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# An Experimental Study on the Effect of Cytoskeletal Changes on Osteoblastic Cell Activities

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The cytoskeleton has been shown to form a network, connecting the extracelluar matrix via integrin with the nucleus and the cytoplasmic constituents of the cell. It is therefore assumed that the cytoskeleton may mediate signals generated by perturbations originating in the matrix. The purpose of this study is to examine the effect of cytoskeletal change on osteoblastic cell activities. The author cultured osteoblastic cells obtained from neonatal mouse calvaria. The cells were treated with cytochalasin B(CB) or colchicine (COL) at four concentrations for 3 hours and after another 24 hours the conditioned media was collected and assayed for prostaglandin $E_2$ ($PGE_2$), interleukin-6(IL-6), tumor necrosis factor-α (TNF-α) and matrix metalloproteinase-1(MMP-1). In addition, the cytoskeletal protein actin were observed by immunofluorescence. The results were as follows:

1. The production of $PGE_2$ showed the tendency to be increased in CB-treated group. $PGE_2$ was increased in COL-treated group dose-dependantly.
2. IL-6 production, in CB-treated group, was increased, except at 1.0 μg/ml. IL-6 was induced in COL-treated group.
3. TNF-α production was increased in CB-treated group, except at 1.0 μg/ml, and in COL-treated group, that was increased.
4. The MMP-1 production was decreased in CB-treated goup and was not changed in COL-treated group, which could be selectively visualized by immunoblotting with monospecific antibody.
5. The cytoskeletal actin stress fibers were disappeared and the cells showed to be rounded in CB-treated group.

These results indicated that there are a relationship between the cytoskeletal rearrangements and osteoblastic cell activities, especially in release of paracrine/autocrine factors, such as $PGE_2$, IL-6, and TNF-α.

**Key Words** : cytoskeletal changes, osteoblastic cells, $PGE_2$, IL-6, TNF-α , MMP-1

Clinical orthodontic treatment is bone-manipulative therapy[29] in the sense that teeth or bones move via bone modeling and remodeling. Thus a better knowledge of the mechanisms involved in orthodontically-induced bone remodeling is fundamentally important. Mechanically induced bone remodeling has been studied mostly by the orthopaedist to determine the optimal condition for physiologic remodeling. Their results have been demonstrated that the mass and architecture in bone are regulated by adaptative mechanisms sensitive to mechanical environment, therefore mechanical stress is essential to maintain skeletal tissues. Wolff's law of bone transformation[12], which proposed that mechanical stresses affect the form of bone, has garnered general acceptance. Also, pressure-tension theory and piezoelectric

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effect etc. have explained orthodontic bone remodelng , but any one theory among them can't work solely. Especially the mechanism of conversion of biophysical force into a cellular response, is not discovered completely[22,35,41,42].

Almost a century of research has been devoted to examing the phenomenon by morphologic methods and so the histological changes have consequently been well documented. In recent, with advents of molecular biology, cell biology and cell kinetics, the efforts to elucidate mechanism of mechanically induced bone remodeling biochemically and physiologically are continued. Understanding of these mechanisms make it possible to control the procedure biochemically and to achieve treatment results successfully as soon as possible within physiologic ranges. The in vivo and in vitro models have been developed to examine the response of connective tissues to mechanical deformations[2,10,16,18-20,24,31,36]. Osteoblasts are now recognized as the cells that control both the resorptive and the formative phases of the remodeling cycle, and receptor studies have shown them to be the target cells for resorptive agents in bone. The osteoblast is percieved as a pivotal cell, controlling many of the responses of bone to stimulation with hormones and mechanical forces. It is apparent that not all the cellular responses induced by mechanically deformed tissues can be explained by the current paradigm emphasizing the importance of prostaglandin[4,5,11,23,41] and cAMP[25,33] elevation; the mobilization of membrane phospholipid giving rising to inositol phosphates[33,34] offers an alternative second messenger pathway. It is also argued from circumstantial evidence that changes in cell shape produce a range of effects mediated by membrane integral proteins(integrins) and the cytoskeleton, which may be improtant intransducing mechanical deformation into a meaningful biologic response.

Mechanotranduction, or the conversion of a biophysical force into a cellular response, is an essestial mechanism for a wide variety of physiological functions that allow living organisms to respond to the mechanical environment. In 1995, Duncan and Tuner[12] have divided the process of mechanotransduction in bone into 4 distinct steps:(1) mechanocoupling, the transduction of mechanical force applied to bone into a local mechanical signal percieved by a sensor cell; (2) biochemical coupling, the transduction of a local signal into a biochemical signal and ultimately, gene expression; (3) transmission of signal from the sensor cell to the effector cell, i.e., the cell that willl accurately form or remove the bone; and (4) effector cell response, the final tissue-level response. Among them, the author were interested in the second step, biochemical coupling and so this study was performed. Though the mechanism for the initial detection and conversion of mechanical force into a biochemical signal has yet to be determined, several candidates have been proposed. One possible transduction pathway is the extracelluar matrix-integrin-cytoskeletal axis.

Bone cells, like most other cell types, attatch to the extracellular matrix through cell-suface receptors for specific matrix protein and many of these receptors belong to integrin superfamily. The cytoskeleton has been shown to form a network, connecting the extracelluar matrix via integrin with the nucleus and the cytoplasmic constituents of the cell. It is therefore assumed that the cytoskeleton may mediate signals generated by perturbations originating in the matrix. The effect of mechanical stress on cytoskeleton have been studied in diffenent types of cells[3,7,17,21,28]. Jones at al.[17] investigated the biochemical signal transduction of mechanical strain in osteoblast-like cells and they concluded that the mechanism is located in the cytoskeleton. Banes et al.[3] showed that tubulin and actin may be altered in vitro in response to cyclic compressionin tendon cells. Brunette[7] examined the influence of mechanical stretching on epitheilal cells and observed the cytoskeleton electromicroscopically.

In addition, various investigations to study the role of actin in connective tissue matrix remodeling indicates that there is a correlation between cytoskeletal architecture and the expression of genes[15,37,38,40]. Though the role of cytoskeleton in other cell types has been well documented, little was known in osteoblastic cells.

The purpose of this study is to examine the effect of cytoskeletal change, which was induced by cytochalasin -B and colchicine, on osteoblastic cell activities by measuring several parameters (prostaglandin, inter-

leukin-6, tumor necrosis factor and matrix metalloproteinase-1) released from cells.

## MATERIALS AND METHODS

### *Materials*

Media, fetal bovine serum(FBS) and other cultural reagents were obtained from Gibco(Grand Island, NY, USA). All disposable culture wares were purchased from Corning Incorporated (Corning, NY, USA). $PGE_2$ EIA system were from Amersham LIFE SCIENCE(Arlington Heights IL, USA) and IL-6 and TNF-α ELISA kit were purchased from Genzyme (Cambridge, MA, USA). Cytochalasin B, clochicine and other reagents were purchased from Sigma(St. Louis, MO, USA). Mouse MMP-1 antibodies were obtained from Calbiochem and western-light plus from Tropix were used.

### *Cell preparation*

Neonatal mouse calvarial osteoblastic cells were obtained as previously described[39]. Briefly described, frontal and parietal bone from two to three- day-old mice were dissected aseptically and digested consecutively six times with enzyme solution containing 0.1% collagenase, 0.05% trypsin and 0.5mM EDTA. At 10, 10, 10, 20, 20 and 20 minute interval(this procedure yields six populations of cells designated as I, II, III, IV, V and VI), released cells of populations IV, V, VI which were characterized as osteoblastic cells. The cells were pooled and cultured α-minimum essential medium supplemented with 10% fetal bovine serum(MEM/FBS) and when confluent, cells were subcultured for following experiments.

### *Treatment with cytochalasin-B and colchicine*

When the cells reached confluency, they were treated with 0.1-2.0 μg/ml cytochalasin-B(CB), 0.1-1.0 μg/ml colchicine(COL) and without chem icals as a control. After 3 hours, media were changed with media containing low concentration of serums and incubated for another 24 hours. Then conditioned media were collected, and used for determining the amount of $PGE_2$, IL-6, TNF-α and MMP-1.

### *Prostaglandin $E_2$ ($PGE_2$) assay*

Levels of prostaglandin $E_2$ in media were determined by enzymeimmunoassay(EIA) system (Amersham LIFE SCIENCE). All assay were perfomed in duplicate according to protocol provided with kit. Briefly described, diluted $PGE_2$ standards and samples were titered into 96-well microtiter plate coated with goat anti-mouse IgG. Then was incubated with diluted lyophilized $PGE_2$ antibody and diluted conjugate for 1hour, washed, incubated with tetramethylbenzidine (TMB) substrates solution for 30 minutes and the reaction was stopped by 1M $H_2SO_4$.

### *Interleukin-6 (IL-6) assay*

Levels of IL-6 in media were determined by enzyme-linked immunoabsorbent assay(ELISA) kit from genzyme. All assay were perfomed in duplicate according to protocol provided with kit. Briefly described, 96-well microtiter plate coated with anti mIL-6 antibody incubated with diluted standards and samples for 1 hour, washed, incubated with biotinylated antibody for 1 hour, washed, and incubated with horseradish peroxidase conjugated streptavidin for 30 min. Finally it was incubated with TMB substrates solution for 15 minutes and the reaction was stopped by 1M $H_2SO_4$.

### *Tumor necrosis factor-α (TNF-α) assay*

Levels of TNF-α in media were determined by ELISA kit from genzyme. All assay were perfomed in duplicate according to protocol provided with kit. Briefly described, 96-well microtiter plate coated with antibody incubated with diluted standards and samples for 2 hour, washed, incubated with horseradish peroxidase conjugated streptavidin for 1 hour. Finally it was incubated with TMB substrates solution for 10 minutes and the reaction was stopped by 1M $H_2SO_4$.

### *Matrix metalloproteinase(MMP) -1 assay*

Cell were cultured in 35mm dish until confluent. After treatment with CB and COL, 1ml medium was precipitated with 3% trichloroacetic acid, and the precipitates were suspended in sample buffer with mercaptoethanol, and electrophoresed on a 8% SDS polyacrylamide gel.

**Table 1.** The effect of Cytochalasin-B on $PGE_2$ production in osteoblastic cells.

| Cytochalasin-B (μg/ml) | PGE2 (ng/dish) |
|---|---|
| 0 | 38.3 ± 6.7 |
| 0.1 | 58.9 ± 20.9 |
| 0.5 | 45.8 ± 4.1 |
| 1.0 | 40.4 ± 16.8 |
| 2.0 | 35.7 ± 21.7 |

Data represent Mean ± SE of replicates
* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$

**Table 2.** The effect of Colchicine on $PGE_2$ production in osteoblastic cells.

| Colchicine (μg/ml) | $PGE_2$ (ng/dish) |
|---|---|
| 0 | 38.3 ± 6.7 |
| 0.1 | 55.8 ± 3.3* |
| 0.2 | 77.0 ± 2.9** |
| 0.5 | 95.6 ± 3.6** |
| 1.0 | 108.0 ± 3.2** |

Data represent Mean ± SE of replicates
* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$

The proteins were transferred to a PVDF membrane, and hybiridized with primary antibody to mouse MMP-1 and secondary antibody conjugated with alkaline phosphatase. After the substrate solution was put onto the blot, the blots were exposed to radiographic flim, and the film was developed.

### *Immunofluroscence staining of actin*

Cells were plated into tissue culture slides well and after 2-day overnight were treated with 2.0 μg/ml CB, 1.0 μg/ml COL for 3 hours and without chemicals as a control. Then the well was removed and the slides were washed with PBS. The cell were fixed in acetone at -20° C for 20 min, dried , and blocked in immunofluorescence blocking buffer for 1 hour at 37° C. Then slide were incubated with diluted primary antibody for 1hour at 37° C in a humid chamber, washed and incubated with the secondary antibody for 1 hour. After washing with PBS, the slides were mounted and examined with fluorescence microscope (OLYMPUS VANOX AHBT3).

### *Statistics*

Data were expressed as Mean±SE of 3 replicates. The statistical significance of the difference was determined by Student's t-test.

**Table 3.** The effect of Cytochalasin-B on IL-6 production in osteoblastic cells.

| Cytochalasin-B (μg/ml) | IL-6 (ng/dish) |
|---|---|
| 0 | 1.4 ± 0.17 |
| 0.1 | 3.6 ± 1.32 |
| 0.5 | 37.5 ± 4.30** |
| 1.0 | 0.7 ± 0.06* |
| 2.0 | 32.9 ± 1.81** |

Data represent Mean ± SE of replicates
* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$

**Table 4.** The effect of Colchicine on IL-6 production in osteoblastic cells.

| Colchicine (μg/ml) | IL-6 (ng/dish) |
|---|---|
| 0 | 1.4 ± 0.17 |
| 0.1 | 102.2 ± 11.2** |
| 0.2 | 178.4 ± 3.63** |
| 0.5 | 171.8 ± 4.24** |
| 1.0 | 169.7 ± 2.30** |

Data represent Mean ± SE of replicates
* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$

## RESULT

In CB-treated group, the $PGE_2$ level showed tendency to be decreased, which was not statistically significant(Table 1). However in COL -treated group $PGE_2$ was increased significantly (Table 2).

**Table 5.** The effect of Cytochalasin-B on TNF-α production in osteoblastic cells.

| Cytochalasin-B (μg/ml) | TNF-α (pg/dish) |
|---|---|
| 0 | 688.5 ± 47.0 |
| 0.1 | 1022.6 ± 419.1 |
| 0.5 | 3384.8 ± 150.2** |
| 1.0 | 1098.4 ± 43.1** |
| 2.0 | 3648.7 ± 225.1** |

Data represent Mean ± SE of replicates
* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$

**Table 6.** The effect of Colchicine on TNF-α production in osteoblastic cells.

| Colchicine (μg/ml) | TNF-α (pg/dish) |
|---|---|
| 0 | 688.5 ± 47.0 |
| 0.1 | 1320.8 ± 86.1** |
| 0.2 | 1175.6 ± 109.5** |
| 0.5 | 1522.2 ± 99.7** |
| 1.0 | 1950.0 ± 113.3** |

Data represent Mean ± SE of replicates
* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$

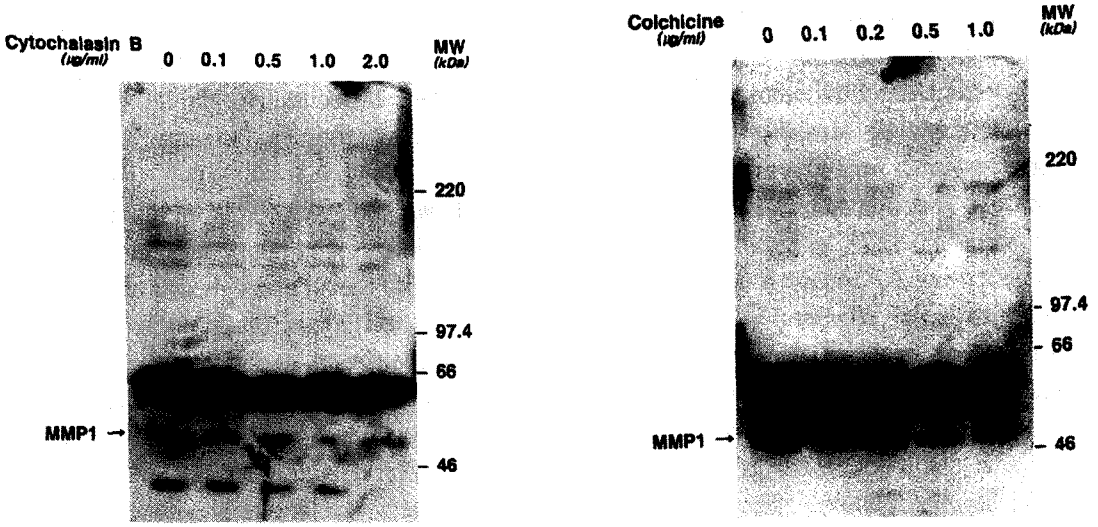


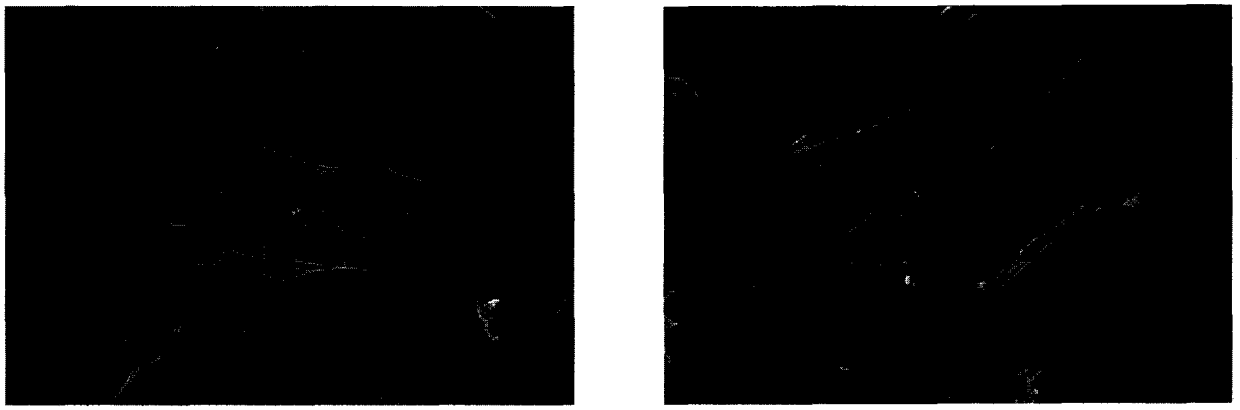

Fig 1. Demonstration of MMP-1 in conditioned culture medium of osteoblastic cells. Molecular weight markers are indicated on the right.

When treated with CB, IL-6 production was increased at 0.5 μg/ml and 2 μg/ml significantly, though at 1.0 μg/ml it was decreased (Table 3). IL-6 was induced in COL-treated group significantly but was not dose-dependant(Table 4).

The assay of TNF-α production showed the same tendency as IL-6. In COL-treated group, they were increased at 0.5 μg/ml and 2 μg/ml significantly, though it was decreased at 1.0 μg/ml (Table 5). IL-6 was induced in COL-treated group significantly but was not dose-dependant (Table 6).

In osteoblastic cells treated with CB, it seems that MMP-1 production was decreased and that was not changed in COL-treated group, which could be selectively visualized by immunoblotting with monospecific antibody(Fig. 1).

According to fluorescence microscopic observations, upon the addition of CB, cells became rounded and stress fibers disappeared from most cells. In untreated cells the stress fibers were intact(Fig. 2).



(a) control group (b) Cytochalasin B treated group

Fig. 2. Localization of actin microfilament organization in mouse calvarial osteoblastic cells by immunofluorescence.

## DISCUSSION

The process of bone apposition and resorption in orthodontic patients are based on the principles that the mechanical environment of clinically applied forces are transferred to bone cells which translate these forces into a cell response.

Several mechanisms have been described for the coupling of an external mechanical signal into an intracellular biochemical signal[12]. These include force transduction from the extracelluar matrix to cytoskeleton and nuclear matrix through the integrins, stretch -activated cation channels within the cell membrane, G protein- dependent pathways in the cell membrane, and linkage between the cytoskeleton and the phospholipase C or phospholipase A pathways. Though each of these signal pathways have been treated as a primary mechanotransduction mechanisms, it should be noted that they have a high degree of association with one another.

One possible transduction pathway is the extracellular-integrin-cytoskeletal axis. Cells attach to the extracellular matrix through binding to membranes spanning glycoprotein called integrins[32]. Integrin attach to the actin cytoskeleton through several actin-associated proteins such as vinculin, talin, tensin, and α-actinin. The cytoskeleton has been shown to form a network, connecting the extracelluar matrix with the nucleus and the cytoplasmic constituents of the cell. The cells generates an internal force through the cytoskeleton which exert a tension on extracelluar matrix. Due to the tension of the cytoskeleton, physical stimulus would be transmitted to the nucleus, possibly altering gene expression. Indeed, cellular attachment to the extracellular matrix has been shown to play an important role in the regulation of cellular proliferation, diffrentiation, morphogenesis, and gene expression.

And also, Sandy et al.[32] pointed out that the cytoskeleton presents a number of possibilities for transducing mechanical forces acting on cells and/or their adjacent matrices. Three main components of the cytoskeleton are microtubules, microfilaments, and intermediate filaments and microfilaments are perhaps the best situated of the three systems to detect these changes. The major subunit protein of microfilaments is actin; there are, however, many associated proteins, such as myosin, tromyosin, vinculin, and talin.

Pender and McCulloch[28] have shown the F-actin change in fibroblasts subjected to mechanical stretch and they concluded that the measured change in F-actin appeared to be global and thus probably occured in the cortical zone of the cell. Brunette[7] examined the influence of mechanical stretching on epitheilal cells and observed the cytoskeleton electromicroscopically. They used epithelial cells derived from the epithelial rests of Malassez stretched

by means of orthodontic screw. Morpholometric analysis of electron micrographs of stretched and unstretched cultures indicated that the stretched cultures had a higher volume fraction of filamentous structures and more desmosomes per unit length of cell membrane than unstrained cutures. Jones et al.[17] and Carvalho et al.[8] investigated the biochemical signal transduction of mechanical strain in osteoblast-like cells and they concluded that the mechanism is located in the cytoskeleton. Banes et al.[3] showed that tubulin and actin may be altered in vitro in response to cyclic compression in tendon cells.

The various investigations to study the role of cytoskeleton in connective tissue matrix remodeling indicates that there is a correlation between cytoskeletal architecture and the expression of genes. Alterations in the cytoskeleton have been observed in cells exposed to stretching and cytochalasin B, which disrupt intracellular microfilaments have shown to induce collagenase synthesis in fibroblasts[38]. Furthermore, colchicine increases $PGE_2$ production by osteoblasts fourfold to fivefold[40]. These responses are accompanied by a rounding-up of the cells. These suggests that increased collagenase systhesis by these cells may also have resulted from disruption of the cytoskeleton.

According to Yeh and Rodan[40], COL, which is microtubule disrupting agents increases $PGE_2$ production by osteoblasts fourfold to fivefold but CB, which disrupts microfilaments, had no effect. Werbs et al.[38] studied the relationship of cytoskeletal changes to the commitment to expression of metalloendopeptidases, collagenase and stromelysin. They used CB or 12-O-tetradecanoylphorbol-13-acetate to alter the morphology of rabbit synovial fibroblast and induced synthesis of these metalloendopeptidases. In this study, fluorescence microscopic observations showed that cells became rounded and actin stress fibers disappeared from the most cells, upon the addition of CB. Some portion of actin filaments might be drawn into clump, or foci. In untreated cells, the stress fibers are intact(Fig. 2). Aggeler et al.[1] explained that catabolic rather than anabolic events are associated with rounded cell shape, so the osteoblastic cells, we observed, are likely to be in catabolic state[32,37,38,42].

How mechanical deformation activates connective tissue cells is not completely understood, although the involvement of prostaglandins and the cyclic AMP pathway is well established[14,25,26]. Somjen et al.[35] have proposed the following scheme : mechanical deformation of the cell initiates $PGE_2$ synthesis from membrane phospholipids ; the subsequent binding of extracellular $PGE_2$ to cell surface receptors activates adenylate cyclase and the cAMP pathway; this mechanism enables $PGE_2$ to activate the cell of origin as well as adjacent cells in an autocrine/paracrine fashion, thereby amplifying the signal.

There are two possible pathways by which a biochemical signal in the sensor cell is propagated to the effector cell after a mechanical stimulus[12]. First, active osteoblasts on the bone surface can sense mechanical strain and also act as the effector cell and which involves second messenger systems, such as cAMP and inositol phosphates. The second means are communication of a biochemical signal from nonproliferative, strain sensing cells(for example, osteocytes and bone lining cells) to osteoprogenitor cells and osteoblasts through paracrine factors. Several cytokine, growth factor and prostaglandins might act as a paracrine factors. In this study IL-6, TNF-α and $PGE_2$ from the medium are measured to determine remodeling activity of osteoblast- like cells.

Prostaglandins may influence local bone cell activity. They may have an initial inhibitory effect on osteoclasts, but their predominant long-term effect is to stimulate the resorption of bone by increasing the proliferation and formation of osteoclasts. Furthermore, the effects of other agents on the resorption of bone may be mediated through their effects on the synthesis of prostaglandins. The precursor of PGs and leukotrienes is arachidonic acid which is released from the phospholipids of the cell membrane by the action of phospholipase A. Arachidonic acid is metabolized by cyclooxygenase enzymes producing PGs and thromboxanes. Metabolism through the lipoxygenase pathway results in production of leukotrienes and hydroxyperoxyeicosatetraenoic acid . The involvement of PGs and leukotriens in mechanically induced bone remodeling

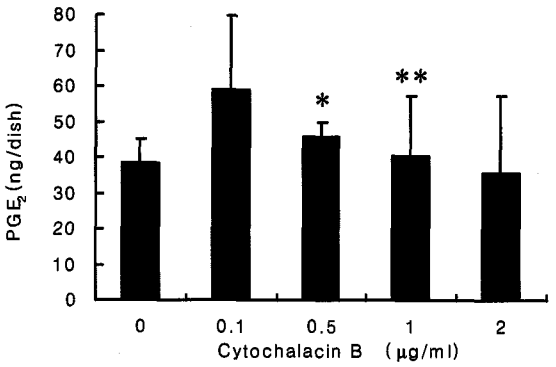



Fig. 3. The effect of Cytochalasin-B on $PGE_2$ production in osteoblastic cells.

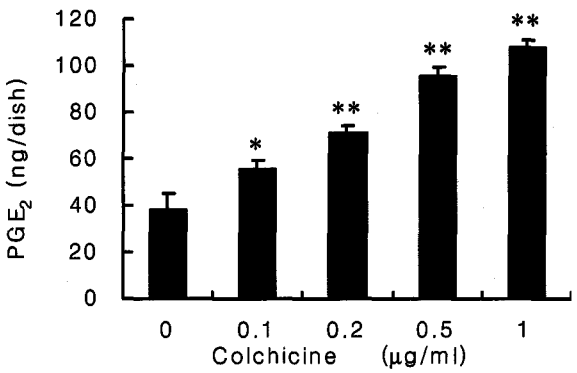



Fig. 4. The effect of Colchicine on $PGE_2$ production in osteoblastic cells.

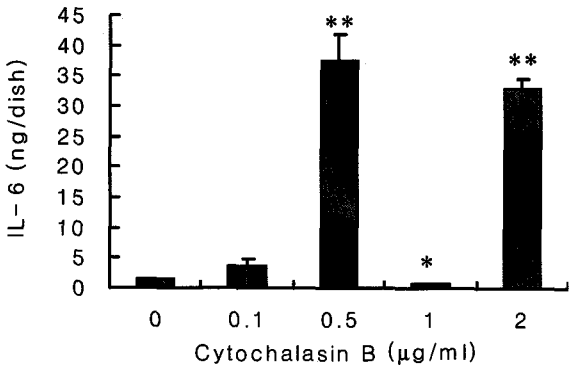



Fig. 5. The effect of Cytochalasin-B on IL-6 production in osteoblastic cells.

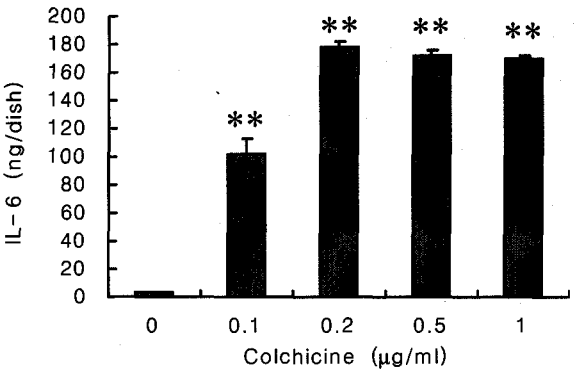



Fig. 6. The effect of Colchicine on IL-6 production in osteoblastic cells.

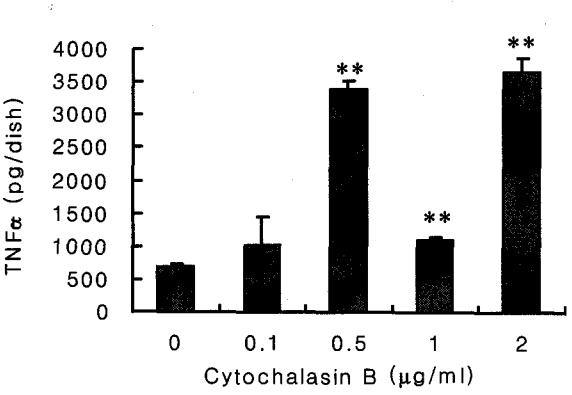



Fig. 7. The effect of Cytochalasin-B on TNF-α production in osteoblastic cells.

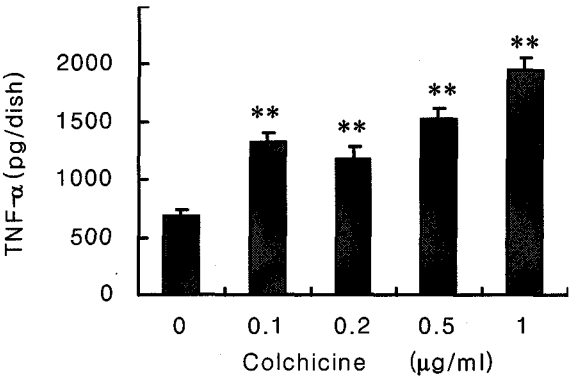



Fig. 8. The effect of Colchicine on TNF-α production in osteoblastic cells.

has been investigated in a number of animal and tissue culture models[30]. In vitro experimentation by Harell et al.[14] suggested that PGs may be important mediators of mechanical stress. In this study, $PGE_2$ was increased in COL-treated group significantly but in CB- treated group, they just have the increased tendency without significance(Fig. 3,4). Yeh and Rodan[40] explained that the effects of COL on PG production were related to microtubule assembly, and so PG were increased upon COL addition , not upon CB addition.

Cytokines can be described as small proteins produced by cells which modify the behavior of other cells. Whether one gets bone formation or bone resorption is dependent upon the cytokines produced locally by mechanically activated cells as well as the functional status of the available target cells. The factors which influence connective tissue remodeling can include the interleukins, the tumor necrosis factors, interferons, peptide growth factors and colony stimulating factors. According to this study results, it could be said that IL-6 and TNF-α were induced by cytoskeletal change(Fig. 5,6,7,8).

Although the effector cell of bone resorption is the osteoclast, studies demonstrating that osteoblasts and not osteocalsts exhibit receptors for PTH, vitamin D metabolites, and prostaglandins indicate that osteoclast recruitment and activity involve cells of the osteoblast lineage. How osteoblasts transmit signals to osteoclasts is not known, but broadly speaking there are two possibilities based on a number of in vitro experiments[9,27]. First, osteoblasts might facilitate bone resorption through mineral exposure by collagenases (MMP-1). Second, the resorptive signal may be trasmitted to the osteoclast by an osteoblast cytokine. IL-6 and TNF-α observed in this study are likely to act as a controlling factor by the second mechanism.

Matrix metalloproteinases(MMP) are a group of highly homologous enzymes(collagenases, stromelysins, gelatinases) believed to play a major role in biologic remodeling of connective tissues in general and the metabolic degradation[6]. The maintenance of normal tissue architecture is dependent not only upon the ability of connective tissue cells to synthesize structutal molecules such as collagen but also on their ability to degrade them[13]. The degradation and remodeling of periodontium are initiated by action of interstitial collagenase and these collagenolysis are involved in tooth movements. The activity of collagenase in osteoblasts and the associated cells were influenced by mechanical stimuli as well as hormones(ex. PTH). Green et al.[13] have stressed coronal suture explants and demonstrated that collagenase production was increased in the area of increased cellularity. They speculated that matrix degradation is an essential prerequisite for cell proliferation as if it creates room to accommodate an increase in cell population. Aggeler[1] found that the cytoskeletal architecture of rabbit synovial fibroblasts in relation to the induction of expression of the two metalloproteinases, collagenases and stromelysin. they also demonstrated that disassembly of actin stress fibers during a critical induction period was required for commitment of cells to expression of these enzymes. Unemori and Werb[37] suggested that the critical factor to induce production of collagenases is reorganization of actin. On the contrary to those results, in this study collagenase production was not induced in spite of the disassembly of actin(Fig 1, 2).

Taken together, it seems like that there are linkage of cytoskeleton with other systems that produce some messengers. Further investigation might have to be focused on the exact mechanism of that linkages in osteoblastic cells.

## CONCLUSION

To investigate the possible involvement of cytoskeleton in bone cell activities, the author cultured osteoblastic cells obtained from neonatal mouse calvaria. The cells were treated with cytochalasin B(CB) or colchicine(COL) at four concentrations, and the conditioned media was assayed for prostaglandin $E_2$ ($PGE_2$) , interleukin -6(IL-6), tumor necrosis factor-α (TNF-α) and matrix metalloproteinase-1(MMP-1). In addition, the cytoskeletal protein actin were observed by immunofluorescence. The results were as follows:

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in COL-treated group dose- dependantly.

2. IL-6 production, in CB-treated group, was increased, except at 1.0 μg/ml. IL-6 was induced in COL-treated group.
3. TNF-α production was increased in CB-treated group, except at 1.0 μg/ml, and in COL-treated group, they was increased.
4. The MMP-1 production was decreased in CB-treated goup and was not changed in COL-treated group, which could be selectively visualized by immunoblotting with monospecific antibody.
5. The cytoskeletal actin stress fibers were disappeared and the cells showed to be rounded in CB-treated group.

These results indicated that there are a relationship between the cytoskeletal rearrangements and osteoblastic cell activities, especially in release of paracrine/autocrine factors, such as $PGE_2$, IL-6, and TNF-α.

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국문초록

# 골모세포유사세포의 cytoskeletal change가 세포 활성에 미치는 영향에 관한 실험적 연구

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Cytoskeleton은 세포핵과 세포외 기질을 연결하고 있어서 기질에 가해지는 물리적 힘에 의해 cytoskeletal change가 유도되고 이에 의해 세포의 개조활성이 영향을 받는다고 생각되어 왔다. 본 연구는 골모세포 활성에 대한 cytoskeletal change의 역할을 규명하기 위한 것으로서, 신생 백서로부터 조골세포양 세포를 분리, 배양하고 네가지 농도의 cytochalasin B(CB) 또는 colchicine(COL)을 3시간 처리하였다. 다시 배양액을 교환하고 24시간 동안 배양하여 prostaglandin $E_2$ ($PGE_2$) , interleukin-6(IL-6), tumor necrosis factor-α(TNF-α) 및 matrix metalloproteinase-1 (MMP-1) 생산을 측정하고 통계적으로 비교하였으며 cytoskeletal protein actin 변화를 관찰하기위하여 면역형광염색하고 형광현미경으로 관찰하여 다음과 같은 결과를 얻었다:

1. CB 처리군에서 $PGE_2$ 생산이 증가되는 경향을 보였고 COL 처리군에서는 약물농도에 비례하여 증가하였다.
2. IL-6 생산은 CB농도 1.0 μg/ml일때를 제외하고 증가되었다.
3. TNF-α도 CB 농도가 1.0 μg/ml 일때를 제외하고 증가하였다.
4. MMP-1 생산은 CB 처리군에서 감소하는 경향을 보이고 COL 처리군에서는 변화되지 않았다.
5. CB처리군에서는 cytoskeletal actin stress fibers가 사라지고 세포모양이 둥글어지는 경향을 보였다.

이상의 결과로 미루어 보아 cytoskeletal rearrangement는 골모세포유사세포의 활성, 특히 $PGE_2$, IL-6, 및 TNF-α 같은 paracrine/autocrine factor의 생산과 관련있는 것으로 보인다.

**주요 단어** : cytoskeletal change, 골모세포유사세포, $PGE_2$, IL-6, TNF-α , MMP-1