in human mast cells (HMC).

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

Results : In BJ treated group, the expression of interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), chemokine (C-X-C motif) ligand 9 (MIG), and interleukin 10 (IL-10) levels were decreased significantly and chemokine (C-C motif) ligand 5 (RANTES), IL-8, interferone-γ (IFN-γ), and tumor necrosis factor alpha (TNF-a) were decreased significantly.

Conclusion : The results of this experiment supposed that the treatment of BJ will ameliorate the secreting levels of some chemokines or cytokines such as IP-10, MCP-1, MIG, IL-10, RANTES, IL-8, IFN-γ, and TNF-a.

Key words : Angelicae Dahuricae Radix, Human Mast Cell, HMC, Cytokine, Chemokine

#*Correspondence to: Kang-Hyun Leem, O.M.D., Ph. D.
College of Korean Medicine, Semyung University, Jechon 390-711: Korea
· Tel: 82-43-649-1341 · E-mail: lkh@semyung.ac.kr
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INTRODUCTION

Angelicae Dahuricae Radix (Korean name is "Baek-Ji"; BJ) is one of most famous drugs in Korean Traditional Medicine. It has effects of Expels Wind and alleviates pain. It used for externally contracted Wind Cold patterns, especially those with headache. It has used for supraorbital pain, nasal congestion, and toothache too. It has used for any problem due to Wind invading the Yang Brightness Channels of the head while primarily a warming herb[1 2]. Nowadays, BJ has been reported various immune reactions such as inhibitory effect of compound 48/80-induced histamine release[3], antimicrobial activity[4], antisepsis[5], and inhibitory effect of cytokine secretion[6].

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)[3,4]. 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[3,5,6].

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[7 10].

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

MATERIALS AND METHODS

1. Cell preparation

Human mast cell line (HMC, 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.

BJ were taken from Prof. Hong in Wonkwang University. 100 g of BJ was extracted with water and filtered. Then evaporated on a rotatory evaporator and finally dried by a freeze drier. The yield of extract was 14.6% (W/W).

3. Cytometric bead array

3-1 Human chemokine array

HMC were placed in 6 well plate and BJ 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 (BD bioscience, USA). At first, 9 step of standards were prepared. 100 ul of Stock Standard buffer were added to 1900 ul of assay diluent buffer to make 2500 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 ul/test) were mixed to make master mix. The master mix were

vortexed and divided to the appropriate assay tubes. And 50 ul of the human chemokine standard dilutions and samples were added. 50 ul of the human chemokine I PE detection reagent were add to the appropriate assay tubes, vortexed and incubated for 3 hours at RT and protected from direct exposure to light. 1ml of wash buffer were added to each assay tube and centrifuged at 200 x g for 5 minutes, then the supernatant were discarded. 300 ul of wash buffer were added to each assay tube and vortexed. Each samples were analyzed on a flow cytometer. Analysis were used BD CBA software. Standard curve were created by using 9 standards and 1 negative control and get the quantity of each chemokine.

3-2 Human Th1/Th2 cytokine array

HMC were placed in 6 well plate and BJ 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 (BD bioscience, USA). 9 step of standards were prepared. 100 ul of Stock standard buffer were added to 1900 ul of assay diluent buffer to make 5000 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 ul/test) were mixed to make master mix. The master mix were vortexed and centrifuged at 200 x g for 5 minutes. The supernatant were discarded and same voluem of serum enhancement buffer were added. The master mix were incubated for 30 minutes at RT and protected from direct exposure to light. After then the master mix were vortexed and divided to the appropriate assay tubes. And 50 ul of the Standard dilutions and Samples were added. 50 ul of the Human Th1/Th2 PE detection reagent were add to the appropriate assay tubes, vortexed and incubated for 3 hours at RT and protected from direct exposure to light. 1ml of wash buffer were added to each assay tube and centrifuged at 200 x g for 5 minutes, then the supernatant were discarded. 300 ul of Wash buffer were added to each assay tube and vortexed. Each samples were analyzed on a flow cytometer. Analysis were used BD CBA software. Standard curve were created by using 9 standards and 1 negative control and 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 BJ treated groups (100 and 10 ug/ml) were 100.0 ± 4.5%, 86.6 ± 3.2% ($P < 0.05$) and 112.9 ± 1.9%, respectively. The MCP-1 expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 3.3%, 81.9 ± 1.9% ($P < 0.05$) and 88.2 ± 3.1% ($P < 0.05$), respectively. The MIG expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 5.1%, 61.8 ± 4.7% ($P < 0.05$) and 93.3 ± 0.4%, respectively. The RANTES expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 2.4%, 112.4 ± 2.7% and 165.4 ± 9.2% ($P < 0.05$), respectively. The IL-8 expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 6.3%, 116.1 ± 0.0% ($P < 0.05$) and 116.6 ± 0.7% ($P < 0.05$), respectively (Figure 2).

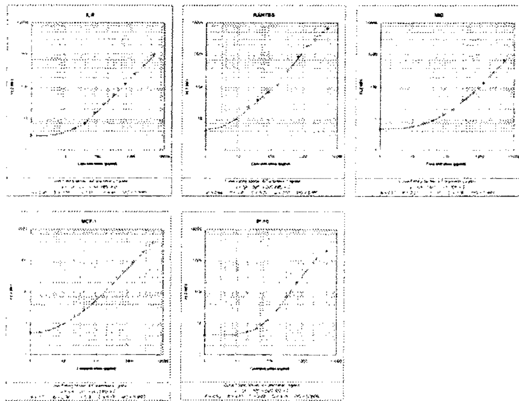

Figure 1. Standard curves of five chemokines calculated by CBA program.

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.

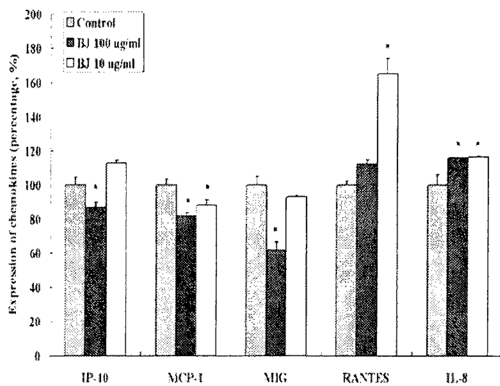

Figure 2. Expression of five chemokines in HMC.

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 ; BJ 100 ug/ml : Angelicae Dahuricae Radix 100 ug/ml treated group ; BJ 10 ug/ml : Angelicae Dahuricae 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 BJ treated groups (100 and 10 ug/ml) were 100.0 ± 8.4%, 129.0 ± 3.4% (P < 0.05) and 121.9 ± 8.8%, respectively. The TNF-a expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 6.6%, 186.2 ± 2.1% (P < 0.05) and 174.5 ± 0.0% (P < 0.05), respectively. The IL-10 expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 5.8%, 79.7 ± 4.2% (P < 0.05) and 111.5 ± 0.7%, respectively. The IL-5 expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 2.6%, 98.2 ± 5.0% and 105.5 ± 6.7%, respectively. The IL-4 expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 4.8%, 94.8 ± 6.6% and 110.1 ± 3.8%, respectively. The IL-2 expression of control and BJ treated groups (100 and 10 ug/ml) were 100.0 ± 8.0%, 113.8 ± 2.9% and 126.0 ± 28.8%, respectively (Figure 4).

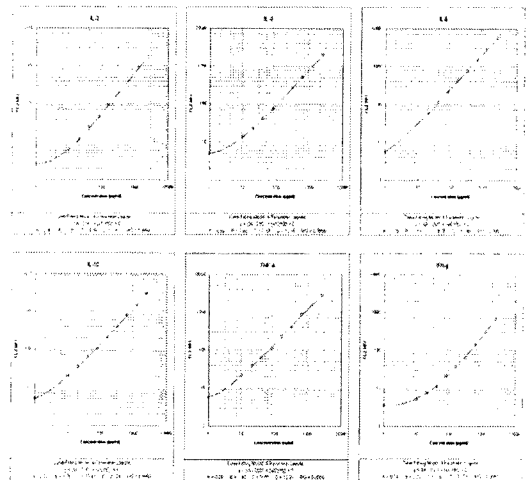

Figure 3. Standard curves of six cytokines calculated by CBA program.

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.

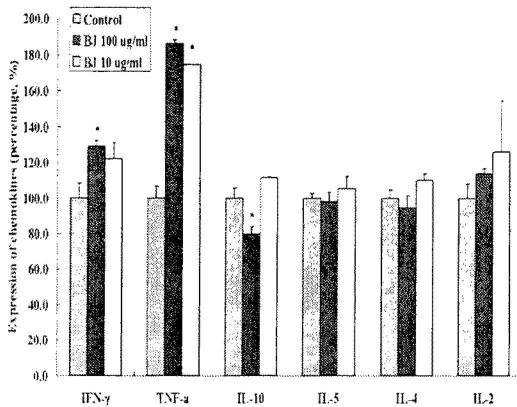

Figure 4. Expression of six cytokines in HMC.
IL-2 : interleukin 2 ; IL-4 : interleukin 4 ; IL-5 : interleukin 5 ; IL-10 : interleukin 10 ; IFN-ɣ : interferon ɣ ; TNF-a : tumor necrosis factor alpha ; Control : PBS treated group ; BJ 100 ug/ml : Angelicae Dahuricae Radix 100 ug/ml treated group ; BJ 10 ug/ml : Angelicae Dahuricae 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).

It is common for different cell types to secrete the same cytokine or for a single cytokine to act on several different cell types. Cytokines are redundant in their activity, meaning similar functions can be stimulated by different cytokines. Cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines. Cytokines can also act synergistically or antagonistically[11-13].

In this study, we treated BJ according to consistency on HMC and measured cytokine and chemokine levels. In BJ treated group, the expression of interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), chemokine (C-X-C motif) ligand 9 (MIG), and interleukin 10 (IL-10) levels were decreased significantly and chemokine (C-C motif) ligand 5 (RANTES), IL-8, interferone-ɣ (IFN-ɣ), and tumor necrosis factor alpha (TNF-a) were decreased significantly. IL-10 has pleiotropic effects in 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 production[18-20]. 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 plays a major role in triggering and sustaining the allergic inflammatory response[21]. TNF-a is an essential cytokine in many pathological conditions such allergic diseases, rheumatoid arthritis, and pulmonary fibrosis. It was reported to induced secretion and release of eosinophil chemotactic factors such as eotaxin and RANTES[22-24]. The results of this experiment is supposed to treatment of BJ will help some of immune diseases like asthma, allergy, and arthritis by means of amiliorating some chemokines or cytokines such as IP-10, MCP-1, MIG, IL-10, RANTES, IL-8, IFN-ɣ, and TNF-a.

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