원 저

# 봉독이 연골육종세포의 유전자 발현에 미치는 영향에 대한 Microarray 연구

인창식 · 고형균

경희대학교 한의과대학 침구학교실

## Microarray Analysis of Gene Expression in Chondrosarcoma Cells Stimulated with Bee Venom

Yin, Changshik · Koh, Hyung-gyun

Department of Acupuncture, College of Korean Medicine, Kyung Hee University

**국문초록**

봉독은 관절염 치료를 비롯한 여러 질환에 그 응용범위가 넓어지고 있으며 기전규명과 새로운 치료효과 개발을 위한 연구가 필요하다. 연골의 파괴는 진행된 각종 관절병증의 공통 병리기전이며 연골세포의 기능이상은 이 기전에 중요한 의미를 지닌다.

사람 연골세포의 특성을 유지하고 있는 HTB-94 연골육종세포를 배양하고 봉독을 처치했을 때의 유전자 발현양상을 microarray를 이용하여 관찰하였다.

대조군에 비해 4배 이상 발현의 차이가 있는 경우를 유의한 것으로 보았을 때 microarray의 344개 유전자중 봉독처치시 발현이 증강되는 유전자는 없었으며 발현이 억제되는 유전자는 interleukin 6 receptor, interleukin 1 alpha, tissue inhibitor of metalloproteinase 1, matrix metalloproteinase 1, tumor necrosis factor (ligand) superfamily, members 4, 8 and 12, and caspases 2, 6, and 10 등 35개가 관찰되었다.

Microarray를 통한 유전자발현 분석을 통해 관절염에 대한 봉독치료의 기전을 시사하는 유용한 자료를 얻을 수 있었으며 앞으로 보다 넓은 범위에 대한 연구가 필요할 것이다.

**Key words :** bee venom, chondrosarcoma, microarray analysis, gene expression

## I. Introduction

The pharmacologic potentials of bee venom(BV) have been investigated by various researchers for some time. Recently, BV, and in particular one of its constituents, melittin, have been reported to possess proinflammatory[1], anti-inflammatory[2], antinociceptive[3], and anticancer effects[4] and therapeutic effect against bacterial diarrhea in piglets[5]. Studies involving gene expression following BV administration have been published[2, 6-7].

Microarray analysis is a technique which has been shown to be of particular utility in areas including simultaneous profiling of global gene expression and uncovering new genes or new functions of known genes[8]. Microarray analysis

※ 교신저자 : 고형균, 서울특별시 동대문구 회기동 1번지
경희대학교 한의과대학 부속한방병원
(Tel : 011-9003-7835, E-mail : acuyin@shinbiro.com)

of gene expressions in a microglia cell line following hypoxic damage[9] and in a human mast cell line following treatment with BV[10] have been previously carried out.

As a therapeutic modality applied to arthritis[11], BV effect needs to be researched in chondrocyte environments. However, there has yet been no report on the effect of BV on chondrocyte or chondrocyte-like cell lines. In the present study, global gene expression profiling was carried out in an effort to understand the effect of BV on HTB-94 chondrosarcoma cells.

## II. Materials and Methods

### 1. Cells of the culture

HTB-94 chondrosarcoma cell lines were cultured in monolayer in Dulbecco's modified Eagle's medium(DMEM ; Gibco BRL, USA) containing 10% heat-inactivated fetal bovine serum (FBS), penicillin (100U/ml), and streptomycin (100ug/ml) (Gibco BRL, USA) at 37°C and in a humidified cell incubator and a atmosphere consisting of 5% $CO_2$-95% room air. The culture medium was changed three times a week.

### 2. MTT assay of cell viability

MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was carried out as per the manufacturer's protocol(Roche, Germany) to investigate the effect of BV on cell viability. MTT assay is based on the cleavage of the yellow tetrazolium salt MTT and the subsequent formation of purple formazan crystals by metabolically active cells in a reaction involving pyridine nucleotide cofactors NADH and NADPH. The formazan product is then solubilized and spectrophotometrically quantified using an ELISA reader. 5 × 104 cells were grown in each well of a 96-well culture plate with 100ul of serum-free medium and the vehicle or BV for 12hours at the following concentrations: (1) 10-1ug/ml, (2) 10-2ug/ml, (3) 10-3ug/ml, and (4) 10-4ug/ml. 10ul MTT solution was added to each well, and cells were then incubated for another 4hours. The purple formazan salts thus produced were solubilized by adding 100ul of solubilization solution to each well and incubating overnight, again at 37°C and in 5%-$CO_2$ supplemented humidified atmosphere. The solubilized solution was colorimetrically assayed using an ELISA reader(Bio-Tek, USA) at a wavelength of 595nm, with a reference wavelength of 690nm. % cell viability was calculated as the absorbance rate of the experimental group over that of the control group. Student's t-test was used for statistic analysis, and a p-value below 0.05 was considered 50 indicate statistical significance.

### 3. Treatment of bee venom and RNA extraction

Cells were washed with the culture medium and incubated in the culture medium with the following agent(s) for 12 hours : vehicle or 10ng/ml BV(Sigma, USA).

After incubation, total RNA was extracted using TRIzol as per the manufacturer's protocol with minor modifications, and spectrophotometrically evaluated for quantity and purity using A260/280 ratio and agarose gel electrophoresis, respectively.

### 4. cDNA synthesis

cDNA synthesis and microarray hybridization was performed with 3DNA™ array 50™(Genisphere, USA) on TwinChip™ Human Cancer 0.4K(Digital Genomics, Korea) as per the manufacturers' protocols. For each set of analysis, the control and treatment cDNA was synthesized from total RNA as follows : 3ul of RT primer, total RNA and additional nuclease-free water were mixed to form a 29ul RNA-RT primer mix, which was microfuged briefly, heated to 80°C for ten minutes, and immediately transferred to ice. 1ul of the RNase inhibitor Superase-In™ was added to the RNA-RT primer mix. 8ul of 5X SuperScript II First Strand Buffer (Gibco BRL, USA), 2ul of dNTP mix(10mM each of dATP, dCTP, dGTP, dTTP), 4ul of 0.1M DTT(dithiotreitol), 2ul of Superscript II enzyme(400units), and 3ul of RNase-free water were mixed in each microtube, and the RNA-RT primer mix was then added. The tubes were then incubated at 42°C for 2 hours, and the reaction was halted by adding 7ul of 0.5M NaOH/50mM EDTA. The microtubes were then incubated

for denaturation at 65°C for 10 minutes, and neutralization was carried out by adding 10ul 1M Tris-HCl at pH 7.5. The contents of 2 tubes were combined to yield a 130ul cDNA solution in one single tube. The original tubes were rinsed with 16ul of 10mM Tris at pH 8.0/1mM EDTA.

Upon completion of the synthesis procedure, the cDNA solution was concentrated by ethanol precipitation. 3ul of thoroughly vortexed 5mg/ml linear acrylamide solution was added to the cDNA solution. 6ul of 5M NaCl and 540ul of 95-100% ethanol was then added and moderately vortexed. The mixture was then incubated at-20°C for 30 minutes, centrifuged at >10,000g for 15minutes, and the supernatant was aspirated. The cDNA pellet was washed with 300ul of 70% ethanol. After centrifuging again at >10,000g for 5 minutes, the supernatant was aspirated, and the cDNA pellet was completely dried at 65°C over a period of 10-30 minutes.

## 5. Microarray hybridization

The concentrated cDNA and 3DNA™ was hybridized on a microarray. 2X formamide-based hybridization buffer was thawed and resuspended by heating at 55°C for 10minutes with intermittent inversions, and then microfuged for 1 minute. 10ul of nuclease-free water was added to the cDNA pellet, and the cDNA was completely resuspended by heating at 65°C for 10-15 minutes and vortexing for 5minutes. 30ul of hybridization mixture was prepared from 10ul of cDNA, 15ul of 2X hybridization buffer, 2ul of Array50 dT Blocker, and 3ul of nuclease-free water. The hybridization mixture was incubated at 80°C for 10 minutes and then at 50°C for 20 minutes while the microarray was pre-warmed at 50°C for 30-60minutes. The hybridization mix was then added to the pre-warmed microarray. After a disposable coverslip was applied, the microarray was incubated overnight in a dark humidified chamber at 50°C. After serial washing, the array was incubated for 2 minutes at room temperature with 95% ethanol. The slide was immediately transferred to a dry 50ml centrifuge tube and dried by centrifugation for 2minutes at 800-1000 RPM. Array50 capture reagent was then thawed in the dark at room temperature over a period of 20minutes and vortexed for 3 seconds. The reagent was evenly resuspended by heating at 55°C for 10minutes and vortexing for 3 seconds. 30ul of hybridization mixture was prepared from 15ul of 2X hybridization buffer, 2.5ul of 3DNA™ capture reagent #1(Cy3), 2.5ul of 3DNA™ capture reagent #2(Cy5), and 10ul of nuclease-free water. Following gentle vortexing and brief microfuging, the hybridization mixture was incubated at 80°C for 10minutes and then at 50°C for 20 minutes. The hybridization mixture was applied to the pre-warmed microarray 1minute after it was removed from the incubator. After a disposable coverslip was applied, the microarray was incubated in a dark humidified chamber at 50°C for 2-3hours. After serial washing, the slide was immediately transferred to a dry 50ml centrifuge tube and dried by centrifugation for 2minutes at 800-1000 RPM, and then transferred to a dark slide box.

## 6. Scanning and data analysis

The hybridized microarray was scanned with a confocal laser scanning microscope(ScanArray 5000 ; Packard Inc, USA) at 532nm for Cy3 and 635nm for Cy5. Image analysis using GenePix(Axon Inc, USA) produced quantitative values for each microarray spot. Pixel intensity of the background was subtracted from those of microarray spots. Spot intensities were normalized using the intensities generated by house-keeping genes. Normalized spot intensities were calculated into gene expression ratios between the control and treatment groups. Greater-than-4 fold changes between two groups were considered to be of significance.

# III. Results

## 1. MTT assay

12hours incubation with BV at the following concentrations produced the respective percent viabilities relative to that of the control group in the chondrosarcoma cell line cultures ; 68.4±1.6%(mean±S.D.) with $10^{-1}$ug/ml BV, 99.0±2.0% with $10^{-2}$ug/ml BV, 97.5±0.8% with 10-3ug/ml BV, and 100.4±4.5% with $10^{-4}$ug/ml BV<Figure 1>. Percent viability with $10^{-1}$ug/ml BV was significantly different from that of the control group.

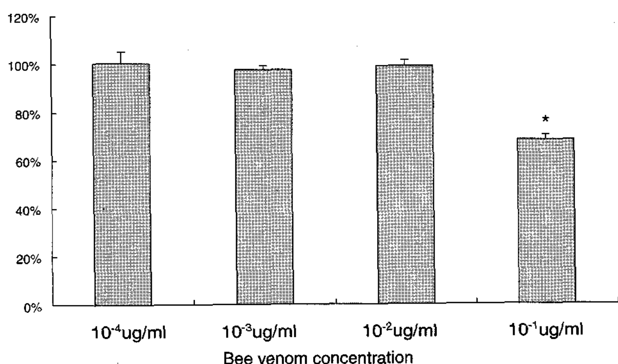


Figure 1. Cell viability(%) after 12hours of incubation with bee venom at the respective concentrations. Chondrosarcoma cells were incubated with different concentrations of bee venom or the vehicle for 12hours, and percent viabilities of the treatment groups were calculated by MTT colorimetric assay relative to that of the control group. Values are given as percent (mean±S.D.). Asterisk(*) indicates statistical difference from the control group.

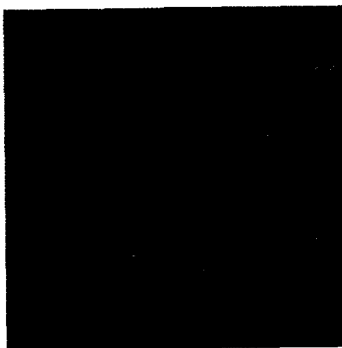

Figure 2. Microarray images of gene expression in chondrosarcoma cells treated for 12 hours with 10ng/ml bee venom or the vehicle.

## 2. Gene expression profiles of bee venom treated cells

Incubation of chondrosarcoma cells with 10ng/ml BV produced no increase(>4 fold) in the expression of the genes examined compared to the vehicle-treated cells.

On the other hand, a decrease(>4 fold) in the expression of the following genes compared to the vehicle-treated cells was observed : IL6R, TIMP1, CDC2, ITGA5, RPA3, ZNF147, SCYA13, AKT2, MMP1, TNFSF4, TNFSF12, IL1A, TNFRSF8, CD83, CHUK, CASP6, ITGB2, CASP10, TERT, TSC1, REL, IGFBP3, ICAM1, IL8, NFKB1, SCYA4, TNFSF8, IL12A, CDC42, TP63, SPN, CASP2, TNFRSF7, WISP2, and ITGA7<Table 1, Figure 2>.

# IV. Discussion

Chondrocytes are the sole constituent cell of the cartilage, living in a low oxygen tension environment. The nutrient/waste exchange of chondrocytes occurs through diffusion. The total volume of chondrocytes is only about 10% of the total tissue volume of the cartilage. The chondrocyte is metabolically active in that it is responsible for the synthesis and turnover of a large volume of extracellular matrix consisting of collagen, proteoglycan, glycoprotein, and hyaluronan which undergoes degradation as a result of the activity of catabolic enzymes such as matrix metalloproteinases (MMPs) and a distintegrin and metalloprotease with thrombospodin motif(ADAM-TS4 and ADAM-TS5)[12]. The normal cartilage is in a state of dynamic equilibrium and under constant remodelling. Major pathologic processes involving the cartilage include loss of extracellular matrix and chondrocyte dysfunction. Arthritic joints display alterations in metabolism and an imbalance between anabolic growth factors and proinflammatory cytokines produced by inflammatory cells, synovial fibroblasts, and chondrocytes. Chondrocytes are prime targets of proinflammatory cytokines in the pathogenesis of arthritis. Tumor necrosis factor(TNF-$\alpha$) and interleukin-1(IL-1) are key proinflammatory cytokines in the pathogenesis of arthritis[13].

The inflammatory condition including arthritis is one of therapeutic applications of BV[2]. Studies involving gene expression following BV administration have been published[2,6-7]. Recent advances in microarray technology has allowed large-scale characterization of coordinated gene expression. The scope of the application of this technique has been expanding in many fields including arthritis research[14], involving known or unknown genes at a certain stage in

Table 1. Genes showing bee venom-induced downregulation in HTB-94 chondrosarcoma cells

| Gene name | Abbreviation | Decrease (times control level) |
|---|---|---|
| interleukin 6 receptor | IL6R | 15.1 |
| tissue inhibitor of metalloproteinase 1(erythroid potentiating activity, collagenase inhibitor) | TIMP1 | 13.1 |
| cell division cycle 2, G1 to S and G2 to M | CDC2 | 12.3 |
| integrin, alpha 5(fibronectin receptor, alpha polypeptide) | ITGA5 | 11.6 |
| replication protein A3(14kD) | RPA3 | 11.0 |
| zinc finger protein 147(estrogen-responsive finger protein) | ZNF147 | 9.2 |
| small inducible cytokine subfamily A(Cys-Cys), member 13 | SCYA13 | 9.2 |
| v-akt murine thymoma viral oncogene homolog 2 | AKT2 | 9.0 |
| matrix metalloproteinase 1(interstitial collagenase) | MMP1 | 8.4 |
| tumor necrosis factor(ligand) superfamily, member 4(tax-transcriptionally activated glycoprotein 1, 34kD) | TNFSF4 | 8.0 |
| tumor necrosis factor(ligand) superfamily, member 12 | TNFSF12 | 8.0 |
| interleukin 1, alpha | IL1A | 7.9 |
| tumor necrosis factor receptor superfamily, member 8 | TNFRSF8 | 7.4 |
| CD83 antigen(activated B lymphocytes, immunoglobulin superfamily) | CD83 | 7.4 |
| conserved helix-loop-helix ubiquitous kinase | CHUK | 6.6 |
| caspase 6, apoptosis-related cysteine protease | CASP6 | 6.2 |
| integrin, beta 2(antigen CD18(p95), lymphocyte function-associated antigen 1; macrophage antigen 1(mac-1) beta subunit) | ITGB2 | 6.0 |
| caspase 10, apoptosis-related cysteine protease | CASP10 | 5.4 |
| telomerase reverse transcriptase | TERT | 5.3 |
| tuberous sclerosis 1 | TSC1 | 5.3 |
| v-rel avian reticuloendotheliosis viral oncogene homolog | REL | 5.1 |
| insulin-like growth factor binding protein 3 | IGFBP3 | 5.1 |
| intercellular adhesion molecule 1(CD54), human rhinovirus receptor | ICAM1 | 5.0 |
| interleukin 8 | IL8 | 4.8 |
| nuclear factor of kappa light polypeptide gene enhancer in B-cells 1(p105) | NFKB1 | 4.7 |
| small inducible cytokine A4 (homologous to mouse Mip-1b) | SCYA4 | 4.5 |
| tumor necrosis factor (ligand) superfamily, member 8 | TNFSF8 | 4.5 |
| interleukin 12A(natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35) | IL12A | 4.4 |
| cell division cycle 42(GTP-binding protein, 25kD) | CDC42 | 4.3 |
| tumor protein 63 kDa with strong homology to p53 | TP63 | 4.3 |
| sialophorin(gpL115, leukosialin, CD43) | SPN | 4.2 |
| caspase 2, apoptosis-related cysteine protease(neural precursor cell expressed, developmentally down-regulated 2) | CASP2 | 4.2 |
| tumor necrosis factor receptor superfamily, member 7 | TNFRSF7 | 4.1 |
| WNT1 inducible signaling pathway protein 2 | WISP2 | 4.0 |
| integrin, alpha 7 | ITGA7 | 4.0 |

* Gene expression was profiled with TwinChip™(Digital Genomics) microarray. A cut-off level of four-fold change was used.

physiologic or pathologic processes[8].

The present study is the first report involving gene expression profiles using microarray analysis in chondrosarcoma cells stimulated with BV. The HTB-94 cell line was obtained from a grade II primary chondrosarcoma in a 72 year-old female Caucasian[15]. It maintains the

chondrocytic phenotype[16] and serves as an appropriate chondrocyte model of human arthritis for various purposes, including interleukin-responsive immediate early gene studies[17], protein kinase gene expression studies[18], and MMP gene expression pathway studies[13]. We used BV as a stimulant at the concentration of 10ng/ml which was shown to be non-toxic from MTT assay and has been adopted in another study in human mast cell line[10].

Using TwinChip™ Human Cancer 0.4K together with 3DNA™ Array 50™, changes in gene expression in human chondrosarcoma cells treated with BV was examined in this study. The microarray carries 344 human cDNA probes for various genes, including MMPs, IL receptors, TNF, TNF receptor superfamilies, and others involved in inflammatory response and apoptosis signaling(full list of genes is available athttp://annotation.digital-genomics.co.kr/excel/h-cancer_v1.xls).

The expressions of the TNF(ligand) superfamily members 4, 8, and 12(TNFSF4, 8, and 12) and TNF receptor superfamily members 7 and 8 were downregulated following administration of BV. BV treatment downregulated the expression of IL-6 receptor, IL-1 alpha, IL-8 and IL-12A genes.

Human cartilage affected by arthritis shows upregulation and spontaneous release of various inflammatory mediators, including NO, prostaglandin E2(PGE2), IL-6, IL-8, and MMPs ; this is induced by the autocrine production of IL-1$\beta$ and TNF$\alpha$. IL-1$\beta$ and TNF$\alpha$ are expressed at high levels in chondrocytes in osteoarthritis(OA) and rheumatoid arthritis (RA) and play major roles in the pathogenesis of cartilage degeneration[19]. Cytokines are extracellular signalling proteins that act as local mediators, binding to receptors and altering cell behavior. The intricate interaction or balance between the pro-inflammatory cytokines TNF-$\alpha$, IL-1, IL-6, IL-8, and IL-11 and the anti-inflammatory cytokines IL-4 and IL-10 has major implication in the pathologic process of various disorders[20]. IL-1 induces the synthesis of MMP-1 and PGE2 which contribute to collagen destruction, periarticular osteopenia, and bone resorption. IL-1$\alpha$ usually remains inside the cell or is expressed on the cell surface, and is believed to function as an autocrine messenger. In contrast, mature IL-1$\beta$ is secreted and exerts its biologic actions on other cells[21]. Human articular chondrocytes stimulated by IL-1$\beta$ and TNF$\alpha$ synthesize large amounts of IL-6 and express both subunits of the IL-6 receptor complex(gp80 and gp130). There exists an autocrine amplification loop between stimulation by oncostatin M(OSM) and/or IL-6 and IL-6 synthesis in chondrocytes. IL-6-stimulated chondrocytes have been reported to synthesize increased amounts of $\alpha$1-antitrypsin, a major inhibitor of serine proteinases, which contributes to an important protective mechanism of articular chondrocytes against cartilage damage in inflammatory joint diseases[22]. Based on elevations in the production of IL-6 observed in RA patients and the close association seen between IL-6 levels and disease activity, IL-6 has been proposed as a possible target for RA therapy. IL-6 receptor antibody has been reported to prevent the development of collagen-induced arthritis[23-4]. In systemic juvenile chronic arthritis(JCA), the levels of soluble IL-6 receptor, IL-6 agonist, were found to be significantly increased[25]. OA-affected chondrocytes exhibit upregulations in TNF receptor expression and are more susceptible to both TNF$\alpha$ and IL-1$\beta$. IL-1$\beta$ and IL-6 are produced by the synovium and increased in the synovial fluid of OA patients. They upregulate chondrocyte p55 TNF receptor expression, with a resultant increase in chondrocyte susceptibility to TNF[26]. By way of mRNA degradation and NF$\kappa$B inhibition, respectively, IL-4 and IL-10 suppress TNF-$\alpha$ and IL-1. IL-4 and IL-10 synergistically suppress arthritic symptoms and enhance proteoglycan synthesis[27].

BV induced a downregulation in the expression of the nuclear factor kappa light polypeptide gene enhancer in B-cells 1(NFKB1). BV also induced a marginal downregulation (3.2-fold) of mitogen-activated protein kinase 3(MAPK3). NF-$\kappa$B is an essential osteoclastogenic transcription complex[28]. The NF-$\kappa$B and MAPK pathways are central in the pathogenesis of RA and OA. Both are coordinately activated by IL-1 and TNF-$\alpha$[17].

MMP-1 and TIMP-1 were downregulated by BV. The balance between MMPs and TIMPs is important in determining cartilage breakdown with resultant tissue destruction[29]. Imbalance between MMPs and TIMPs is suggested to be responsible for cartilage matrix degradation[30]. TIMPs exist in four types : 1, 2, 3, and 4. They inhibit activated MMPs by tightly binding to them with 1:1

stoichiometry. TIMPs also bind to proMMPs. TIMPs 1 and 2 can stimulate mesenchymal cells to proliferate. MMPs are classified into 5 subgroups : (a) collagenases such as tissue collagenase(MMP-1), neutrophil collagenase(MMP-8), and collagenase 3(MMP-13) ; (b) gelatinases such as gelatinases A(MMP-2) and B(MMP-9) ; (c) stromelysins such as stromelysins 1(MMP-3) and 2(MMP-10) ; (d) membrane-type MMPs such as MT1-MMP(MMP-14), MT2-MMP (MMP-15), MT3-MMP(MMP-16), MT4-MMP(MMP-17), and MT5-MMP(MMP-18) ; (e) others, including matrilysin (MMP-7), stromelysin 3(MMP-11), metalloelastase(MMP-12), enamelysin(MMP-20), MMP-19, and MMP-23[31]. MMPs are key mediators in the resorption of cartilage, bone, arthritic synovial fluid, and adjacent soft tissue during pathological destruction of joint tissue[29]. Of the 27 known MMPs, MMPs 1, 8, 13, and 14 are considered to be more active in cartilage degradation[32]. In OA, proteinases produced by chondrocytes play a major role. In RA, where inflammation is more prominent than in OA, many types of cells including synoviocytes produce proteinases and mediators[29]. MT2-MMP can degrade fibronectin, tenascin, nidogen, aggrecan, perlecan, and laminin. MT2-MMP processes pro-TNF$\alpha$ to its mature form and activates proMMP-2. MT2-MMP is reported to be exclusively associated with RA synovitis[33]. MT1-MMP is capable of digesting fibrillar type collagens I, II, and III into the characteristic three-quarter and one-quarter fragments, preferentially cleaving type I collagen, and of degrading other extracellular components, including gelatin, proteoglycan, fibronectin, and laminin[34]. In RA and OA, the interstitial collagens(types I, II, and III) are the principal targets of destruction. Secreted collagenases(MMP-1 and MMP-13) play major roles in this destruction. Their expression involves the nuclear factor $\kappa$B(NF-$\kappa$B) and mitogen-activated protein kinase(MAPK) pathways[17].

Caspases 2, 6, and 10 were downregulated by BV. Apoptosis involves the sequential activation of a proteolytic cascade of enzymes called caspases. At least 10 caspases have been described, which have been divided into initiators and effectors[35]. Apoptosis signalling involves a family of receptors known as "death receptors"(DRs). The TNF-family DRs(TNFR1, Fas, DR3, DR4, and DR5) link via their cytosolic death domains(DD) to adaptor proteins such as FADD. Adaptor proteins in turn bind via their death effector domains(DED) to initiator caspases(caspases 8 and 10). Recruitment of initiator caspases in turn leads to activation of downstream effector caspases such as caspases 3, 6, 7, 9 and various other proteins[36].

Treatment with BV induced a downregulation of integrins alpha 5, alpha 7, and beta 2. BV also induced a marginal downregulation(3.9-fold) of integrin alpha 4. Integrins are heterodimeric transmembrane proteins formed by non-covalent association of $\alpha$ and $\beta$ subunits. Both subunits are type I membrane proteins with large extracellular ectodomains and short cytoplasmic tails. In mammals, the integrin family contains at least 18 $\alpha$ subunits that associate with at least nine and $\beta$ subunits. The $\alpha$ and $\beta$ subunits assemble into at least 24 distinct receptors. Integrins function both as cell adhesion receptors and as intracellular signalling receptors[30]. Recently, Integrin $\alpha V\beta 3$ began drawing attention as a possible therapeutic target in the treatment of RA[37]. In the normal articular cartilage, integrin heterodimers $\alpha V\beta 1$, $\alpha 5\beta 1$, and $\alpha V\beta 5$ are expressed strongly ; $\alpha 3$, $\beta 3$, and $\beta 4$ subunits are also expressed. Osteoarthritic cartilage is reported to express the $\alpha 2$, $\alpha 4$, and $\beta 2$ subunits in addition[38]. Integrin $\beta 1$ is overexpressed in the cartilage in OA. Integrin $\beta 1$-mediated cell-matrix interactions were reported to provide survival signals for chondrocytes[30].

BV treatment induced a downregulation in insulin like growth factor binding protein(IGFBP)-3. Recently, IGFBP-3 has been reported to be increased in severe OA cartilage, due to autocrine production. It was proposed to be a renderer of cartilage insensitivity to IGF action in OA[39]. The rate of IGFBP-3 synthesis is reported to be 3 times higher in the cartilage affected by OA than in the normal cartilage[40]. The pro-inflammatory cytokines TNF-$\alpha$, IL-1$\alpha$, and IL-1$\beta$ were demonstrated to induce IGFBP3 and IGFBP5 on chondrocytes. This induction was shown to lead to the suppression of insulin like growth factor-1(IGF-1)-induced proteoglycan synthesis, with a consequent imbalance between synthesis and degradation of cartilage matrix[41].

Changes in gene expression caused by BV treatment in chondrosarcoma cells suggest some possible mechanisms of BV behind the antiarthritic effects of BV. However, further

research appears to be necessary in clarifying the cytokine, enzymatic, or apoptosis-modulatory effects of BV.

## V. Conclusion

The following change in the gene expression profile was observed during microarray analysis of the cDNA of HTB-94 cells treated with BV, by a cut-off level of four-fold change: BV induced the downregulation of 35 genes, including interleukin 6 receptor, interleukin 1 alpha, tissue inhibitor of metalloproteinase 1, matrix metalloproteinase 1, tumor necrosis factor(ligand) superfamily, members 4, 8 and 12, and caspases 2, 6, and 10. Further research will be necessary in clarifying the cytokine, enzymatic, or apoptosis-modulatory effects of BV.

## VI. References


1. Sumikura H, Andersen OK, Drewes AM, Arendt-Nielsen L. A comparison of hyperalgesia and neurogenic inflammation induced by melittin and capsaicin in humans. Neurosci Lett. 2003 ; 337(3) : 147-50
2. Nam KW, Je KH, Lee JH, Han HJ, Lee HJ, Kang SK, Mar W. Inhibition of COX-2 activity and proinflammatory cytokines(TNF-alpha and IL-1beta) production by water-soluble sub-fractionated parts from bee(Apis mellifera) venom. Arch Pharm Res. 2003 ; 26(5) : 383-8
3. Kim HW, Kwon YB, Ham TW, Roh DH, Yoon SY, Lee HJ, Han HJ, Yang IS, Beitz AJ, Lee JH. Acupoint stimulation using bee venom attenuates formalin-induced pain behavior and spinal cord fos expression in rats. J Vet Med Sci. 2003a ; 65(3) : 349-55
4. Orsolic N, Sver L, Verstovsek S, Terzic S, Basic I. Inhibition of mammary carcinoma cell proliferation in vitro and tumor growth in vivo by bee venom. Toxicon. 2003 ; 41(7) : 861-70
5. Choi SH, Cho SK, Kang SS, Bae CS, Bai YH, Lee SH, Pak SC. Effect of apitherapy in piglets with preweaning diarrhea. Am J Chin Med. 2003 ; 31(2) : 321-6
6. Jang MH, Shin MC, Lim S, Han SM, Park HJ, Shin I, Lee JS, Kim KA, Kim EH, Kim CJ. Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. J Pharmacol Sci. 2003 ; 91(2) : 95-104
7. Hwang D, Kim H, Kim C, Kim E. Bee venom induces apoptosis and inhibits COX-2 in human osteosarcoma cell line MG-63. Journal of Korean Acupuncture & Moxibustion Society. 2003 ; 20(3) : 63-74
8. Thornton S, Sowders D, Aronow B, Witte DP, Brunner HI, Giannini EH, Hirsch R. DNA microarray analysis reveals novel gene expression profiles in collagen-induced arthritis. Clin Immunol. 2002 ; 105(2) : 155-68
9. Kim BS, Seo JC. Microarray analysis of hypoxia-induced changes in gene expression in BV-2 microglial cells. Journal of Korean Acupuncture & Moxibustion Society. 2003b ; 20(4) : 85-92
10. Lee W, Kang S, Koh H. Microarray analysis of CD/cytokine gene expression in human mast cell treated with bee venom. Journal of Korean Acupuncture & Moxibustion Society. 2003c ; 20(5) : 50-62
11. Lee S, Lee H, Baek Y, Kim S, Park J, Hong S, Yang H, Kim K, Lee J, Choi D, Lee D, Lee Y. Effects of bee venom on the pain, edema, and acute inflammatory reactant of rheumatoid arthritis patients. Journal of Korean Acupuncture & Moxibustion Society. 2003b ; 20(2) : 77-84
12. Archer CW, Francis-West P. The chondrocyte. Int J Biochem Cell Biol. 2003 ; 35(4) : 401-4
13. Liacini A, Sylvester J, Li WQ, Huang W, Dehnade F, Ahmad M, Zafarullah M. Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. Exp Cell Res. 2003 ; 288(1) : 208-17
14. Ibrahim SM, Koczan D, Thiesen H-J. Gene-expression profile of collagen-induced arthritis. J Autoimmun. 2002 ; 18(2) : 159-67
15. ATCC(American Type Culture Collection). [cited 2003 Oct 5] ; available from : URL : http://www.atcc.org/

SearchCatalogs/longview.cfm?view=ce,5339760,HTB-94&text=sw%20%26%201353&max=20
16. Ah-Kim H, Zhang X, Islam S, Sofi JI, Glickberg Y, Malemud CJ, Moskowitz RW, Haqqi TM. Tumor necrosis factor α enhances the expression of hydroxyl lyase, cytoplasmic antiproteinase-2 and a dual specificity kinase TTK in human chondrocyte like cells. Cytokine. 2000 ; 12(2) : 142-50
17. Vincenti MP, Brinckerhoff CE. Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1β. Arthritis Res. 2001 ; 3 : 381-8
18. Islam S, Kermode T, Sultana D, Moskowitz RW, Mukhtar H, Malemud CJ, Goldberg VM, Haqqi TM. Expression profile of protein tyrosine kinase genes in human osteoarthritis chondrocytes. Osteoarthritis Cartilage. 2001 ; 9(8) : 684-93
19. Lee DA, Frean SP, Lees P, Bader DL. Dynamic mechanical compression influences nitric oxide production by articular chondrocytes seeded in agarose. Biochem Biophys Res Commun. 1998 ; 251(2) : 580-5
20. Lee MS, Ikenoue T, Trindade MC, Wong N, Goodman SB, Schurman DJ, Smith RL. Protective effects of intermittent hydrostatic pressure on osteoarthritic chondrocytes activated by bacterial endotoxin in vitro. J Orthop Res. 2003a ; 21(1) : 117-22
21. Amin AR. Gene mining, bioinformatics and functional genomics in human arthritis and inflammatory diseases ex vivo. Drug Dev Res. 2000 ; 49 : 22-9
22. Piecyk M, Anderson P. Signal transduction in rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2001 ; 15(5) : 789-803
23. Dayer J-M. The saga of the discovery of IL-1 and TNF and their specific inhibitors in the pathogenesis and treatment of rheumatoid arthritis. Joint Bone Spine. 2002 ; 69(2) : 123-32
24. Fischer DC, Siebertz B, van de Leur E, Schiwy-Bochat KH, Graeve L, Heinrich PC, Haubeck HD. Induction of α 1-antitrypsin synthesis in human articular chondrocytes by interleukin-6-type cytokines : evidence for a local acute-phase response in the joint. Arthritis Rheum. 1999 ; 42(9) : 1936-45
25. Mihara M, Kotoh M, Nishimoto N, Oda Y, Kumagai E, Takagi N, Tsunemi K, Ohsugi Y, Kishimoto T, Yoshizaki K, Takeda Y. Humanized antibody to human interleukin-6 receptor inhibits the development of collagen arthritis in cynomolgus monkeys. Clin Immunol. 2001 ; 98(3) : 319-26
26. Naka T, Nishimoto N, Kishimoto T. The paradigm of IL-6 : from basic science to medicine. Arthritis Res. 2002 ; 4(Supp 3) : S233-42
27. Keul R, Heinrich PC, Muller-newen G, Muller K, Woo P. A possible role for soluble IL-6 receptor in the pathogenesis of systemic onset juvenile chronic arthritis. Cytokine. 1998 ; 10(9) : 729-34
28. Webb GR, Westacott CI, Elson CJ. Osteoarthritic synovial fluid and synovium supernatants up-regulate tumor necrosis factor receptors on human articular chondrocytes. Osteoarthritis Cartilage. 1998 ; 6(3) : 167-76
29. Lubberts E, Joosten LA, Helsen MM, van den Berg WB. Regulatory role of interleukin 10 in joint inflammation and cartilage destruction in murine streptococcal cell wall(SCW) arthritis. More therapeutic benefit with IL-4/IL-10 combination therapy than with IL-10 treatment alone. Cytokine. 1998 ; 10(5) : 361-9
30. Lam J, Abu-Amer Y, Nelson CA, Fremont DH, Ross FP, Teitelbaum SL. Tumour necrosis factor superfamily cytokines and the pathogenesis of inflammatory osteolysis. Ann Rheum Dis. 2002 ; 61 : ii82-3
31. Cawston T. Matrix metalloproteinases and TIMPs : properties and implications for the rheumatic diseases. Mol Med Today. 1998 ; 4(3) : 130-7
32. Goggs R, Carter SD, Schulze-Tanzil G, Shakibaei M, Mobasheri A. Apoptosis and the loss of chondrocyte survival signals contribute to articular cartilage degradation in osteoarthritis. Vet J. 2003 ; 166(2) : 140-58
33. Yoshihara Y, Nakamura H, Obata K, Yamada H, Hayakawa T, Fujikawa K, Okada Y. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. Ann Rheum Dis.

2000 ; 59 : 455-461

34. Poole AR, Kobayashi M, Yasuda T, Laverty S, Mwale F, Kojima T, Sakai T, Wahl C, El-Maadawy S, Webb G, Tchetina E, Wu W. Type II collagen degradation and its regulation in articular cartilage in osteoarthritis. Ann Rheum Dis. 2002 ; 61(Suppl II) : ii78-81
35. Konttinen YT, Ainola M, Valleala H, Ma J, Ida H, Mandelin J, Kinne RW, Santavirta S, Sorsa T, Lopez-Otin C, Takagi M. Analysis of 16 different matrix metalloproteinases(MMP-1 to MMP-20) in the synovial membrane : different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis. 1999 ; 58 : 691-7
36. Mort JS, Billington CJ. Articular cartilage and changes in arthritis matrix degradation. Arthritis Res. 2001 ; 3 : 337-341
37. Mobasheri A. Role of chondrocyte death and hypocellularity in ageing human articular cartilage and the pathogenesis of osteoarthritis. Med Hypotheses. 2002 ; 58(3) : 193-7
38. Pettersen I, Figenschau Y, Olsen E, Bakkelund W, Smedsrod B, Sveinbjornsson B. Tumor necrosis factor-related apoptosis-inducing ligand induces apoptosis in human articular chondrocytes in vitro. Biochem Biophys Res Commun. 2002 ; 296(3) : 671-6
39. Wilder RL. Integrin alpha V beta 3 as a target for treatment of rheumatoid arthritis and related rheumatic diseases. Ann Rheum Dis. 2002 ; 61(Suppl II) : ii96-9
40. Ostergaard K, Salter DM, Petersen J, Bendtzen K, Hvolris J, Andersen CB. Expression of $\alpha$ and $\beta$ subunits of the integrin superfamily in articular cartilage from macroscopically normal and osteoarthritic human femoral heads. Ann Rheum Dis. 1998 ; 57 : 303-8
41. Morales TI. The insulin-like growth factor binding proteins in uncultured human cartilage : increases in insulin-like growth factor binding protein 3 during osteoarthritis. Arthritis Rheum. 2002 ; 46(9) : 2358-67
42. Eviatar T, Kauffman H, Maroudas A. Synthesis of insulin-like growth factor binding protein in vitro in human articular cartilage cultures. Arthritis Rheum. 2003 ; 48(2) : 410-7
43. Heemskerk VH, Daemen MA, Buurman WA. Insulin-like growth factor-1(IGF-1) and growth hormone(GH) in immunity and inflammation. Cytokine Growth Factor Rev. 1999 ; 10(1) : 5-14