

Original Articles

Effects of *Bambusae concretio Silicea* on Suppression of Collagenolysis and Bone Resorption in Mouse Calvarial Osteoblasts

Seong-Choon Lee, Cheol-Ho Yoon, Ji-Cheon Jeong

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

Objective : We studied the effect of *Bambusae concretio Silicea* (BCS) on bone metabolism.

Methods : At first, we treated PTH, 1,25(OH)₂D₃, mononuclear cell conditioned medium (MCM) and IL-1 to osteoblast cells derived from mouse calvarial bone explants *in vitro*, and then investigated the activities of collagenolysis and bone resorption factors.

Results : BCS extracts have no cytotoxicities in concentrations of 1-150 μ g/ml. BCS had protective activity against PTH (5 units/ml), MCM (5%, v/v), 1,25(OH)₂D₃ (20 ng/ml), IL-1 α (2 ng/ml) and IL-1 β , (1 ng/ml)-induced collagenolysis in the mouse calvarial cells. And, pretreatment of BCS for 1 hr significantly reduced the collagenolysis. Furthermore, it was much more expressed at 16 hrs after BCS (50 μ g/ml)-pretreatment. And, BCS significantly protected against enhanced collagenolysis induced by IL-1 α and IL-1 β .

Conclusion : BCS extracts inhibited the bone resorption in mouse calvarial bone cell, thus BCS could be used clinically for bone diseases.

Key Words: *Bambusae concretio Silicea* (BCS), collagenolysis, bone resorption

Introduction

Bone resorption is known to be affected by both circulating and locally produced factors. Parathyroid hormone, vitamin D metabolites and calcitonin are the major circulating hormones affecting bone resorption^{1,2)}. The cytokines of interleukin-1(IL-1), tumor necrosis

factor, and certain prostaglandins are locally produced factors which have been shown to stimulate bone resorption *in vitro*. Bone resorption is sometimes mediated by the synergistic activities of those factors³⁾. Research during the past decade indicates that two interrelated mechanisms are involved in the proteolytic stages of bone resorption⁴⁾. The latter findings support the view that osteoblasts play a major role in bone resorption by degrading the surface osteoid layer, thereby exposing the underlying mineralized matrix for osteoclastic action⁵⁾.

It was demonstrated that one such cytokine, IL-1, can modulate several aspects of the activity of various bone

Received 13 May 2004; received in revised from 15 October 2004; accepted 20 October 2004

Correspondent to : Cheol-Ho Yoon.

Oriental Medical Hospital, Dongguk University 357,
Yong-Gang-Dong, Kyung-Ju city Kyung-Buk, 780-140, Korea
Tel : +82-54-770-1254, E-mail : dryoon@dongguk.ac.kr

cell types. IL-1 is a potent stimulator of bone resorption *in vitro*⁶⁾, an action apparently partially mediated via the stimulation of osteoclast cell formation, indicating the modulation of osteoblast cell activity by IL-1⁷⁾. Previous studies have demonstrated that IL-1 β , regulates several aspects of the functional activity of human osteoblast-like cells *in vitro*^{8,9)}. It is also a potent inducer of prostanoids^{10,11)}. 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 prostaglandin-independent stimulation of bone resorption followed by a prolonged increase in resorption, which was prostaglandin-dependent¹²⁾. Osteocalcin and alkaline phosphatase, as phenotype markers of the osteoblast cells⁷⁾, are inducible factors of vitamin D. Elevated production of IL-1 β , has been implicated in the pathogenesis of osteoporosis and with humoral hypercalcemia associated with some calcinomas.

It was reported that the extract of *Bambusae concretio Silicea* (BCS) is specifically effective for cerebrovascular lesions and aphasia during the treatment of wind-heat syndrome and heat-phlegm in oriental medicine¹³⁾. The pharmacological mechanism for BCS was attributed to anti-aging and sexual-reinforcing activities in experimental *in vitro* and *in vivo* systems¹⁴⁾, although little is yet known about the pharmacological effects or active ingredients. It was investigated regarding the effect of BCS on cultured astrocytes, lipid peroxidation and antioxidative enzyme activities in A β ,25-35 treated conditions¹⁵⁾.

It is generally known that inflammation induces bone resorption and osteoporosis. Therefore, anti-bone resorption activity may be assessed by the effect on osteoblastic cells. In this study, the authors have examined whether these effects are exhibited by IL-1 β , on mouse calvarial osteoblast cells derived from fetal mice. IL-1 β , 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-1 β , in a dose-dependent manner. Also, the BCS extract was tested for the inhibitory effects against IL-1 β , -induced PGE₂ production and IL-1 β , -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 resorption¹⁶⁾.

Materials and Methods

1. Extraction of *Bambusae concretio Silicea*

Bambusae concretio Silicea (300 g) was obtained from the Oriental Medical Hospital, College of Oriental Medicine, Dongguk University and extracted with 500 ml of boiling water for 3 hrs. 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 °C in aliquots. Depending upon the experimental group, BCS was added (at 2% volume in culture medium) to or omitted from flasks. After 16~18 hrs, 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 described⁷⁾. The cells obtained have been routinely characterized and shown to express an osteoblast-like phenotype in culture. Recombinant pure human IL-1 β , (specific activity 5×10^5 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, cycloheximide (CHM), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), indomethacin, dexamethasone and other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA). PGE₂ antibody was purchased from Immunoassay Co. (Tokyo, Japan). [³H]-prostaglandin was purchased from New England Nuclear (Seoul, Korea). Other radiochemicals were obtained from New England Nuclear Corp. (Boston, MA). Tissue culture media and reagents, Fetal bovine serum (FBS) were from Gibco (Chagrin Falls, OH). Recombinant pure human IL-1 β , (specific activity 5 \times 10⁶ U/mg) was our deposit¹⁷), which was a generous gift of Dr. S. H. Park, Korea Research Institute of Bioscience and Biotechnology (Taejon, Korea) or was obtained from Genzyme Corp. (Cambridge, MA, USA).

3. MTT cytotoxicity of the BCS water extracts on the isolated mouse calvarial bone cells.

Cytotoxicity of the BCS extracts on the isolated calvarial cells was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-formazan assay, as follows: the cell suspension was plated (200 μ l 1 \sim 2 \times 10⁴ cells/ well) in a 96 well-microculture plate (flat bottom; Falcon 3027) (Becton Dickinson and Company, New Jersey, USA). After 24 hrs culture, 30 μ l of varying concentrations of each sample solution was added to the wells and cultured for 3 days. Finally, 50 μ l of MTT solution (5 mg/ml in DMEM or RPMI 1640 medium) was added to the wells and further incubated for 4-6 h. After incubation, the culture supernatants were discarded by aspirating and then 250 μ l of dimethyl sulfoxide (DMSO) was added. The optical density (O.D) was measured in 50 mM glycine buffer with enzyme-linked immunosorbent assay (ELISA) using by a microplate reader MPR-A4 at

540 nm. The mean value of O.D of 5-6 wells was used for the calculation of the % cytotoxicity and the equation was as follows: % cytotoxicity = (1-O.D treated well/O.D control well) \times 100.

4. Osteoblasts isolation and culture

Mouse calvarial osteoblasts were isolated from neonatal BALB/c mice by enzymatic digestion, as described for rat osteoblasts^{11,18}). Explants of mouse calvarial bone were cultured and the cells obtained have been routinely characterized and shown to express an osteoblast-like phenotype in culture. The population released during the last three digestions was highly enriched in cells expressing two markers of the osteoblast lineage, alkaline phosphatase and osteocalcin⁹). Cells released by collagenase digestions were washed and grown to confluent in 75 cm² culture flasks (Falcon) in (DMEM) supplemented with antibiotics (penicillin and streptomycin) and 10% fetal calf serum (FCS; Gibco, BRL, Bethesda, MD, USA). Incubations were carried out at 37 ^\circ C in a humidified atmosphere of 5% CO₂/95% air; the medium was changed every 2-3 days. Cells were grown to confluence at 37 ^\circ C and cultured in duplicate or triplicate wells for an additional 24 hrs in serum-free medium supplemented with Polymixin B sulfate to prevent endotoxin effects prior to treatment.

5. Measurement of collagenolysis with PTH, 1,25(OH)₂D₃, mononuclear cell conditioned medium (MCM), IL-1 α - and IL-1 β ,

Calvarial osteoblasts were isolated from neonatal BALB/c mice by enzymatic digestion, as for mouse osteoblasts⁹). Cells released by collagenase digestions were washed and grown to confluent in 75 cm² culture flasks (Falcon) in DMEM supplemented with antibiotics and 10% FCS. Incubations were carried out at 37 ^\circ C in a humidified atmosphere of 5% CO₂/95% air;

the medium was changed every 2-3 days. Radiolabelled collagen films were prepared as described⁴⁾. Aliquots of [¹⁴C]acetylated collagen [mouse skin type I; 150 μ g in 300 μ l of 10 mM phosphate buffer (pH 7.4), containing 300 mM NaCl and 0.02% sodium azide] were dispensed into tissue culture wells and dried at 37°C. The collagen was washed twice with sterile distilled water and once with DMEM prior to use. First passage cells (10⁵/well) were settled onto the collagen films and cultured in 1 ml DMEM with 10% FCS for 24 hrs. After a wash in serum-free DMEM, the cells were cultured for either 72 or 120 hrs in DMEM (500 μ l) with 2% acid treated mouse serum (this contains no α_2 -macroglobulin or other detectable proteinase inhibitors). Cells were stimulated with either PTH (2 units/ml), or MCM (5%, v/v), or IL-1 β , or rhIL-1 α (1 ng/ml) or 1,25(OH)₂D₃ (10 ng/ml). PTH (1-84) and recombinant pure human (rh) IL-1 α were supplied by Funabashi Co., (Tokyo, Japan). MCM was partially purified from cultured pig leucocytes on Ultrogel ACA-54 as described by Heasman⁸⁾. At the end of the culture period the media were centrifuged (10 min, 1200 \times g) to remove any collagen fibrils, and the radioactivity released during collagen degradation quantified by liquid scintillation counting.

6. Measurement of BCS-treatment on PTH, 1,25(OH)₂D₃, MCM, IL-1 α and IL-1 β -induced collagenolysis in calvarial osteoblast cells

Two different assays were carried out to assess the anti-collagenolysis activity of BCS extracts (each 100 μ g/ml) on PTH, 1,25(OH)₂D₃, MCM, IL-1 α and IL-1 β -induced collagenolysis in calvarial osteoblast cells, as follows:

1) Posttreatment

- The mouse calvarial osteoblast cells were treated with PTH, or 1,25(OH)₂D₃, or MCM, IL-1 α and

IL-1 β , to induce the collagenolysis for 56 hrs, and the treated cells were further treated with BCS with time courses of 1, 2, 4, 8 and 16 hrs, and the reduced collagenolysis was assayed.

2) Pretreatment

- The mouse calvarial osteoblast cells were initially treated with BCS for 1 hr and further treated with each agents such as PTH, or 1,25(OH)₂D₃, or MCM) IL-1 α and IL-1 β , to induce the collagenolysis for 46 and 56 hr. Finally, the reduced collagenolysis was assayed.

7. Analytical methods

Protein content was determined by the method of Lowry¹⁹⁾ with bovine serum albumin as the standard. Protein in the cell culture medium was routinely followed by the absorbance at 280 nm.

8. Bone resorption assay

The fetal mouse long bone organ tissue culture system was based on that described by Raisz²⁰⁾. Fetal bones were labeled with ⁴⁵Ca by injecting the mother with 200 μ Ci ⁴⁵Ca (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, Grand Island, NY) containing 1.0 ml/ml bovine serum albumin, 100 units/ml penicillin G, and 1 μ g/ml polymyxin B for 1 day to reduce exchangeable ⁴⁵Ca. One bone from a pair (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°C and 95% humidity with one change of media after 2 days. The percentage of ⁴⁵Ca released from a bone into the medium during the 5-day culture was determined by measuring the radioactivity in medium 1, medium 2, and the trichloroacetic acid solubilized bone using a liquid scintillation counter. Stimulated resorption was expressed as the paired difference between treatment and control bone percent ⁴⁵Ca released from during the 5-day culture. Dead bone ⁴⁵Ca release in this system was approximately 10%. BGJb control ⁴⁵Ca release was 16-20% and maximum IL-1b ⁴⁵Ca release was 60-80%. Since “stimulated” release is expressed as the mean difference between paired BGJb control bones (C%) and treated bones (T%), the T%-C% for an inactive treatment is zero, and a maximum IL-1β, response is approximately 40-60%. Each bone was labeled with approximately 20,000 CPM ⁴⁵Ca.

9. Statistics

Standard procedures were used to calculate means and standard error. Values were compared using a student's *t*-test with on SigmaPlot 2000 for Windows program (Version 6.0, SPSS Inc.). *p*<0.05 was considered significant.

Results

1. MTT test of BCS extracts on mouse calvarial bone cells

Author examined the effects of various medicinal agents on MTT reduction in mouse calvarial cells. Whereas the LDH release assay is an index of membrane damage, the MTT reduction assay reflects intracellular redox state. Thus, inhibition of MTT is not necessarily accompanied by complete cell lysis. The

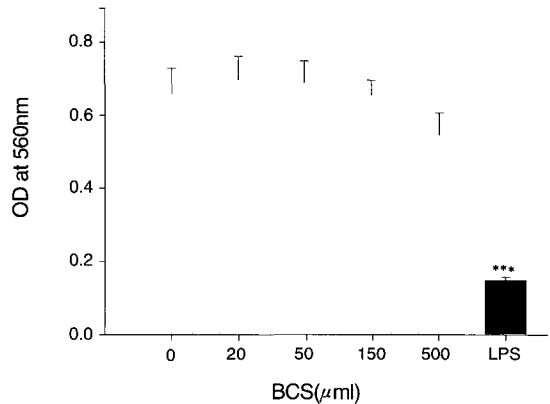


Fig. 1. MTT test of BCS water extracts on calvarial osteoblasts. As a negative control, 200 μg/ml LPS gave significant inhibition of the activity. Each point represents the mean ± S.E. of 3 experiments. ### *p*<0.001 ; Significantly different from BCS-untreated group.

results of *in vitro* cytotoxicities showed that BCS-water extracts have no any cytotoxicities in concentrations of 1-150 μg/ml and furthermore there is no any cytotoxicity even in concentration of 500 μg/ml on mouse calvarial bone cells (Fig. 1).

In contrast, 200 μg/ml of LPS, a cytotoxic and inflammatory control reagent, showed the severe cytotoxicity on the mouse calvarial bone cells, resulting in 75% death of the cells.

These results indicated that the BCS-water extracts are highly stable and applicable to clinical uses. However, for 100 μg/ml of each extract was used for the next experiments. When BCS-ethanol extract was assayed, no cytotoxicity was observed up to 160 μg/ml concentration (Fig. 2).

2. Effects of BCS extract on PTH, 1,25(OH)₂D₃, MCM-induced collagenolysis

The calvarial osteoblasts were isolated from neonatal BALB/c mice by enzymatic digestion, and cells were

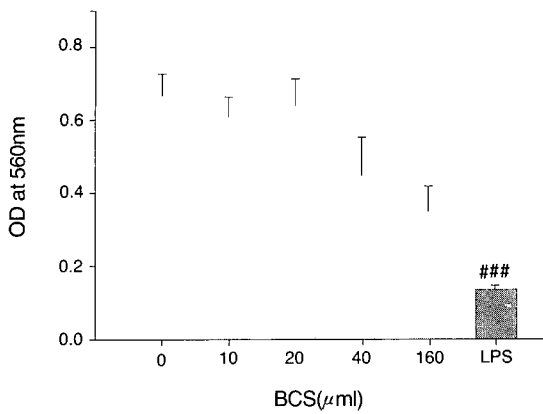


Fig. 2. MTT test of BCS ethanol extracts on mouse calvarial osteoblasts. As a negative control, 200 μg/ml LPS gave significant inhibition of the activity. Each point represents the mean ± S.E. of 5 experiments. ### $p < 0.001$; Significantly different from BCS-untreated group.

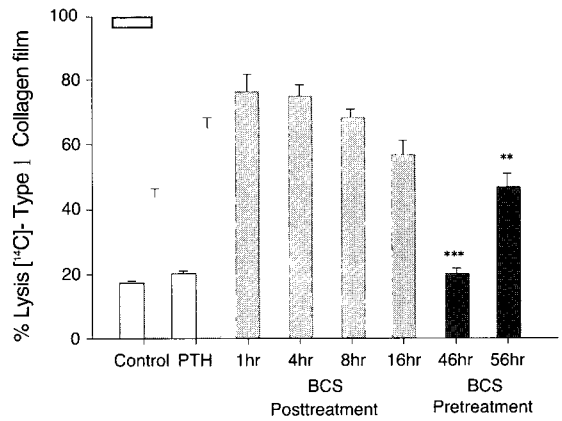


Fig. 3. Effects of BCS extracts on PTH-induced collagenolysis by means of ¹⁴C-labelled type I collagen films. Collagen degradation was expressed as a percentage radioactivity released from the films (mean ± S.E.) for four wells. ** $p < 0.01$, *** $p < 0.001$; Significantly different from PTH-56hr-treated group.

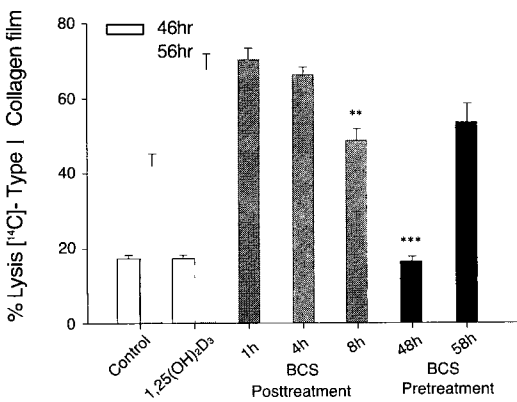


Fig. 4. Effects of BCS extracts on 1,25(OH)₂D₃-induced collagenolysis by means of ¹⁴C-labelled type I collagen films. Collagen degradation was expressed as a percentage radioactivity released from the films (mean ± S.E.) for four wells. ** $p < 0.01$, *** $p < 0.001$; Significantly different from 1,25(OH)₂D₃-56hr-treated group.

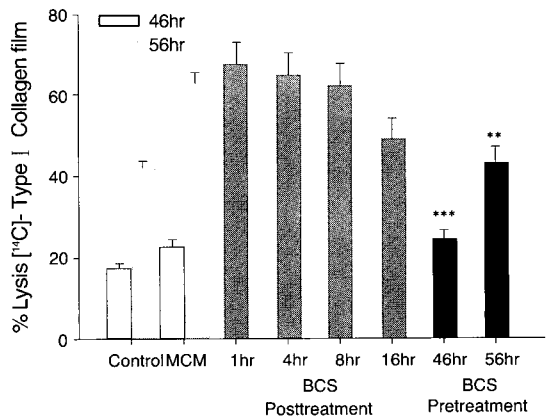


Fig. 5. Effects of BCS extracts on MCM-induced collagenolysis by means of ¹⁴C-labelled type I collagen films. Collagen degradation was expressed as a percentage radioactivity released from the films (mean ± S.E.) for four wells. ** $p < 0.01$, *** $p < 0.001$; Significantly different from MCM-56hr-treated group.

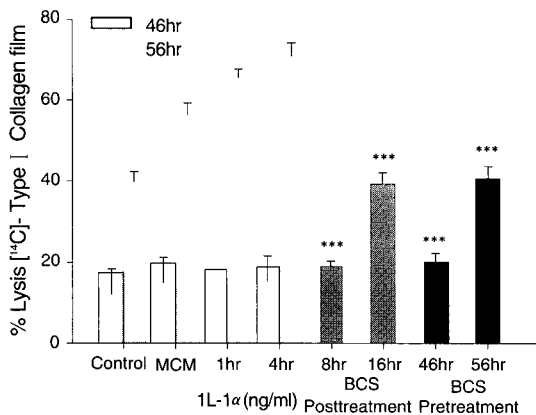


Fig. 6. Effect of BCS extracts on IL-1 α (2.0 ng/ml)-induced collagenolysis. Collagen degradation was expressed as a percentage radioactivity released from the films (mean \pm S.E.) for four wells. *** $p < 0.001$; Significantly different from IL-1 α (2.0 ng/ml)-56hr-treated group.

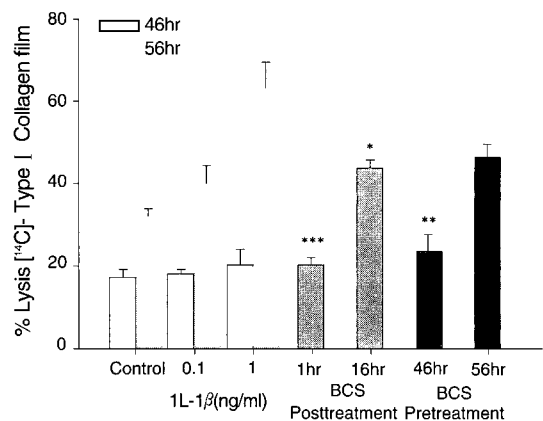


Fig. 7. Effect of IL-1 β on lysis of ¹⁴C-labelled type I collagen films and effects of BCS extracts on IL-1 β , (1.0 ng/ml)-induced collagenolysis. Collagen degradation was expressed as a percentage radioactivity released from the films (mean \pm S.E.) for four wells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Significantly different from IL-1 β , (1.0 ng/ml)-56hr-treated group.

stimulated with either parathyroid hormone (PTH; 2 units/ml), or MCM (5%, v/v) or 1,25-hydroxyvitamin D3 [1,25(OH)₂D₃; 10 ng/ml].

Then, the radioactivity released during collagen degradation was quantified by liquid scintillation counting and collagen degradation was expressed as a percentage radioactivity released from the films. There were small but statistically significant increase in collagenolysis with PTH, 1,25(OH)₂D₃ and MCM treatment after 56 hrs (Fig. 3-5).

3. Effects of BCS extract on IL-1 α and IL-1 β -induced collagenolysis

To examine the anti-collagenolysis of the BCS extracts on IL-1 α and IL-1 β -induced collagenolysis in calvarial osteoblast cells, various concentrations of the BCS extracts were tested whether they could protect against IL-1 α (2 ng/ml) or IL-1 β , (1 ng/ml)-induced collagenolysis in the mouse calvarial cells (Fig. 6-7).

Since interleukin-1 (IL-1) is a major constituent of MCM, author tested the ability of rhIL-1 (IL-1 α ; 1.0-5.0 ng/ml) to stimulate collagen degradation by the cells; maximal collagenolysis (69%) was again only achieved after 56 hrs with the optima dose of 10 ng/ml (Fig. 6). Also, IL-1 β , was tested for stimulation of collagen degradation (0.1 - 1.0 ng/ml). The maximal collagenolysis was obtained after 56 hrs with the optima dose of 1 ng/ml (Fig. 7).

4. Effects of BCS extracts on IL-1 α and IL-1 β -induced bone resorption

Now, author examined the effects of the BCS extracts on IL-1 α and IL-1 β -induced bone resorption in the mouse calvarial cells. BCS extracts were shown to have the inhibiting effects against IL-1 α and IL-1 β -induced bone resorption and the effect of the pretreatment with the BCS extracts were significant (Fig. 8-9).

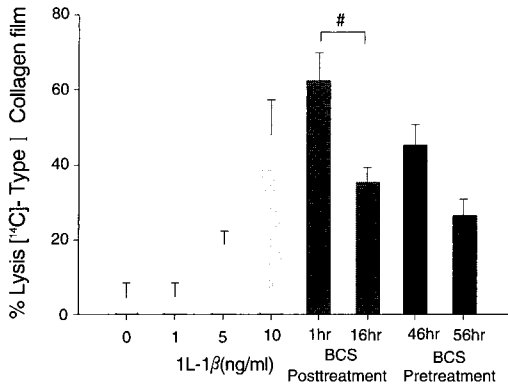


Fig. 8. Effects of BCS extracts on IL-1 α -induced bone resorption. Bone resorption was measured as percent release of ⁴⁵Ca during 5 days of culture. Each point is the mean paired difference \pm S.E. for 5 treatment-control bone pairs. Analysis of covariance indicated no significant differences in slopes of the increasing portions of the two curves. # $p < 0.05$; Significantly different from control group.

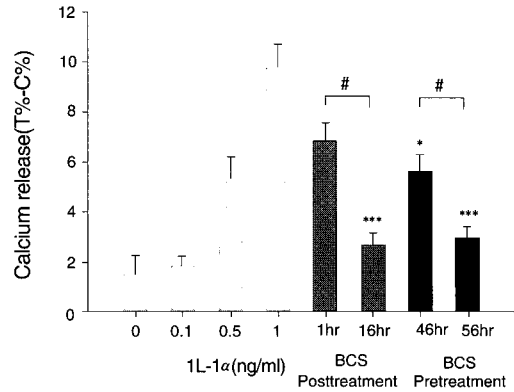


Fig. 9. Effects of BCS extracts on IL-1 β -induced bone resorption. Bone resorption was measured as percent release of ⁴⁵Ca during 5 days of culture. Each point is the mean paired difference \pm S.E. for 5 treatment-control bone pairs. Analysis of covariance indicated no significant differences in slopes of the increasing portions of the two curves. Variance ratio testing showed highly significant differences. * $p < 0.05$, *** $p < 0.001$; Significantly different from IL-1 β , (1 ng/ml)-treated group. # $p < 0.05$, ## $p < 0.01$; Significantly different from control group.

Discussion

Bambusae concretio Silicea (BCS) has anti-inflammatory properties. It is used in the treatment of cerebrovascular lesion and aphasia during the treatment of wind-heat syndrome and heat-phlegm in oriental medicine¹³. It was investigated on the effect of BCS on cultured astrocytes, lipid peroxidation and antioxidative enzyme activities in A β ,25-35 treated conditions¹⁵. Thus, it still occupies an important place in traditional oriental medicine.

IL-1 in stimulating bone resorption was examined using fetal mouse long bone organ culture. IL-1 stimulated bone resorption and produced marked resorption when present simultaneously. The cells were

used in all experiments and characterized as osteoblasts by the following criteria; staining for alkaline phosphatase positivity; osteocalcin synthesis in response to 1,25(OH)₂D₃; type I collagen production; and accumulation of cAMP in response to PTH treatment¹⁰. On the other hand, to examine the inhibitory effect of the BCS extracts on the bone resorption and collagenolysis induced by PTH, MCM, 1,25(OH)₂D₃, IL-1 α and IL-1 β , in the mouse calvarial bone cells, author have screened and assayed the inhibitory activities of BCS extracts.

When calvarial osteoblasts were stimulated with either PTH, or MCM, or IL-1(1 ng/ml) or 1,25(OH)₂D₃, IL-1 α and IL-1 β , there were statistically significant

increase in collagenolysis with PTH, 1,25(OH)₂D₃ and MCM treatment (Fig.3-5). These levels of degradation were comparable to those achieved by rabbit calvarial osteoblasts after 120 hrs¹⁾. The BCS extracts were shown to have the protective effects against collagenolysis induced by the bone resorption agents of IL-1 α and IL-1 β . However, their effects were not stringent to protect the collagenolysis. The collagenolysis-induction agents has been known to increase the susceptibility of the calvarial cells against collagenolysis²⁾, although there are some controversies. Thus, author examined the effect of the pretreatment with a various concentrations of the BCS extracts then treated the collagenolysis-induction agents. Pretreatment of the BCS extracts for 1 hr, which by itself had little effect on cell survival, did not enhance the collagenolysis, nor significantly reduced the collagenolysis by pretreatment (Fig. 6,7).

It is well known in cellular and molecular aspects that IL-1 α and IL-1 β , have similar dose dependent responses in most biological systems²⁾. However, the dose response for stimulating bone resorption differed significantly in the fetal mouse long bone organ tissue culture, as shown in Fig. 9. Human IL-1 β , is approximately 10 times more potent than human IL-1 α in stimulating bone resorption as measured by means of calcium release when each is normalized to nano gram of amounts. Analysis of covariance indicated no significant difference in the slopes of the increasing portions of the two curves. Variance ratio tests showed highly significant difference ($p < 0.01$) between the adjusted (for nano gram) means for the different IL-1s. These results are similar to that obtained from fetal rat long bone organ cultures³⁾. It was known that IL-1 α and IL-1 β , generally have the same potency and biological activity and bind to the same receptor³⁾. My result showed IL-1 β is significantly less potent than human IL-1 β , in stimulating bone resorption. Thus, the

differences in relative activity between IL-1 α and IL-1 β , in different assays would be not unusual.

The assays for the inhibition of bone resorption and collagenolysis are composed of *in vitro* cytotoxicities on mouse calvarial bone cells, collagenolysis, gelatinase activities, and bone resorption activity with a pretreatment and posttreatment of the BCS extracts. From these results, it was concluded that the BCS extracts are highly stable and applicable to clinical uses in osteoporosis.

Conclusion

Bambusae concretio Silicea (BCS) is a medicinal material used in Korea for the treatment of various symptoms accompanying hypertension and cerebrovascular disorders. The authors studied the protective effect of the BCS extracts on the bone resorption of the mouse calvarial bone cells. Interleukin-1 β , (IL-1 β), regulates the osteoblast cells derived from mouse calvarial bone explants *in vitro*.

Mouse osteoblasts, which were stimulated by PTH, 1,25(OH)₂D₃, mononuclear cell conditioned medium (MCM) and IL-1 as bone resorption agents, showed increased collagenolysis by producing the active gelatinase. The results of *in vitro* cytotoxicities showed that BCS-water extracts have no any cytotoxicities in concentrations of 1-150 $\mu\text{g/ml}$ and furthermore there is no any cytotoxicity even in concentration of 500 $\mu\text{g/ml}$ on mouse calvarial bone cells. BCS extracts had protective activity against PTH, MCM, IL-1 α , 1,25(OH)₂D₃ and IL-1 β ,-induced collagenolysis in the mouse calvarial cells.

Pretreatment of the BCS extracts for 1 hr, which by itself had little effect on cell survival, did not enhance the collagenolysis, nor significantly reduced the collagenolysis by pretreatment. Furthermore, the BCS extracts were shown to have the protective effects

against collagenolysis induced by IL-1 α and IL-1 β . Pretreatment of the extracts for 1 hr significantly reduced the collagenolysis. BCS extracts were shown to have the inhibiting effects against IL-1 α and IL-1 β , stimulated bone resorption and the effect of the pretreatment of the BCS extracts were significant. These results indicated that the BCS extracts are highly stable and applicable to clinical uses in osteoporosis.

References

1. Aubauch GD, Marx SJ, Speigel AM. Parathyroid hormone, calcitonin, and calciferols. In Williams, R. H. ed) Textbook of endocrinology. 6th ed. Philadelphia: W.B. Saunders Co. 1981:932-1031.
2. Vaughan J. The physiology of bone, 3rd ed. Oxford:Clarendon Press. 1981:139-172.
3. Dewhirst F, Ago JM, Peros WJ, Stashenko P, Synergism between parathyroid hormone and interleukin 1 in stimulating bone resorption in organ culture. *J. Bone and Mineral Res.* 1987;2:127-34.
4. Meikle MC, Bord S, Hembry RM, Reynolds JJ. Rabbit calvarial osteoblasts in culture constitutively synthesize progelatinase-A, and TIMP-1 and TIMP-2. *Biochim. Biophys. Acta.* 1994;1224:99-102.
5. Kuroki Y, Shiozawa S, Sugimoto T, Fujita T. Constitutive expression of c-fos gene inhibits type-I collagen synthesis in transfected osteoblasts, *Biochem. Biophys. Res. Comm.* 1992;182:1389-94.
6. Hazuda DJ, Strickler J, Hueppers F, Simon PL, Young PR. Processing of precursor Interleukin 1 β , and inflammatory disease. *J. Biol. Chem.* 1990;265:6318-22.
7. Evans DB, Bunning RAD, Russell RGG. The effects of recombinant human interleukin-1 β , on cellular proliferation and the production of prostaglandin E $_2$, plasminogen activator, osteocalcin and alkaline phosphatase by osteoblast-like cells derived from human bone. *Biochem. Biophys. Res. Comm.* 1990;166:208-16.
8. Heasman PA, Collins JG, Offenbacher, Changes in crevicular fluid levels of interleukin-1 β , leukotriene B $_4$, prostaglandin E $_2$, thromboxane B $_2$ and tumor necrosis factor α in experimental gingivitis in humans. *J Periodont Res.* 1993;28:241-7.
9. 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 E $_2$ in cultured neonatal mouse calvariae. *Endocrinology.* 1994;135:1157-64.
10. Harrison JH, Lorenzo JA, Kawaguchi H, Raisz LG, Pilbeam CC. Stimulation of prostaglandin E $_2$ production by interleukin-1 α and transforming growth factor α in osteoblastic MC3T3-E1 cells. *J. Bone Miner. Res.* 1994;9:817-23.
11. Sato K, Fuji Y, Kasano K, Saji M, Tsushima T, Shizume K. Stimulation of prostaglandin E $_2$ and bone resorption by recombinant human interleukin-1 α in fetal mouse bone. *Biochem. Biophys. Res. Comm.* 1986;138:618-24.
12. 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.
13. Lee SI. Clinical Application of Oriental Medicinal Herbs, Seoul:Sungbo Publishing Co. 1986;453-97.
14. Chen X, Jia Y, Wang B. Inhibitory effects of the extract of *Bambusae concretio* Silicea on monoamine oxidase in aged mice. *Chung Kuo Chung Yao Tsa Chih.* 1992;17:107-10.
15. Lee WH. Effects of *Bambusae concretio* Silicea on amyloid- β , -induced neuronal cell toxicity, lipid peroxidation and antioxidative enzymes in rat neuronal astrocytes, Thesis of Doctor's degree, Dongguk University, 1999.
16. Kim SJ. Effects of *Bambusae concretio* Silicea on mouse calvarial bone resorption, Thesis of Master's degree, Dongguk University, 2000.
17. Park C, Kim JR, Shim JK, Kang BS, Park YG, Nam KS, Lee YC, Kim CH., Inhibitory effects of streptozotocin, tumor necrosis factor- α , and interleukin-1 β , on glucokinase activity in pancreatic islets and gene expression of GLUT2 and glucokinase. *Arch Bioche Biophys.* 1999;362:217-24.
18. Kawaguchi H, Nemoto K, Raisz LG, Harrison JR, Vosnesensky OS, Alander CB, Pilbeam CC.

- Interleukin-4 inhibits prostaglandin G/H synthase-2 and cytosolic phospholipase A2 induction in neonatal mouse parietal bone cultures. *J. Bone Miner. Res.* 1996;11:358-66.
19. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951;193:265-75.
 20. Raiz LG, Niemann I. Effect of phosphate, calcium and magnesium on bone resorption and hormonal responses in tissue culture. *Endocrinology.* 1969;85:446-52.
 21. Pilbeam C, Harrison JR, Raiz LG. Prostaglandins and bone metabolism. In Bilezikian, J.P., Raiz, L. G., Rodan, G.A. (eds.) *Principles of Bone Biology.* San Diego, USA: Academic Press. 1996;715-28.
 22. Wood DM, Bayne EK, Goldring MB, Gowen M, Hamerman D, Humes JL, Ihrle EJ, Lipsky PE, Staruch MJ. The four biochemically distinct species of human interleukin 1 all exhibit similar biologic activities. *J. Immunol.* 1985;134:895-903.