대한한방내과학회지 제25권 3호(2004년 9월)
Korean J.Orient.Int. Med. 2004;25(3):398-407

마우스의 Calvarial Bone Resorption에 미치는 天竺黃의 영향

김성재, 윤철호, 정지천

동국대학교 한의과대학 내과학교실

Effects of *Bombusae concretio* Salicea on Mouse Calvarial Bone Resorption

Seong-Jae Kim, Cheol-Ho Yoon, Ji-Cheon Jeong

Department of Internal Medicine, College of Oriental Medicine, Dongguk University

목적 : 天竺黃이 골대사에 미치는 영향을 검토하고자 생쥐의 골세포를 이용하여 골흡수 억제 효과를 관찰하였다.

방법 : Interleukin-1β (IL-1β)는 시험관내에서 osteoblast를 조절하는 것으로 알려져 있는데, 天竺黃이 IL-1β로 유발된 PGE_2 생성에 관한 영향을 관찰하였다.

결과 : 天竺黃는 osteoblast에 독성을 나타내지 않았으며, PGE_2 생성을 억제하였다. 특히, 天竺黃을 1시간동안 전처리한 경우 PGE_2의 합성을 억제하여 골세포 보호효과가 인정되었으며, 또한 골 흡수인자인 IL-1β에 의해 유발된 alkaline phosphatase의 활성도 억제하였는데, 天竺黃 전처리후 16시간째에 훨씬 높은 억제효과를 나타내었다. 유사하게 天竺黃을 1시간 동안 전처리한 경우 osteocalcin의 생성이 증가되었다. 또한, calcitonin이 나타내는 osteoclast 및 osteoblast 함유 세포에서 골 흡수를 억제하였다.

결론 : 天竺黃은 osteoclast가 매개된 골흡수를 억제하는 효과가 인정되었다.

중심단어 : 天竺黃, interleukin-1β, PGE_2, alkaline phosphatase, osteoblast

Ⅰ.緖 論

Several reports have suggested that natural dietary plants may play an antioxidative role in the prevention of aging and carcinogenesis and may offer effective protection from lipid peroxidative damage *in vitro* and *in vivo*[1,2]. Therefore, much attention has been focused on natural antioxidants[3,4]. In particular, it was reported that the extract of *Bombusae concretio* Salicea (BCS) is specifically effective for cerebrovascular lesion and aphasia during the treatment of Wind-heat syndrome and heat-phlegm in Oriental medicine[5]. In recent study, the pharmacological mechanism for BCS was attributed to antioxidative activities in experimental in vitro and in

· 접수 : 2004년 6월 1일 · 채택 : 2004년 7월 15일
· 교신저자 : 윤철호, 경북 경주시 용강동 357 동국대 한방병원 5내과
(Tel. 054-770-1254, E-mail : dryoon@dongguk.ac.kr)

vivo systems[6,7], although little is yet known about the pharmacological effects or active ingredients.

It was demonstrated that one such as cytokine, interleukin-1 (IL-1), can modulate several aspects of the activity of various bone cell types. IL-1 is a potent stimulator of bone resorption *in vitro*[8], an action apparently partially mediated via the stimulation of osteoclast cell formation indicating the modulation of osteoblast cell activity by IL-1[9]. Previous studies have demonstrated that interleukin-1β (IL-1β) regulate several aspects of the functional activity of human osteoblast-like cells *in vitro*[10,11]. It is also a potent inducer of prostanoids[12,13]. Both prostaglandin-dependent and prostaglandin-independent effects on bone metabolism have been reported. For example, injection of IL-1 above the calvariae of mice caused a short-term prostagladin-independent stimulation of bone resorption followed by a prolonged increase in resorption, which was prostaglanding-dependent[14]. Osteocalcin and alkaline phosphatase, as phenotype markers of the osteoblast cells[9], are inducible factors of vitamine D. Elevated production of IL-1β has been implicated in the pathogenesis of osteoporosis and with humoral hypercalcemia associated with some calcinomas.

In this study, we have examined whether these effects are exhibited by IL-1β on mouse calvarial osteoblast cells derived from fetal mouse. IL-1β suppressed the osteocalcin and alkaline phosphatase, as phenotype markers of the osteoblast cells, in association with the stimulation of cell proliferation and the effects of these phenotype markers were strongly antagonized by IL-1β in a dose-dependent manner. Also, the medicinal extracts of BCS was tested for the inhibitory effects against IL-1β-induced PGE_2 production and IL-1β-stimulated bone resorption in the mouse calvarial bone cells having both of the osteoblast and osteoclast cells. The inhibitory effect of BCS extracts was highly similar to that of calcitonin treatment, indicating these two subjects play some key roles in inhibition of the osteoclast-mediated bone resorption.

II. 材料 및 方法

1. *Bombusae concretio* Salicea and the extraction

Bombusae concretio Salicea (OHC-B-3 in harbarium record) (300 g) was obtained from Oriental Medical Hospital, College of Oriental Medicine, Dongguk University and extracted with 500 ml of boiling water for 3 hr. After the extract was centrifuged at 7500 rpm for 30 min, the supernatant was lyophilized. For direct use, the extract solution was stored at 4℃ in aliquots. Depending upon the experimental group, BCS was added (at 2% volume in culture medium) to or omitted from flasks. After 16~18 hr, cells were washed twice with warm phosphate-buffered saline (PBS) and serum-free medium added to the flask.

2. Materials and Mouse bone cell culture

Explants of mouse calvarial bone were cultured as described[9]. The cells obtained have been routinely characterized and shown to express an osteoblast-like phenotype in culture. Recombinant pure human IL-1β (specific activity 5 x 10^5 U/mg) was a our deposti. Salmon calcitonin was obtained from Armour Pharmaceutical Co. (IL, USA).

Dulbecco's modified Eagle's medium (DMEM), bovine serum albumin and other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA). PGE_2 antibody was purchased from Immunoassay Co. (Tokyo, Japan).

3. Osteocalcin assay

Osteocalcin released into the culture media, over a 72hr incubation period was measured using a specific radioimmunoassay with an antibody raised in rabbits

against purified bovine osteocalcin. Results are expressed as ng osteocalcin per μg cell protein.

4. Alkaline phosphatase assay

Alkaline phosphatase activity in the solubilized cell layer was measured by monitoring the release of p-nitrophenol from disodium p-nitrophenyl phosphate. The assay buffer consisted of 0.1 M diethanolamine, supplemented with 0.5 M magnesium chloride (pH 10.5). Results are expressed as μmoles per μg cell protein per hr.

5. Prostaglandin assay

Prostaglandin E_2 (PGE_2) released into the culture medium over a 72hr incubation period was measured by radioimmunoassay using an antiserum with specificity towards PGE_2 (Immunoassay, Co., Tokyo Japan) as descrived by Ruch *et al*[1]. Results are expressed as ng PGE_2 per μg cell protein.

6. Effects of BCS-treatment on IL-1β-induced PGE_2-production, osteocalcin production, alkaline phosphatase activity and bone resorption in calvarial bone cells.

Two different assays were carried out to assess the activities of BCS extracts (each μg/ml) on IL-1β-induced PGE_2-production, osteocalcin production, alkaline phosphatase activity and bone resorption in the cells, as follows ;

1) Experiment-1 (post treatment) : The mouse calvarial bone cells were treated with IL-1β to induce PGE_2-production, osteocalcin production, alkaline phosphatase activity and bone resorption for 24 hr, and the treated cells were further treated with BCS (50 μ g/ml) with time courses of 1 and 16 hr, and each activity was assayed.

2) Experiment-2 (pretreatment) : The mouse calvarial bone cells were initially treated with BCS (50 μg/ml) for 1 hr and further treated with IL-1β to induce for 46 and 56 hr. Finally, each activity was assayed.

7. Bone resorption assay

The fetal mouse long bone organ tissue culture system was based on that described by Raisz[15]. Fetal bones were labeled with ^{45}Ca by injecting the mother with 200 μCi ^{45}Ca (NEN, Boston, MA) on the eighteenth day of gestation. Radii and ulnae bone shafts were obtained from 19 day fetuses by microdissection. The shafts were cut just beyond the calcified zone and therefore contained short lengths of cartilage at the ends. Bones were cultured on sunk Millipore filter dots in 24-well Limbro plates. The shafts were first cultured in 0.5 ml BGJ_b medium(Gibco Laboratories, Grans Islan, NY) containing 1.0 ml/ml bovine serum albumin, 100 units/ml penicillin G, and 1 μg/ml polymyxin B for 1 day to reduce exchangeable ^{45}Ca. One bone from a fair (right and left radii or right and left ulnae from a single fetus) was then transferred into medium containing agonist(s) (treatment) and the contralateral bone was placed into identical medium without agonist(s) (control). A typical test group consisted of 5 pairs of bones. Bones were cultured for 5 days in a 95% air / 5% CO2 incubator at 37℃ and 95% humidity with one change of media after 2 days. The percentage of ^{45}Ca released from a bone into the medium during the 5-day culture was determiend by measuring the radioactivity in medium1, medium 2, and the trichloroacetic acid solubilized bone using a liquid scintillation counter. Stimulated resorption was expressed as the paired difference bwtween treatment and control bone percent ^{45}Ca released from during the 5-day culture. Dead bone ^{45}Ca release in this system was apprximately 10%. BGJ_b control ^{45}Ca release was 16-20% and maximum IL-1b ^{45}Ca release was 60-80%. Since "stimulated" release is expressed as the mean

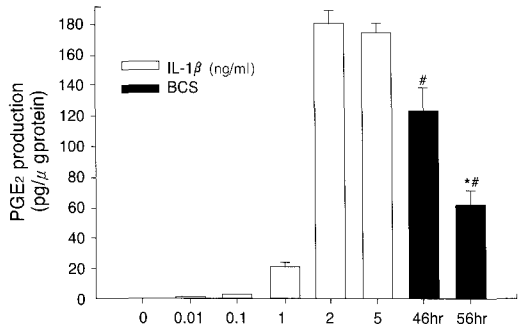

Fig. 1. The Effects of IL-1β on the Prodcution of PGE2 by Mouse Bone Cells Including Osteoblasts and Osteoclast Cells, and Inhibitory Effect of Pretreatment of BCS Extracts on IL-1β-induced PGE_2 Production. After 1 hr of BCS treatment, IL-1β (2.0 ng/ml) was added and PGE_2 production was observed after 46 and 56 hr. PGE_2 released into the culture media was measured as described in Materials and Methods. Values represent mean $\pm$ S.E (n=3). * $p<0.05$ compared with 46 hr-treated group ; # $p<0.05$ compared with IL-1β (2 ng/ml)-treated group.

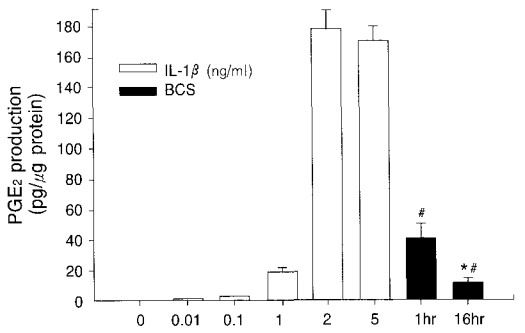

Fig. 2. The Effects of IL-1β on the Prodcution of PGE2 by Mouse Bone Cells Including Osteoblasts and Osteoclast Cells and Inhibitory Effect of Posttreamnet of BCS Extracts on IL-1β-induced PGE_2 Production.PGE_2 released into the culture media was measured as described in Materials and Methods. After 56 hr of IL-1β (2.0 ng/ml) treatment, BCS was further treated to that, and then PGE_2 production was observed after 1 and 16hr. Values represent mean $\pm$ S.E (n=3). * $p<0.05$ compared with 1 hr-treated group ; # $p<0.05$ compared with IL-1β (2 ng/ml)-treated group.

difference betwaeen paired BGJ_b control bones (C%) and treated bones (T%), the T%-C% for an inactive treatment is zero, and a maximum IL-1b response is approximately 40-60%. Each bone was labeled with approximately 20,000 CPM ^{45}Ca.

8. Statistics

Standard procedures were use to calculate means and standard error. Values were compared using student' s t-test with on SigmaPlot for Windows program (Version 3.02, Jandel Corporation). $P<0.05$ was considered significant.

III. 結 果

1. Inhibitory effect of BCS extracts (50 μg/ml) on IL-1β-induced PGE_2 production in the mouse osteoblasts

IL-1β stimulated the production of PGE_2 in a dose-dependent manner over the concentration range of 0.01 ng - 5 ng/ml with a maximal effect being observed at 2 ng/ml (Fig. 1). The stimulation of cell proliferation was most pronounced at 2.0 ng/ml, while concentrations below 1.0 ng/ml exhibited no detectable activity on the synthesis of PGE_2. To examine the inhinitory effect of BCS, the medicinal extracts of BCS was tested for whether they could inhibit IL-1β-induced PGE_2 production (Fig. 1). Cell viability was not significantly affected by treatment with the indicated concentration of the extracts alone (data not shown). The medicinal extracts were shown to have the inhibitory effects against the synthesis of PGE_2. This result indicates that the BCS extracts could inhibit the cyclooxygenase-2

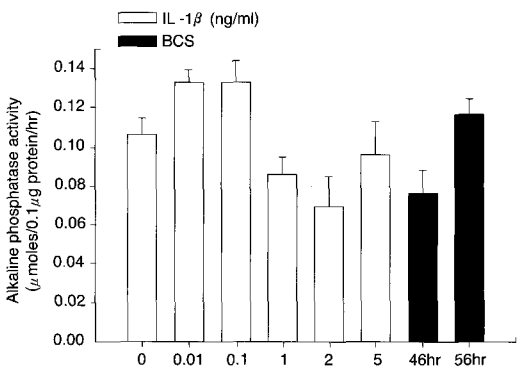

Fig. 3. Effect of IL-1β on Alkaline Phosphatase Activity in - the Mouse Osteoblast Cells and BCS Pretreatment.- The alkaline phosphatase activity of the solubilized cell layer of the mouse osteoblast cells was measured as described in Materials and Methods. Values represent mean ± S.E (n=3).

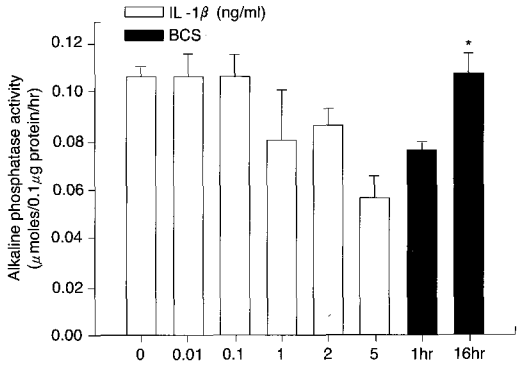

Fig. 4. Effect of IL-1β on Aalkaline Phosphatase Activity in the Mouse Osteoblast Cells and BCS Posttreatment. The alkaline phosphatase activity of the solubilized cell layer of the mouse osteoblast cells was measured as described in Materials and Methods. Values represent mean ± S.E (n=3). * $p<0.05$ compared with 1 hr-treated group.

activity or gene expression of cyclooxygenase-2, which is a mediator of the synthesis of PGE_2 from arachidonic acid. However, their effects were not stringent to protect the synthesis of PGE_2. The PGE_2-induction agents has been known to increase the susceptibility of the calvarial cells against bone resorption, although there are some controversies. Thus, we examined the effect of the pretreatment and posttreatment with a concentrations of the BCS extracts (50 μg/ml) then the PGE_2-induction agent was added. Pretreatment of the BCS extracts for 1 hr, which by itself had little effect on cell survival, did not enhance the synthesis of PGE_2, nor significantly reduced the synthesis of PGE_2 by pretreatment. Treatment group for 56 hr after preteatment of BCS (50 μg/ml) showed much higher inhibitory effects (Fig. 1).

Also, after 56 hr of IL-1β (2.0 ng/ml) treatment, BCS was further treated to that, and then PGE_2 production was observed after 1 and 16 hr to examine the effect of BCS posttreatment. The effects of posttreatment was lower than that of pretreatment (Fig. 2).

2. Inhibitory effect of BCS extracts (50 μg/ml) on IL-1β-alkaline phospahatse activity and osteocalcin production stimulated by vitamin D in the mouse calvarial bone cells

To examine the effects of IL-1β on alkaline phosphatase activity in the mouse calvarial bone cells, it is important to know the cell differentiation. When various concentrations IL-1β were treated to the cells and then alkaline phosphatase activities were assayed, the basal alkaline phosphatase activity of the mouse osteoblast cells was decreased by IL-1β over the dose range of 0.01 - 5.0 ng/ml (Fig. 3).

Next, the effect of the pretreatment and posttreatment

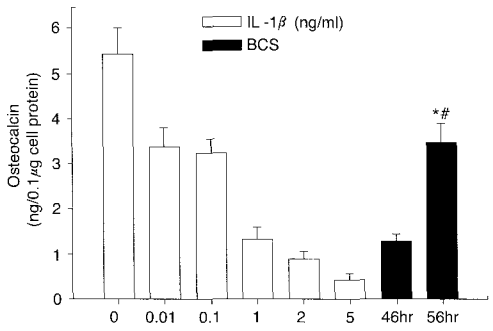

Fig. 5. Effect of IL-1β on Osteocalcin Production Stimulated by Vitamin D in the Mouse Osteoblast Ccells, and Eeffect of BCS Pretreatment on IL-1β-Aantagonized Osteocalcin Production in Osteoblast Cells Stimulated by Vitamin D. Vitamin D (5 μg/ml) was treated to cells and a series of concentration of IL-1β was added to the vitamin D-treated cells. The osteocalcin production of the solubilized cell layer of the mouse osteoblast cells was measured as described in Materials and Methods. Values represent mean ± S.E (n=3). * $p<0.05$ compared with 46 hr-treated group ; # $p<0.05$ compared with IL-1β (2 ng/ml)-treated group.

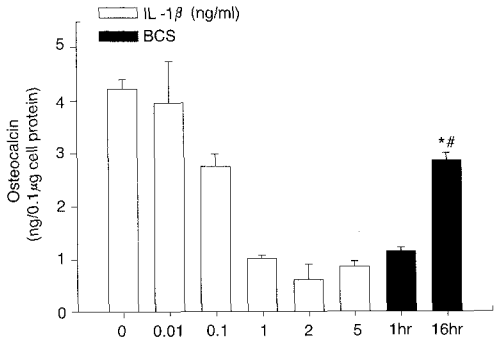

Fig. 6. Effect of IL-1β on Osteocalcin Production Stimulated by Vitamin D in the Mouse Osteoblast Cells and Effect of BCS Posttreatment on IL-1β-Antagonized Osteocalcin Production in Osteoblast Ccells Stimulated by Vitamin D. Vitamin D (5 μg/ml) was treated to cells and a series of concentration of IL-1β was added to the vitamin D-treated cells. The osteocalcin production of the solubilized cell layer of the mouse osteoblast cells was measured as described in Materials and Methods. Values represent mean ± S.E (n=3). * $p<0.05$ compared with 1 hr-treated group ; # $p<0.05$ compared with IL-1β (2 ng/ml)-treated group.

with BCS extracts (50 μg/ml) was examined after the addition of IL-1β. Treatment group for 46 hr after pretreatment of BCS extracts did not show any change of alkaline phosphatase activity, while treatment group for 56 hr after pretreatment of BCS (50 μg/ml) extract showed much higher inhibitory effects (Fig. 3). Also, after IL-1β (2.0 ng/ml) treatment, BCS was further treated to that, and then alkaline phosphatase activity was observed to examine the effect of BCS posttreatment. The effects of posttreatment was lower than that of pretreatment without significance (Fig. 4).

When the production of osteocalcin by osteoblast cells were assayed after stimulation by vitamin D, osteocalcin production was significantly antagonized by IL-1β in range of 0.01 - 5.0 ng/ml (Fig. 5). However, pretreatment group of BCS extracts (50 μg/ml) for both 46 and 56 hr elevated the osteocalcin production (Fig. 5). Also, after IL-1β (2.0 ng/ml) treatment, BCS was further treated to that, and then osteocalcin production was observed to examine the effect of BCS posttreatment. The effects of posttreatment was lower than that of pretreatment with significant difference (Fig. 6).

3. Stimulation of IL-1β on bone resorption and inhibition of IL-1β-stimulated bone resorption by calcitonin and BCS extracts in the mouse calvarial bone cells

Table 1. Inhibition of IL-1β-Mediated Bone Resorption by Calcitonin and BCS Treatment.

Addition to bone culture	Bone resorbing activity[a] (Calcium release (T% - C%)
None	8.9±1.4
Calcitonin (0.5 U/ml)	4.4±0.7
Calcitonin (0.5 U/ml) + BCS	2.9±0.4
IL-1β (100 ng/ml)	13.6±0.6
Calcitonin + IL-1β	7.4±0.6*
Calcitonin + IL-1β + BCS	5.1±1.2**
Devitalized bone	2.1±0.12

Bone resorption was measured as percent release of ^{45}Ca during 5 days of culture. Each point is the mean paired difference ± S.E. for 5 treatment-control bone pairs.

[a]Data shown are means ± S.E for quadruplicate determinations.

Bone were devitalized by three cyclyes of freeze-thawing.

* Significantly different from bone treated with IL-1β. *p<0.05. **p<0.01.

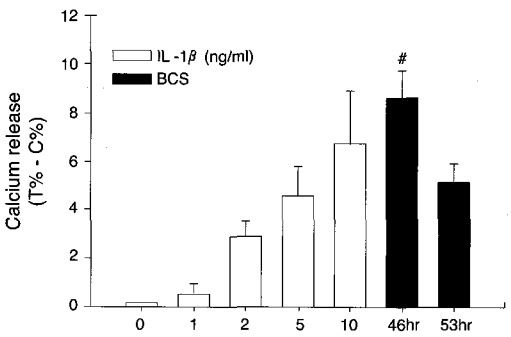

Fig. 7. Inhibitory Effect of Pretreatment of BCS Extracts on IL-1β-Induced Bone Resorption in Mouse Calvarial Cells. Bone resorption was measured as percent release of ^{45}Ca during 5 days of mouse calvarial bone cell culture. Each point is the mean paired difference ± S.E. for 5 treatment-control bone pairs. # p<0.05 compared with IL-1β (2 ng/ml)-treated group.

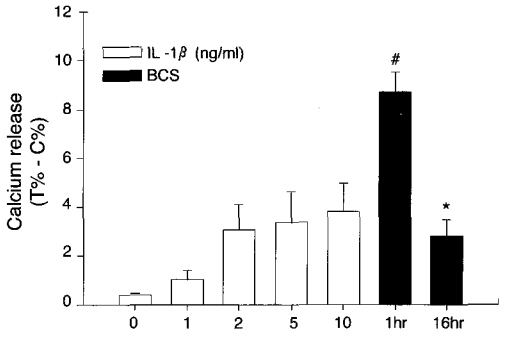

Fig. 8. Inhibitory Effect of Posttreatment of BCS Extracts on IL-1β-Induced Bone Resorption in Mouse Calvarial Cells. Bone resorption was measured as percent release of ^{45}Ca during 5 days of mouse calvarial bone cell culture. Each point is the mean paired difference ± S.E. for 5 treatment-control bone pairs. * p<0.05 compared with 1 hr-treated group ; # p<0.05 compared with IL-1β (2 ng/ml)-treated group.

Treatment of IL-1β resulted in stimulating of bone resorption. As shown in Fig. 7, IL-1β is a potent in stimulating bone resorption as measured by means of calcium release when each is normalized to nano gram of amounts. Interestingly, BCS extracts were shown to have the inhibiting effects against IL-1β-stimulated bone resorption in cells having both of the osteoblast and osteoclast cells. When various concentrations of BCS extracts were pretreated and then the agents was treated, pretreatment of the BCS extracts did not enhance the bone resorption, nor significantly reduced the bone resorption by pretreatment (Fig. 7), while its posttreatment of BCS was lower than that of pretreatment (Fig. 8). The bone resorption induced by

IL-1β appears to be osteoclast-mediaed, since it was largely inhibited by calcitonin treatment, as shown in Table 1. These results are similar to the results from calcitonin treatment (Table 1) and the BCS extracts play key role in inhibition of the osteoclast-mediated bone resorption induced by IL-1β.

IV. 討 議

It was known that IL-1β is reactive to progressive degradation of bone by activating osteoblast cells and by causing the progeniror cells to mature cells[16]. IL-1β stimulated the plasminogen activator activity of the mouse osteblast cells in a dose-dependent manner. The stimulation of plasminogen activator activity by IL-1β has been observed in several connective tissue cell types, indicating human osteoblast-like cells[9]. IL-1β is a potent stimulator of bone resorption both *in vitro*[16] and *in vivo* through an action which may be mediated primarily via the osteoblast[8,9]. The observation that IL-1β stimulates the plasminogen activator activity of the mouse osteoblast cells may indicate a potential mechanism for the osteoblast-mediation of bone recorption.

The stimulation of prostaglandin E_2 production by IL-1β allows bone breakdown by bone resorption and by stimulating the plasminogen activator activity of osteoblast-cell like cells[9]. The synthesis of prostaglandin E_2 production by IL-1β and resulting stimulation of bone resorption can occur partially via prostaglandin E_2-dependent mechanism. Elevated production of IL-1β has been implicated in the pathogenesis of osteoporosis and with humoral hypercalcemia associated with squamous cell carcinomas. Osteocalcin and alkaline phosphatase are widely accepted phenotype markers of the osteoblast cells[9] and the induction of these two factors in response to vitamine D was strongly antagonized by IL-1β.

When the medicinal extracts of BCS was tested for whether they could inhibit IL-1β-induced PGE_2 production, cell viability was not significantly affected by treatment with the indicated concentration and the BCS extracts were shown to have the inhibitory effects against the synthesis of PGE_2, indicating that the BCS extracts could inhibit the cyclooxygenase-2 activity or gene expression of cyclooxygenase-2, which is a mediator of the synthesis of PGE_2 from arachidonic acid. Pretreatment of the BCS extracts for 1 hr, which by itself had little effect on cell survival, did not enhance the synthesis of PGE_2, nor significantly reduced the synthesis of PGE_2 by pretreatment. Treatment group for 46 hr after pretreatment of BCS extracts did not show any change of alkaline phosphatase activity, while treatment group for 56 hr after pretreatment of BCS (50 μg/ml) extract showed much higher inhibitory effects. However, the effect of posttreatment was lower than that of pretreatment without significance. Pretreatment of BCS extracts (50 μg/ml) for 1 hr elevated the osteocalcin production.

Cell viability was not significantly affected by treatment with the indicated concentration of the extracts. Also, the BCS extracts were shown to have the protective effects against plasminogen dependent fibrinolysis induced by IL-1β. BCS extracts showed the inhibiting effects against IL-1β-stimulated bone resorption. When the effect of the pretreatment with the BCS extracts was assayed, the extracts strongly reduced the bone resorption. The absolutely same result was also observed in case of calcitonin treatment. Thus, these results suggested that the BCS extracts inhibit the bone resorption and osteoporosis by inhibiting the osteoclast-mediated bone resorption reaction, which is usually induced by IL-1β.

V. 結 論

Bombusae concretio Salicea (BCS) is a medicinal plant used in Korea for the treatment of various symptoms accompanying hypertension and cerebrovascular disorders. We studied the protective effect of the BCS extracts on the bone resorption of the mouse calvarial bone cells. Interleukin-1β (IL-1β) regulates the osteoblast cells derived from mouse calvarial bone explants in vitro. When the medicinal extracts of BCS was tested for whether they could inhibit IL-1β-induced PGE_2 production. Cell viability was not significantly affected by treatment with the indicated concentration of the extracts. The BCS extracts were shown to have the inhibitory effects against the synthesis of PGE_2. The effect of the pretreatment with the BCS extracts and then treated the PGE_2-induction agents, pretreatment of the BCS extracts for 1 hr, which by itself had little effect on cell survival, did not enhance the synthesis of PGE_2. Pretreatment of the BCS (50 μg/ml) extracts for 1 hr did not show any change of alkaline phosphatase activity, while treatment group for 56 hr after BCS pretreatment showed much higher inhibitory effects. However, the effect of posttreatment was lower than that of pretreatment. Pretreatment of BCS extracts (50 μg/ml) for 1 hr elevated the osteocalcin production. Finally, calcitonin showed the inhibitory activity the IL-1β-stimulated bone resorption in the mouse calvarial bone cells having both of the osteoblast and osteoclast cells. Seemingly, pretreatment of the BCS extracts for 1 hr reduced the bone resorption. These results clearly indicated that calcitonin and BCS extracts play key roles in inhibition of the osteoclast-mediated bone resorption.

參 考 文 獻

1. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from chinese green tea. *Carcinogenesis.* 1989;10:1003-8.
2. Tsuda T, Watanabe M, Ohshima K, Norinobu S, Choi SW, Kawakishi S et al. Antioxidative activity of the anthocyanin pigments cyanidin 3-O-β-D-Glucoside and cyanidin. *J. Agric. Food Chem.* 1994;42:2407-10.
3. Wang BX, Zhao XH, Qi SB, Kaneko S, Hattori M, Namba T et al. Effects of repeated administration of deer antler extract on biochemical changes related to aging in senescence-accelerated mice. *Chem. Pharm. Bull.* 1988a;36:2587-92.
4. Wang BX, Zhao XH, Qi SB, Yang XW, Kaneko S, Hattori M *et al.* Stimulating effect of deer antler extract on protein synthesis in senescence accelerated mice *in vivo*. *Chem. Pharm. Bull.* 1988b;36:2593-8.
5. Lee SI. Clinical Application of Oriental Medicinal Herbs. South Korea:Sungbo Publishing Co;1986;p.453-97.
6. Chen X, Jia Y, Wang B. Inhibitory effects of the extract of *Bombusae concretio* Salicea on monoamine oxidase in aged mice. *Chung Kuo Chung Yao Tsa Chih.* 1992; 17:107-10.
7. Lee WH, Jeong JC. Effects of *Bombusae concretio* Salicea on Amyloid-β-induced neuronal cell toxicity and lipid peroxidation in cultured rat astrocytes. *J. Kor. Oriental Internal Medicine.* 1998;19:381-91.
8. Hazuda DJ, Strickler J, Hueppers F, Simon PL, Young PR. Processing of precursor Interleukin 1β and inflammatory disease. *PA. J. Biol. Chem.* 1990;265:6318-22.
9. Evans DB, Bunning RAD, Russell RGG. The effects of recombinant human interleukin-1β on cellular proliferation and the production of prostaglandin E2, plasminogen activator, osteocalcin and alkaline phosphatase by osteoblast-like cells derived from human bone. *Biochem. Biophys. Res. Comm.* 1990;166:208-16.
10. Heasman PA, Collins JG, Offenbacher. Changes in crevicular

fluid levels of inteleukin-1β, leukotriene B_4, prostaglandin E_2, thromboxane B_2 and tumor necrosis factor α in experimental gingivities in humans. *J Periodont Res.* 1993;28:241-7.

11. Kawaguchi H, Raisz LG, Vosnesensky OS, Alander CB, Hakeda Y, Pilbeam CC. Regulation of the two prostaglandin G/H synthases by parathyroid hormone, interleukin-1, cortisol and prostaglandin E2 in cultured neonatal mouse calvariae. *Endocrinology.* 1994;135:1157-64.
12. Harrison JH, Lorenzo JA, Kawaguchi H, Raisz LG, Pilbeam CC. Stimulation of prostaglandin E2 production by interleukin-1α and transforming growth factor α in osteoblastic MC3T3-E1 cells. *J. Bone Miner. Res.* 1994;9:817-23.
13. Sato K, Fuji Y, Kasono K, Saji M, Tsushima T, Shizume K. Stimulation of prostaglandin E2 and bone resorption by recombinant human interleukin-1α in fetal mouse bone. *Biochem. Biophys. Res. Comm.* 1986;138:618-24.
14. Boyce BF, Aufdemorte TB, Garrett IR, Yates AJP, Mundy GR. Effects of interleukin-1 on bone turnover in normal mice. *Endocrinology.* 1989:125:1142-50.
15. Raiz LG, Niemann I. Effect of phosphate, calcium and magnesium on bone resorption and hormonal responses in tissue culture. *Endocrinology.* 1969:85:446-52.
16. Baron R, Vignery A, Horowitz M. In Bone and Mineral Research Annual 2 (W. A. Peck, ed), Amsterdam. Elsevier;1983:175-243.