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Effects of complex extracts having Drynariae Rhizoma on suppression of collagenolysis and bone resorption in mouse calvarial osteoblasts

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【Abstract】 Anti-bone resorption properties of the Korean herbal medicine, CEDR, which is comprised 5 herbs of [*Drynariae Rhizoma, Loranthi Ramus, Cibotii Rhizoma, Amydae carapax, Psoraleae semen*], were investigated. Mouse calvarial osteoblast cells were isolated and cultured. Mouse osteoblasts, which were stimulated by PTH, $1,25(OH)_2D_3$, TNF-α and IL-1 as bone resorption agents, showed increased collagenolysis by producing the active gelatinase. 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 results of *in vitro* cytotoxicities showed that CEDR extracts have no any cytotoxicities in concentrations of 1-60 μg/ml and furthermore there is no any cytotoxicity even in concentration of 120 μg/ml on mouse calvarial bone cells. CEDR extracts had protective activity against PTH (5 units/ml), or IL-1α (1 ng/ml) or TNF-α or $1,25(OH)_2D_3$ (20 ng/ml), IL-1α and IL-1β -induced collagenolysis in the mouse calvarial cells. Pretreatment of the CEDR extracts for 1 h, which by itself had little effect on cell survival, did not enhance the collagenolysis, nor significantly reduced the collagenolysis by pretreatment. Furthermore, the medicinal extracts were shown to have the protective effects against collagenolysis induced by IL-1α and IL-1β. Pretreatment of the extracts for 1 h significantly reduced the collagenolysis. Interestingly, the CEDR extracts were shown to have the inhibiting effects against gelatinase enzyme and processing activity induced by the bone resorption agents of PTH, $1,25(OH)_2D_3$, TNF-α, IL-1β and IL-1α with strong protective effect in pretreatment with the extracts. CEDR extracts were shown to have the inhibiting effects against IL-1α- and IL-1β-stimulated bone resorption and the effect of the pretreatment with a various concentrations of the medicinal extracts were significant. These results indicated that the CEDR extracts are highly stable and applicable to clinical uses in osteoporosis.

Key words : *Drynariae Rhizoma,* bone resorption, osteophoresis, calvarial bone cells, interleukin-1α, interleukin-1β

I. Introduction

It is well known that Korean herbal medicine, complex extracts having *Drynariae Rhizoma* [CEDR], is effective for the treatment of inflammation, hyperlipemia, arteriosclerosis, and gynecological diseases such as osteophorosis and bone resorption. according to the ancient

Chinese and Korean medicinal and herbal literature [1]. The CEDR is a formula of Korean herbal medicines applied in Korea and China as an effective biological response modifier for augmenting host homeostasis of body circulation [1]. CEDR is consisted of crude ingredients from 5 medicinal herbs, [*Drynariae Rhizoma, Loranthi Ramus, Cibotii Rhizoma, Amydae carapax, Psoraleae semen*]. The pharmacological action of CEDR has been limitedly studied in regard to gynecological diseases. This herbal medicine has been shown to express diverse activities such as immunomodulating, anti-infarction, anti-allergic and anti-inflammatory effects. It is a haemostatic agent, promoting blood coagulation and it is also a cardiac tonic and diuretic; these actions are probably effected by producing renal vasodilation [2]. In addition, the herb has anti-inflammatory properties. It is used in the treatment of Yin deficiency of liver and kidney, hectic fever, night sweat, and dizziness [3]. Thus, it still occupies an important place in traditional Oriental medicine.

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 [4,5]. The cytokines of inteleukin-1, tumor necrosis factor, and certain prostaglandins are locally produced factors which have been shown to stimulate bone resorption in vitro. The bone resoprtion is sometimes mediated by the synergistic activities by those factors [6]. Research during the past decade indicates that two interrelated mechanisms are involved in the proteolytic stages of bone resoprtion [7]. 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 action [8].

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 $1,25(OH)_2D_3$; type I collagen production; and accumulation of cAMP in response to PTH treatment [9]. Bone resorption activity was examined using fetal mouse long bone organ culture by IL-1, together with the effects of indomethacin and dexamethasone. On the other hand, to examine the inhibitory effect of some oriental medicinal extracts on the bone resorption and collagenolysis induced by PTH, $1,25(OH)_2D_3$, TNF-α, IL-1α and IL-1β in the mouse calvarial bone cells, we have screened and assayed the inhibitory activities of CEDR extracts. Although the effectiveness of CEDR for inflammatory diseases has been widely demonstrated by clinical administration, the scientific and acting mechanisms for those are not understood and elucidated. It is generally known that inflammation induces bone resorption and osteophorosis. Therefore, anti-bone resorption activity may be assessed by the effect on osteoblastic cells. The present paper reports the effect of extracts obtained from CEDR on cytokine-induced experimental bone resorption in mouse calvarial cells. 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 CEDR. From the results, it was concluded that the CEDR is highly applicable to clinical uses in osteoporosis.

Ⅱ. Materials and Methods

1. Materials

CEDR is consisted of each herb such as *Drynariae Rhizoma, Loranthi Ramus, Cibotii Rhizoma, Amydae carapax, Psoraleae semen.* The aqueous extracts of CEDR and its seven composed Korean herbs, which was massproduced as for clinical use, were kindly supplied by the Oriental Medical Hospital of Dongguk University (Kyungju, Korea).

Dulbecco's modified Eagle's medium (DMEM), bovine serum albumin, cycloheximide (CHM), TNF-α, 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). Fetal bovine serum (FBS) were from Gibco (Chagrin Falls, OH). Recombinant pure human IL-1β (specific activity 5 x 10^5 U/mg) was our deposit [10], 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).

2. MTT cytotoxicity of the CEDR water extracts on the isolated mouse calvarial bone cells.

Cytotoxicity of the medicinal herb 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 μl; 1~2 x 10^4 cells/ well) in a 96 well-microculture plate (flat bottom; Falcon 3027) (Becton Dickinson and Company, New Jersey, USA). After 24 h culture, 30 μl of varying concentrations of each sample solution was added to the wells and cultured for 3 days. Finally, 50 μl 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 incunation, the culture supernatants were discarded by aspirating and then 250 μl of dimethyl sulfoxide (DMSO) was added. The optical density (O.D) was measured in 50 mMglycine 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) x 100.

3. Osteoblasts isolation and culture

Mouse calvarial osteoblasts were isolated from neonatal BALBc mice by enzymatic digestion, as described for rat osteoblasts [11,12]. 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 osteocalcin [13]. Cells released by collagenase digestions were washed and grown to confluent in 75㎠ 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℃ in a humidified atmosphere of 5% CO_2/95% air; the medium was changed every 2-3 days. Cells were grown to confluence at 37℃ and cultured in duplicate or triplicate wells for an additional 24 h in serum-free medium

supplemented with Polymixin B sulfate to prevent endotoxin effects prior to treatment.

4. Measurement of collagenolysis with PTH, 1,25(OH)$_2$D$_3$, IL-1α and IL-1β.

Calvarial osteoblasts were isolated from neonatal BALBc mice by enzymatic digestion, as for mouse osteoblasts [7]. Cells released by collagenase digestions were washed and grown to confluent in 75㎠ culture flasks (Falcon) in DMEM supplemented with antibiotics and 10% FCS. Incubations were carried out at 37℃ in a humidified atmosphere of 5% CO_2/95% air; the medium was changed every 2-3 days. Radiolabelled collagen films were prepared as described [7]. Aliquots of [^{14}C]acetylated collagen [mouse skin type I; 150μg in 300 μl 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℃. The collagen was washed twice with sterile distilled water and once with DMEM prior to use. First passage cells (10^5/well) were settled onto the collagen films and cultured in 1 ml DMEM with 10% FCS for 24h. After a wash in serum-free DMEM, the cells were cultured for either 72 or 120 h in DMEM (500μl) with 2% acid treated mouse serum (this contains no α2-macroglobulin or other detectable proteinase inhibitors). Cells were stimulated with either PTH (2 units/ml), or IL-1β, or IL-1α (1 ng/ml) or 1,25(OH)$_2$D$_3$(10 ng/ml). PTH (1-84) and IL-1α were supplied by Funabashi Co., (Tokyo, Japan). At the end of the culture period the media were centrifuged (10 min, 1200 xg) to remove any collagen fibrils, and the radioactivity released during collagen degradation quantified by liquid scintillation counting.

5. Measurement of CEDR-treatment on PTH, TNF-α, 1,25(OH)$_2$D$_3$, IL-1α and IL-1β-induced collagenolysis in calvarial osteoblast cells.

Two different assays were carried out to assess the anti-collagenolysis activity of CEDR extracts (each 100 μg/ml) on PTH, 1,25(OH)$_2$D$_3$, IL-1α and IL-1β-induced collagenolysis in calvarial osteoblast cells, as follows: 1) Experiment-1: The mouse calvarial osteoblast cells were treated with PTH, or 1,25(OH)$_2$D$_3$, IL-1α and IL-1β to induce the collagenolysis for 56 hr, and the treated cells were further treated with CEDR with time courses of 1, 2, 4, 8, and 16 hrs, and the reduced collagenolysis was assayed. 2) Experiment-2: The mouse calvarial osteoblast cells were initially treated with CEDR for 1 h and further treated with each agents such as PTH, or 1,25(OH)$_2$D$_3$, IL-1α and IL-1β to induce the collagenolysis for 46 and 56 hr. Finally, the reduced collagenolysis was assayed.

6. Analytical methods

Protein content was determined by the method of Lowry [14] with bovine serum albumin as the standard. Protein in the cell culture medium was routinely followed by the absorbance at 280 nm.

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% CO_2 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 between treatment and control bone percent ^{45}Ca released from during the 5-day culture. Dead bone ^{45}Ca release in this system was approximately 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 difference between paired BGJ_b 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 ^{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 2000 for Windows program (Version 6.0, SPSS Inc.). $P<0.05$ was considered significant.

Ⅲ. Results and Discussion

1. MTT test of CEDR-water extracts on mouse calvarial bone cells.

When we 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 results of in vitro cytotoxicities showed that CEDR-water extracts have no any cytotoxicities in concentrations of 1-20 μg/ml and furthermore there is no any cytotoxicity even in concentration of 60 μg/ml on mouse calvarial bone cells (Fig. 1). However, in higher concentration of the extracts, the MTT reduction observed and the degree of inhibition was increased in a dose-dependent manner from 0.15 mg/ml concentrations.

In contrast, 100 μg/ml of LPS, a cytotoxic and inflammatory control reagent, showed the severe cytotoxicity on the mouse calvarial bone cells, resulting in 75% of cell death of the cells. These results indicated that the CEDR-water extracts are highly stable and applicable to clinical uses. However, for 60 μg/ml of each extract was used for the next experiments. Cell viability was not significantly affected by treatment with the indicated concentration of the extracts alone, as examined also in MTT assays.

2. Effects of oriental medicinal extracts such as CEDR on PTH, $1,25(OH)_2D_3$, TNF-α, IL-1α and IL-1β-induced collagenolysis in calvarial osteoblast cells.

When calvarial osteoblasts were isolated from neonatal BALBc mice by enzymatic digestion, and cells were stimulated with either PTH (2 units/ml) or $1,25(OH)_2D_3$ (10 ng/ml) or TNF-α, IL-1α and IL-1β. 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 (mean ± S.E.) for five wells.

There were small but statistically significant increase in collagenolysis with parathyroid hormone (PTH), 1,25-hydroxy vitamin D3 ($1,25(OH)_2D_3$), TNF-α treatment after 56 h (Fig. 2-4). With all three agents, however, 56 h of culture were required to reach levels of degradation comparable to those achieved by rabbit calvarial osteoblasts after 120 h [4].

On the other hand, various medicinal extracts were tested for whether they could protect against TNF-α, IL-1α and IL-1β -induced collagenolysis in the mouse calvarial cells (Fig. 4-6). Cell viability was not significantly affected by treatment with the indicated concentration of the extracts alone, as examined also in MTT assays (Fig. 1). All these medicinal extracts were shown to have the protective effects against collagenolysis induced by the bone resorption agents. 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 collagenolysis [16], although there are some controversies. Thus, we examined the effect of the pretreatment with a various concentrations of the medicinal extracts then treated the collagenolysis-induction agents. Pretreatment of the extracts for 1 h, which by itself had little effect on cell survival, did not enhance the collagenolysis, nor significantly reduced the collagenolysis by pretreatment (Fig.2-4).

3. Effects of oriental medicinal extracts such as CEDR on IL-1α and IL-1β -induced collagenolysis in calvarial osteoblast cells.

We tested the ability of recombinant human IL-1 (IL-1α; 0.1-20.0 ng/ml) to stimulate collagen degradation by the cells; maximal collagenolysis (69%) was only achieved after 56 h with the optima dose of 5 ng/ml (Fig. 5). Also, IL-1$\beta$was tested for stimulation of collagen degradation (0.1 - 2.0 ng/ml). The maximal collagenolysis was obtained after 56 h with the optima dose of 2 ng/ml (Fig. 6). Therefore, it was suggested that IL-1α and IL-1β were bone resorptive agent because target cells are likely to be exposed in vivo to mixtures rather than individual cytokines, particularly during inflammation.

To examine the anti-collagenolysis of the medicinal extracts of CEDR on IL-1α and IL-1β-induced collagenolysis in calvarial osteoblast cells, various concentrations of the CEDR extracts were tested for whether they could protect against IL-1α (2 ng/ml) or IL-1β(1 ng/ml)-induced collagenolysis in the mouse calvarial cells (Fig. 5 and 6).

Furthermore, the CEDR extracts were shown to have the protective effects against collagenolysis induced by the bone resorption agents of IL-1α and IL-1β. 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 collagenolysis [16], although there are some controversies. Thus, we examined the effect of the pretreatment with a various concentrations of the CEDR extracts then treated the collagenolysis-induction agents. Pretreatment of the CEDRextracts for 1 h, which by itself had little effect on cell survival, did not enhance the collagenolysis, nor significantly reduced the collagenolysis by pretreatment (Fig. 5,6).

4. Bone resorption activity of IL-1 and effects of CEDR extracts on IL-1 α - and IL-1β-induced bone resorption mouse calvarial osteoblasts

It is well known in cellular and molecular aspects that IL-1 α - and IL-1β have similar dose dependent responses in most biological systems [17]. However, the dose response for stimulating bone resorption differed significantly in the fetal mouse long bone organ tissue culture, as shown in Fig. 7 and 8. Human IL-1β 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 cultures [6]. It was known that IL-1 α - and IL-1β generally have the same potency and biological activity and bind to the same receptor [6]. Our result showed IL-1 α is significantly less potent than human IL-1β in stimulating bone resorption. Thus, the differences in relative activity of IL-1 α - and IL-1β in different assays would be not unusual. CEDR 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 with a various concentrations of the medicinal extracts were significant (Fig. 7,8).

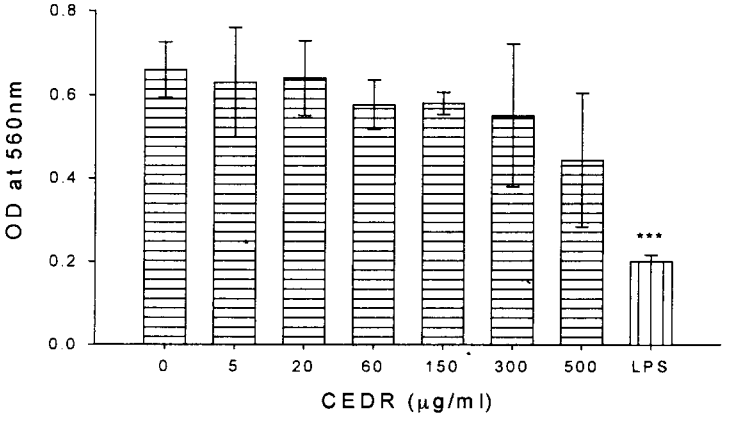

Fig. 1. MTT test of CEDR water extracts on calvarial osteoblasts.
As a negative control, 100 μg/ml LPS gave significant inhibition of the activity. Each point represents the mean± S.E. of 3 experiments from separate joints. *** $p<0.001$ compared with CEDR-untreated group.

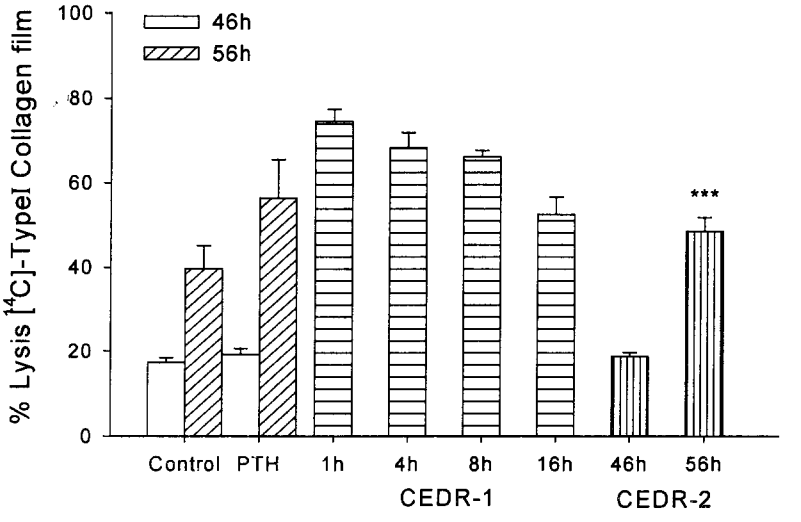

Fig. 2. Lysis of ^{14}C-labelled type I collagen films by mouse calvarial osteoblasts and effects of CEDR extracts on PTH-induced collagenolysis.

Collagen degradation was expressed as a percentage radioactivity released from the films (mean ± S.E.) for four wells. ** $p<0.01$ compared with PTH-56 h-treated group. CEDR-1 : posttreatment, CEDR-2 : pretreatment

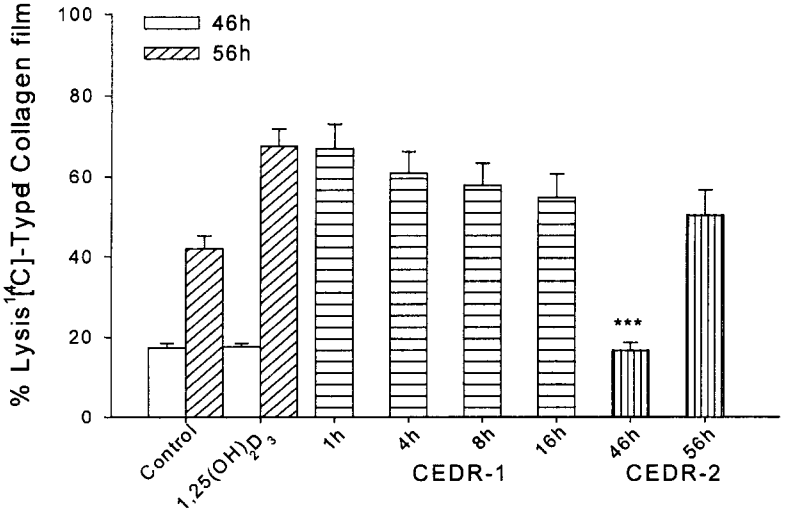

Fig. 3. Lysis of ^{14}C-labelled type I collagen films by mouse calvarial osteoblasts and effects of CEDR extracts on $1,25(OH)_2D_3$-induced collagenolysis..

Collagen degradation was expressed as a percentage radioactivity released from the films (mean ± S.E.) for four wells. *** $p<0.001$ compared with $1,25(OH)_2D_3$-56 h-treated group. CEDR-1 : posttreatment, CEDR-2 : pretreatment

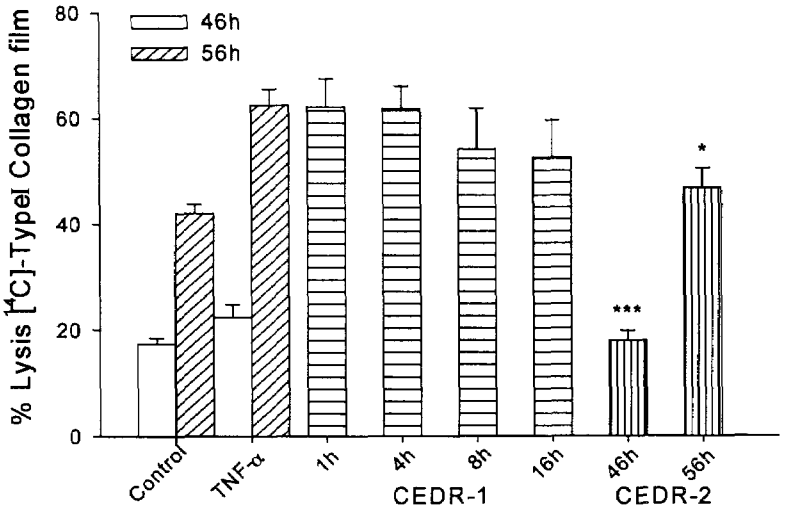

Fig. 4. Lysis of ^{14}C-labelled type I collagen films by mouse calvarial osteoblasts and effects of CEDR extracts on TNF-α-induced collagenolysis.

Collagen degradation was expressed as a percentage radioactivity released from the films (mean ± S.E.) for four wells. * $p<0.01$, *** $p<0.001$ compared with TNF-α-56 h-treated group. CEDR-1 : posttreatment, CEDR-2 : pretreatment

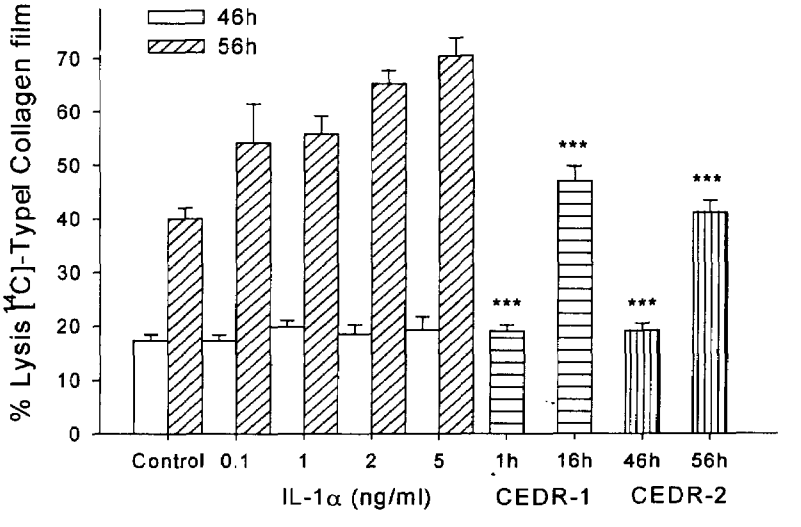

Fig. 5. Effect of CEDR extracts on IL-1α (2.0 ng/ml)-induced collagenolysis by mouse calvarial osteoblasts.

Collagen degradation was expressed as a percentage radioactivity released from the films (mean ± S.E.) for four wells. *** $p<0.001$ compared with IL-1α (5 ng/ml)-56 h-treated group. CEDR-1 : posttreatment, CEDR-2 : pretreatment

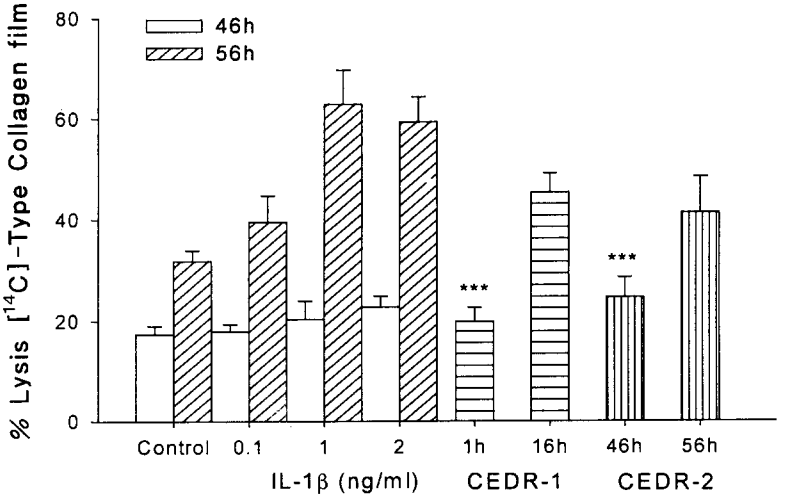

Fig. 6. Effect of IL-1β (0.1-5 ng/ml) on lysis of ^{14}C-labelled type I collagen films by mouse calvarial osteoblasts and effects of CEDR extracts on IL-1β (1.0 ng/ml)-induced collagenolysis.

Collagen degradation was expressed as a percentage radioactivity released from the films (mean ± S.E.) for four wells. ** $p<0.01$, *** $p<0.001$ compared with IL-1β(1 ng/ml)-56 h-treated group. CEDR-1 : posttreatment, CEDR-2 : pretreatment

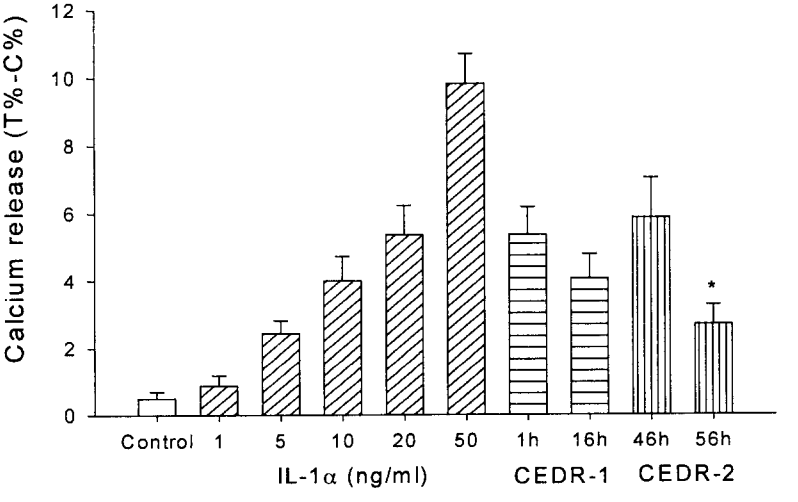

Fig. 7. Dose-dependent responses for IL-1α-stimulated bone resorption and effects of CEDR extracts on IL-1α-induced bone resorption.

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. * $p<0.05$ compared with IL-1α (20 ng/ml)-treated group. CEDR-1 : posttreatment, CEDR-2 : pretreatment

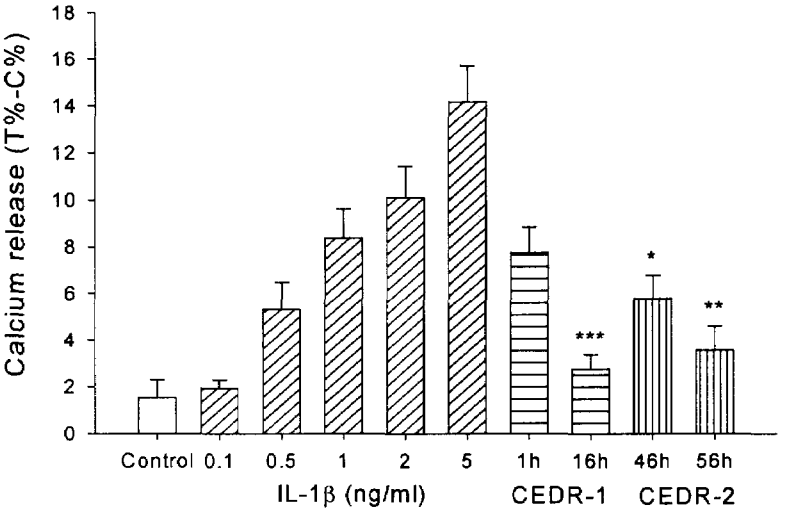

Fig. 8. Dose-dependent responses for IL-1β-stimulated bone resorption and effects of CEDR extracts on IL-1β-induced bone resorption.

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. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with IL-1β(2 ng/ml)-treated group. CEDR-1 : posttreatment, CEDR-2 : pretreatment

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骨碎補 복합제제가 생쥐의 calvarial osteoblast에서 collagen 용해와 골 재흡수에 미치는 영향

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한방에서 建强骨시키는 효능이 있는 5가지 약물(骨碎補, 桑寄生, 金毛狗脊, 別甲, 法破古紙)로 구성된 처방(CEDR로 약칭)으로 실험을 하였다. 생쥐의 calvarial osteoblast를 분리하고 배양한 후 실험을 행하였는데, 골 재흡수 인자인 PTH, $1,25(OH)_2D_3$, TNF-α, IL-1에 자극받은 osteoblasts는 활동적인 gelatinase를 생산하므로서 collagen 용해를 증가시켰다. 암컷 생쥐의 long bone organ을 culture하여 골 재흡수를 자극하는 IL-1를 실험한 결과, IL-1은 골의 재흡수를 자극하였으며 동시에 존재해있을 때에는 뚜렷한 재흡수를 보였다. 더욱이, indomethacin과 dexamethasone이 IL-1α의 투여량 증가에 대한 영향을 관찰하였을 때에는 indomethacin과 dexamethasone은 IL-1의 투여량에 대한 곡선 그래프를 오른쪽으로 이동시켰다. 시험관내에서 세포 독성에 대하여 관찰하였을 때, CEDR 추출물은 1-60 μg/ml의 농도에서 아무런 세포 독성이 나타나지 않았으며, 뿐만 아니라 생쥐의 두개골 세포에서는 120 μg/ml 농도에서 전혀 세포독성이 관찰되지 않았다.

CEDR 추출물은 생쥐의 calvarial cells에서 PTH (5 units/ml), IL-1α (1 ng/ml), TNF-α, $1,25(OH)_2D_3$ (20 ng/ml) 및 IL-1α, IL-1β에 유도되는 collagen 용해를 보호하는 효과가 있었다. CEDR 추출물을 1시간동안 전처리하였을 때, 그 자체로는 세포 생존에 영향이 없었으며, collagen 용해를 증가시키지도 않았으며 전처치로 collagen 용해를 유의성있게 감소시키지도 않았다. 게다가, 추출물은 IL-1α와 IL-1β에 의해 유도되는 collagen 용해를 방지하는 효과가 있었다. 1시간 동안 전처리로 한 결과, collagen 용해를 유의성있게 감소시켰다.

흥미로운 것은 CEDR 추출물이 gelatinase 활성과 PTH, $1,25(OH)_2D_3$, TNF-α, IL-1β 및 IL-1α의 재흡수 인자에 유도되어 진행되는 활성을 억제하였으며, CEDR 전처리시에는 강력한 보호 효과를 나타내었다. CEDR 추출물은 IL-1α와 IL-1β에 자극되는 골 재흡수를 억제하는 효과를 나타내였으며, 또한 농도를 다양하게 한 CEDR의 전처리시에는 유의성이 있었다. Indomethacin과 dexamethasone의 비 스테로이드성 항 염증 인자에 의한 IL-1에 자극받은 골 재흡수를 억제하는 정도와 현상은 CEDR 추출물을 생쥐의 두개골 배양 시스템에 적용시켜 얻은 결과와 유사하였다. 이러한 결과들로 보건데, CEDR 추출물은 임상적으로 골다공증의 치료에 매우 안정적으로 적용할 수 있음을 제시하고 있다.

중심 낱말 : 骨碎補, 골 재흡수, 골다공증, 두개골세포, IL-1α, IL-1β