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

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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, anti-inflammatory, antinoceptive, and anticancer effects, and therapeutic effect against bacterial diarrhea in piglets. Studies involving gene expression following BV administration have been published and.

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. Microarray analysis
of gene expressions in a microglia cell line following hypoxic damage and in a human mast cell line following treatment with BV have been previously carried out.

As a therapeutic modality applied to arthritis, 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 (100μg/ml) (Gibco BRL, USA) at 37°C and in a humidified cell incubator and a atmosphere consisting of 5% CO2-95% 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 100μl of serum-free medium and the vehicle or BV for 12 hours at the following concentrations: (1) 10-1ug/ml, (2) 10-2ug/ml, (3) 10-3ug/ml, and (4) 10-4ug/ml. 10μl MTT solution was added to each well, and cells were then incubated for another 4 hours. The purple formazan salts thus produced were solubilized by adding 100μl of solubilization solution to each well and incubating overnight, again at 37°C and in 5%-CO2 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: 3μl of RT primer, total RNA and additional nuclease-free water were mixed to form a 29μl RNA-RT primer mix, which was microfuged briefly, heated to 80°C for ten minutes, and immediately transferred to ice. 1μl of the RNase inhibitor Superase-In™ was added to the RNA-RT primer mix. 8μl of 5X SuperScript II First Strand Buffer (Gibco BRL, USA), 2μl of dNTP mix (10mM each of dATP, dCTP, dGTP, dTTP), 4μl of 0.1M DTT (dithiotreitol), 2μl of Superscript II enzyme (400units), and 3μl 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 7μl 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 10 minutes 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 5 minutes. 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 30-60 minutes. 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 slide was immediately transferred to a dry 50ml centrifuge tube and dried by centrifugation for 2 minutes at 800-1000 RPM. Array50 capture reagent was then thawed in the dark at room temperature over a period of 20 minutes and vortexed for 3 seconds. The reagent was evenly resuspended by heating at 55°C for 10 minutes 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 10 minutes and then at 50°C for 20 minutes. The hybridization mixture was applied to the pre-warmed microarray 1 minute 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 2-3 hours. After serial washing, the slide was immediately transferred to a dry 50ml centrifuge tube and dried by centrifugation for 2 minutes 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

12 hours 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 10μg/ml BV, 99.0±2.0% with 100μg/ml BV, 97.5±0.8% with 10-3μg/ml BV, and 100.4±4.5% with 100μg/ml BV<Figure 1>. Percent viability with 10μg/ml BV was significantly different from that of the control group.
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 distintegration and metalloprotease with thrombospondin motif(ADAM-TS4 and ADAM-TS5)20. 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-α) and interleukin-1(IL-1) are key proinflammatory cytokines in the pathogenesis of arthritis20.

The inflammatory condition including arthritis is one of therapeutic applications of BV20. Studies involving gene expression following BV administration have been published20. 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 research20, involving known or unknown genes at a certain stage in
Table 1. Genes showing bee venom-induced downregulation in HTB-94 chondrosarcoma cells

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

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

physiologic or pathologic processes. 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. It maintains the
chondrocytic phenotype and serves as an appropriate chondrocyte model of human arthritis for various purposes, including interleukin-responsive immediate early gene studies, protein kinase gene expression studies, and MMP gene expression pathway studies. 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.

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 at http://annotation.digital-genomics.com.co/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-1β and TNFα. IL-1β and TNFα are expressed at high levels in chondrocytes in osteoarthritis(OA) and rheumatoid arthritis (RA) and play major roles in the pathogenesis of cartilage degeneration. 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-α, 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. IL-1 induces the synthesis of MMP-1 and PGE2 which contribute to collagen degradation, periarticular osteopenia, and bone resorption. IL-1α 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-1β is secreted and exerts its biologic actions on other cells.

Human articular chondrocytes stimulated by IL-1β and TNFα 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 α1-antitrypsin, a major inhibitor of serine proteinases, which contributes to an important protective mechanism of articular chondrocytes against cartilage damage in inflammatory joint diseases. 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. In systemic juvenile chronic arthritis(JCA), the levels of soluble IL-6 receptor, IL-6 agonist, were found to be significantly increased. OA-affected chondrocytes exhibit upregulations in TNF receptor expression and are more susceptible to both TNFα and IL-1β. IL-1β 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 TNFα. By way of mRNA degradation and NF-κB inhibition, respectively, IL-4 and IL-10 suppress TNF-α and IL-1. IL-4 and IL-10 synergistically suppress arthritic symptoms and enhance proteoglycan synthesis.

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-κB is an essential osteoclastogenic transcription complex. The NF-κB and MAPK pathways are central in the pathogenesis of RA and OA. Both are coordinately activated by IL-1 and TNF-α.

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. Imbalance between MMPs and TIMPs is suggested to be responsible for cartilage matrix degradation. TIMPs exist in four types: 1, 2, 3, and 4. They inhibit activated MMPs by tightly binding to them with 1:1
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 stromelysin 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), enamelysins(MMP-20), MMP-19, and MMP-23. MMPs are key mediators in the resorption of cartilage, bone, arthritic synovial fluid, and adjacent soft tissue during pathological destruction of joint tissue. Of the 27 known MMPs, MMPs 1, 8, 13, and 14 are considered to be more active in cartilage degradation. 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. MT2-MMP can degrade fibronectin, tenascin, nidogen, aggrecan, perlecan, and laminin. MT2-MMP processes pro-TNFα to its mature form and activates proMMP-2. MT2-MMP is reported to be exclusively associated with RA synovitis. 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. In RA and OA, the interstitial collagens(types I, II, and III) are the principal targets of destruction. Secreeted collagenases(MMP-1 and MMP-13) play major roles in this destruction. Their expression involves the nuclear factor κB(NF-κB) and mitogen-activated protein kinase(MAPK) pathways.

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. 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 to adaptor proteins such as FADD. Adaptor proteins in turn bind via their death effector domains to initiator caspases (caspase 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.

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 α and β 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 α subunits that associate with at least nine and β subunits. The α and β subunits assemble into at least 24 distinct receptors. Integrins function both as cell adhesion receptors and as intracellular signalling receptors. Recently, Integrin αVβ3 began drawing attention as a possible therapeutic target in the treatment of RA. In the normal articular cartilage, integrin heterodimers αVβ3, αVβ1, and αVβ5 are expressed strongly; α3, β3, and β4 subunits are also expressed. Osteoarthritic cartilage is reported to express the α2, α4, and β2 subunits in addition. Integrin β1 is overexpressed in the cartilage in OA. Integrin β1-mediated cell-matrix interactions were reported to provide survival signals for chondrocytes.

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. The rate of IGFBP-3 synthesis is reported to be 3 times higher in the cartilage affected by OA than in the normal cartilage. The pro-inflammatory cytokines TNF-α, IL-1α, and IL-1β 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.

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


17. Vincenti MP, Brinckerhoff CE. Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1β. Arthritis Res. 2001; 3: 381-8


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