대한한방부인과학회지
THE JOURNAL OF ORIENTAL OBSTETRICS & GYNECOLOGY
VOL.15, NO.4 : 061 - 075 (2002)

A Study on Anti-Bone Resorption & Osteoporosis by Taeyoungjon-Jahage Extracts

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ABSTRACT

유전자 재조합으로 제조한 사람 interleukin-1β (rhIL-1β)는 생쥐의 calvarial 골세포계에서 분리한 골아세포에 여러 가지 조절기능을 갖는 것으로 알려져 있다. 본 연구에서 rhIL-1β가 농도의존적으로 골세포에 영향을 주는지 해명하기 위하여 배양된 골아세포의 세포증식과 prostaglandin E_2합성 그리고 plasminogen activator활성에 대한 영향을 검토한 결과 이들을 촉진하였다. 그러나 비타민D에 따라 반응하는 골아세포의 특징으로 알려진 osteocalcin생합성과 alkaine phosphatase활성의 유도생성은 rhIL-1β에 의해 오히려 길항적이었다. 이러한 결과는 골세포대사의 병리학적인 조절과정에서 IL-1β가 골다공증의 병리학적 역할을 규명하는 새로운 결과이다. IL-1β에 의한 골흡수현상이 생쥐의 calvarial골세포에서 calcitonin처리로 크게 억제되어, 결과적으로 이러한 결과는 IL-1β에 의해 유발되는 골재흡수란 osteoclast에 의한다는 사실을 시사하였다. 한편, 한방에서 골다공증치료와 예방에 사용되는 대영전-자하거추출물의 기능을 해명하기 위하여, IL-1β-유발 PGE_2 생산에 대한 저해정도를 시험한 결과 세포의 생존에는 크게 영향을 미치지 않고 PGE_2합성만을 특이적으로 저해하였다. 또한, 대영전-자하거 extract을 1시간 동안 여러 가지 농도로 전처리하고 다음으로 PGE_2-유도시약을 처리한 결과, PGE_2합성을 억제하였으며 동시에 IL-1β에 의해 유도된 plasminogen 의존적인 fibrinolysis을 억제하는 보호효과가 인정되었다. 한편, calcitonin처리가 IL-1β-촉진 골재흡수에 대한 저해활성을 보였으며 이러한 결과들은 calcitonin과 대영전-자하거 extract이 osteoclast매개성 골재흡수의 억제에 핵심적인 역할을 함을 시사하며 한방치료제로서의 근거를 제시하였다고 사료된다.

접수 : 2002.10.31 채택 2002.11.12
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Recombinant human interleukin-1β (rhIL-1β) regulates several activities of the osteoblast cells derived from mouse calvarial bone explants in vitro. rhIL-1β stimulated cellular proliferation and the synthesis of prostaglandin E_2(PGE_2) and plasminogen activator activity in the cultured cells in a dose-dependent manner. However, the induction of osteocalcin synthesis and alkaine phosphatase activity in response to vitamine D, two characteristics of the osteoblast phenotype, were antagonized by rhIL-1β over a similar dose range. This study supports the role of IL-1β in the pathological modulation of bone cell metabolism, with regard to implication in the pathogenesis of osteoporosis by IL-1β. When the mouse calvarial bone cells were used, the bone resorption induced by IL-1β was strongly inhibited by calcitonin treatment, indicating osteoclast-mediated bone resorption. On the other hand, the medicinal extracts of Taeyoungjon-Jahage (T.Y.J-J.H.G extracts) 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 T.Y.J.-J.H.G. extracts were shown to have the inhibitory effects against the synthesis of PGE_2. We also examined the effect of the pretreatment with a various concentrations of the T.Y.J.-J.H.G. extracts then treated the PGE_2-induction agents. Pretreatment of the T.Y.J.-J.H.G. extracts for 1 h, which by itself had little effect on cell survival, did not enhance the synthesis of PGE_2. Furthermore, the T.Y.J.-J.H.G. extracts were shown to have the protective effects against plasminogen dependent fibrinolysis induced by the bone resorption agents of IL-1β. Pretreatment of the T.Y.J.-J.H.G. extracts for 1 h did not enhance the plasminogen dependent fibrinolysis. 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 T.Y.J.-J.H.G. extracts for 1 h reduced the bone resorption. These results clearly indicated that calcitonin and T.Y.J.-J.H.G. extracts play key roles in inhibition of the osteoclast-mediated bone resorption.

I. Introduction

The potential contribution of resident immune cells and their products to the localized remodeling processes involved in bone metabolism is receiving increasing attention[1]. Studies have demonstrated that one such cytokine, interleukin-1 (IL-1), can modulate several aspects of the activity of various bone cell types. IL-1 is a potent stimmulator of bone resorption in vitro[2], an action apparently partially mediated via the stimulation of osteoclast cell formation indicating the modulation of osteoblast cell activity by IL-1[3]. Previous studies have demonstrated that IL-1β regulate several aspects of the functional activity of human osteoblast-like cells in vitro[3,4].

It is also a potent inducer of prostanoids [5,6]. 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-depndent[7]. It has also been shown to be a potent stimulator of prostaglanding G/H synthase-2 (PGHS-2) expression in murine osteoblastic cells[8].

PGHS-2 is generally expressed at low levels in most tissues but can be rapidly and transiently induced to high levels by multiple factors, including cytokines, growth factors, and tumor promoters, while PGHS-1 is constitutvely expressed[9]. Many of the important regulators of bone metabolism including cytokines, such as IL-1[10], IL-6[11], and tumor necrosis factor-α[12], growth factors, such as transforming growth factor-α[10], transforming growth factor-β[10,13], and basic fibroblast growth factor[14], hormonse, such as parathyroid hormone[15] and cortisol [8,13], prostaglandins themselves[8,13], and mechanical forces[16] have been shown to regulate prostaglanding production in rodent osteoblastic cells largely by changes in expression of PGHS-2[17]. Osteocalcin and alkaline phosphatase, as phenotype markers of the osteoblast cells[3], 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 recombinant human IL-1β (rhIL-1β) on mouse calvarial osteoblast cells derived from fetal mouse. rhIL-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 rhIL-1β in a dose-dependent manner. Also, the medicinal extracts of T.Y.J.-J.H.G. was tested for the inhibitory effects against IL-1β-induced PGE_2 production, plasminogen dependent fibrinolysis, and IL-1β-stimulated bone resorption in the mouse calvarial bone cells having both of the osteoblast and osteoclast cells. The inhibitory effect of T.Y.J.-J.H.G. 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. Materials and Methods

1. Materials and Mouse bone cell culture

Explants of mouse calvarial bone were cultured as described[3]. 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 generous gift of Dr. S. H. Park, Korea Research Institute of Bioscience and Biotechnology, Taejon, KIST. Salmon calcitonin was obtained from Armour Pharmaceutical Co. (IL, USA).

Dulbecco's modified Eagle's medium (DMEM), bovine serum albumin, cycloheximide

(CHM), indomethacin and other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA). PGE_2 antibody was purchased from Immunoassay Co. (Tokyo, Japan). [^{3}H]-prostaglandin was purchased from New England Nuclear (Seoul, Korea). The mouse β-actin cDNA was our deposit. Human PGHS-2 electrophoresis standard and rabbit polyclonal antibody directed against human P GHS-2 utilized for western analysis were obtained from Cayman Chemical Company (USA).

2. Cell proliferation

Cell proliferation was assessed by the incorporation of [^{14}C]-thymidine into materials precipitable by trichloroacetic acid. Cells were pulsed for the final 24 h of a 46h incubation period.

3. Osteocalcin assay

Osteocalcin released into the culture media, over a 72h incubation period was measured using a specific radioimmunoassay with an antibody raised in rabbits against purified bovine osteocalcin (unpublished data). 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 h.

5. Prostaglandin assay

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

6. Effects of T.Y.J.-J.H.G.-treatment on IL-1β-induced PGE_2-production, plasminogen activity, osteocalcin production, alkaline phosphatase activity and bone resorption in calvarial bone cells.

Two different assays were carried out to assess the activities of T.Y.J.-J.H.G. extracts (each 100 μg/ml) on IL-1β-induced PGE_2-production, plasminogen activity, 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, plasminogen activity, osteocalcin production, alkaline phosphatase activity and bone resorption for 24 hr, and the treated cells were further treated with T.Y.J.-J.H.G. with time courses of 1 and 16 hrs, and each activity was assayed.

2) Experiment-2 (pretreatment): The mouse calvarial bone cells were initially treated with T.Y.J.-J.H.G., T.Y.J.-J.H.G., and T.Y.J.-J.H.G. for 1 h 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[18]. 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 i nto 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 mediuml, 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 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 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-1b response is approximately 40-60%. Each bone was labeled with approximately 20,000 CPM ^{45}Ca.

8. Statistics

Statistical differences between treatments were determined using analysis of variance.

Ⅲ. Results

1. Stimulation of cell proliferation of mouse osteoblasts by rhIL-1β

The proliferation of the mouse osteoblasts was stimulated in a dose-dependent manner by rhIL-1β over the concentration range of 0.01 ng -2 ng/ml (Fig. 1). The stimulation of cell proliferation was maximal at 2.0 ng/ml, while concentrations below 0.1 ng/ml had no detectable effect.

2. Effects on PGE_2 production of the mouse osteoblasts by IL-1β and inhibitory effect of T.Y.J.-J.H.G. extracts on IL-1β-induced PGE_2 production.

rhIL-1β stimulated the production of PGE_2 in a dose-dependent manner over the concentration range of 0.01 ng -2 ng/ml with a maximal effect being observed at 2 ng/ml (Fig. 2). The stimulation of cell proliferation was most pronounced at 2.0 ng/ml, while

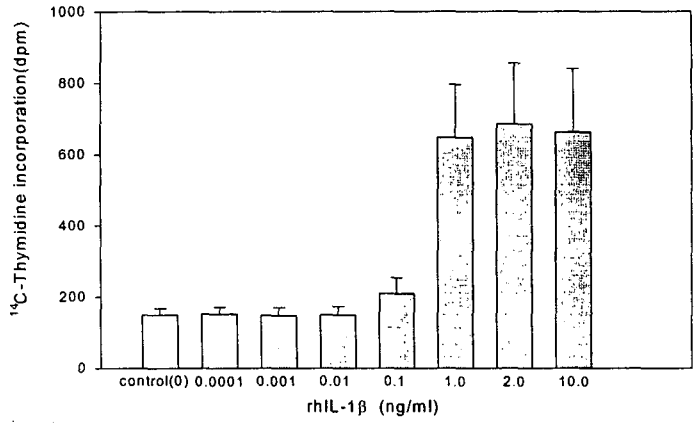

Fig. 1. Stimulation of cell proliferation of the mouse bone cells including osteoblasts and osteoclasts by IL-1β.
Cell proliferation was assessed by [^{14}C]-thymidine incorporation as described in Materials and Methods. Values represent mean ± S.E.M (n=5). Significant difference from control, *<0.05, *** P<0.001.

concentrations below 1.0 ng/ml exhibited no detectable activity on the synthesis of PGE_2.

On the other hand, the medicinal extracts of T.Y.J.-J.H.G. was tested for whether they could inhibit IL-1β-induced PGE_2 production (Fig. 2). 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 T.Y.J.-J.H.G. 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. However, their effects were not stringent to protect the synthesis of PGE_2. The 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 with a various concentrations of the T.Y.J.-J.H.G. extracts then treated the PGE_2-induction agents. Pretreatment of the T.Y.J.-J.H.G. extracts for 1 h, 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 (Fig. 2).

3. Effect on plasminogen activator activity of mouse osteoblasts by rhIL-1β and inhibitory effect of T.Y.J.-J.H.G. extracts on IL-1β-induced plasminogen activator activity

The plasminogen activator activity of the mouse osteoblast was also stimulated by rhIL-1β in a dose-dependent manner over the dosage range of 0.01 ng -2 ng/ml with a

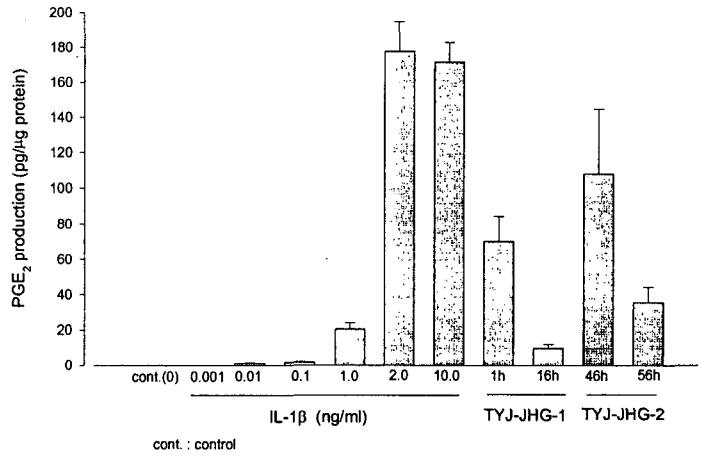

Fig. 2. The effects of rhIL-1β on the prodcution of PGE_2 by mouse bone cells including osteoblasts and osteoclast cells, and inhibitory effect of Y.J.-J.H.G. extracts on IL-1β-induced PGE2 production.

PGE_2 released into the culture media was measured as described in Materials and Methods. Values represent mean ± S.E.M (n=5).
Significant difference from control, *<0.05, *** P<0.001.
T.Y.J-J.H.G.-1 , The cells were treated with IL-1β(2,0ng/ml), and then after 56 hrs, T.Y.J.-J.H.G. extracts were further treated to the cells, then IL-1β(2,0ng/ml) induced collagenolysis were assayed with time courses of 1 and 16 hrs.
T.Y.J-J.H.G.-2 , First, the cells were treated with T.Y.J.-J.H.G. extracts for 1 hr, and then the cells were further treated with IL-1β (2,0ng/ml) and after 46 and 56 hrs, IL-1β (2,0ng/ml)-induced collagenolysis were assayed to see T.Y.J.-J.H.G. pretreatment effect.

maximal effect being observed at 2 ng/ml (Fig. 3). The plasminogen activator activity was significantly stimulated compared to that control. Concentrations below 0.1 ng/ml exhibited no detectable activity on the plasminogen activator activity.

To examine the anti-plasminogen dependent fibrinolysis of the T.Y.J.-J.H.G. extracts on IL-1β-induced plasminogen activator activity in calvarial osteoblast cells, the medicinal extracts were tested for whether they could protect against IL-1β(1 ng/ml)-induced plasminogen dependent fibrinolysis in the mouse calvarial cells (Fig. 3). Cell viability was not significantly affected by treatment with the indicated concentration of the extracts alone.

Furthermore, the T.Y.J.-J.H.G. extracts were shown to have the protective effects against plasminogen dependent fibrinolysis induced by the bone resorption agents of IL-1β. However, their effects were not stringent to protect the plasminogen dependent fibrinolysis. We also examined the effect of the pretreatment with a various concentrations of

the T.Y.J.-J.H.G. extracts then treated the agents. Pretreatment of the T.Y.J.-J.H.G. extracts for 1 h, which by itself had little effect on cell survival, did not enhance the plasminogen dependent fibrinolysis, nor significantly reduced the plasminogen dependent fibrinolysis by pretreatment (Fig. 3).

4. Effect of IL-1β on alkaline phosphatase activity and osteocalcin production stimulated by vitamin D in the mouse calvarial bone cells

To examine the effects of rhIL-1β on alkaline phosphatase activity in the mouse calvarial bone cells, various concentrations IL-1 β 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 -2.0 ng/ml (Fig. 4).

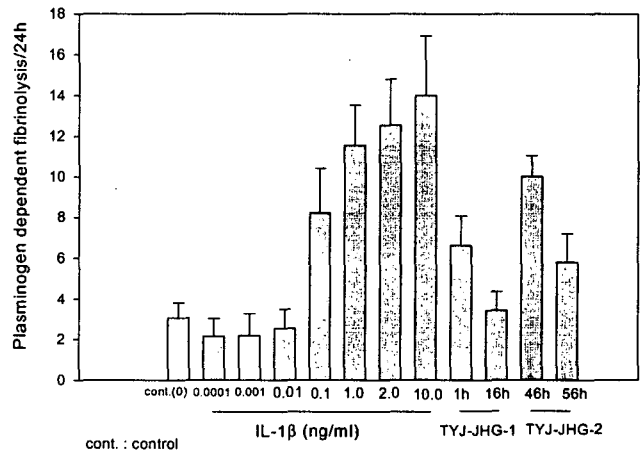

Fig. 3. Stimulation of the plasminogen activator activity of mouse bone cells including osteoblasts and osteoclasts by IL-1β and inhibitory effect of T.Y.J.-J.H.G. extracts on IL-1β-induced plasminogen activator activity
Plasminogen activator activity of mouse osteoblasts induced by rhIL-1β was measured as described in Materials and Methods. The data shown represents the plasminogen dependent fibrinolysis of [^{125}I] -fibrin substrate. Values represent mean ± S.E.M (n=5). Significant difference from control, *<0.05, *** P<0.001.

T.Y.J-J.H.G.-1 , The cells were treated with IL-1β(2,0ng/ml), and then after 56 hrs, T.Y.J.-J.H.G. extracts were further treated to the cells, then IL-1β(2,0ng/ml) induced collagenolysis were assayed with time courses of 1 and 16 hrs.

T.Y.J-J.H.G.-2 , First, the cells were treated with T.Y.J.-J.H.G. extracts for 1 hr, and then the cells were further treated with IL-1β(2,0ng/ml) and after 46 and 56 hrs, IL-1β(2,0ng/ml)-induced collagenolysis were assayed to see T.Y.J.-J.H.G. pretreatment effect.

Furthermore, to examine the effects of rhIL-1β on alkaline phosphatase activity stimulated by vitamin D in the mouse osteoblast and osteoclast cells, cells were treated with vitamin D and then the cells were further stimulated by rhIL-1β in a dose-dependent manner. The basal alkaline phosphatase activity of the mouse osteoblast cells was decreased by rhIL-1β over the dose range of 0.01 -1.0 ng/ml (Fig. 5). Concentration below 0.01 ng/ml exhibited no marked inhibitory action on basal enzyme levels (Fig. 5). The induction of alkaline phosphatase activity by vitamin D was antagonized by rhIL-1β in a dose depdendent manner over a concentration range of 0.01 ng -2 ng/ml. Lower doses had no obvious effect on the enzyme activity (Fig. 5). Concentrations below 1.0 ng/ml exhibited no detectable activity on the enzyme activity.

On the other hand, when the production of osteocalcin by osteoblast cells were assayed, the productin of osteocalcin stimulated by vitamin D was significantly antagonized by rhIL-1β over the same dose range of 0.01 - 2.0 ng/ml in a dose depdendent manner (Fig. 6).

5. Stimulation of IL-1β on bone resorption and inhibition of IL-1β-stimulated bone res-o rption by calcitonin and T.Y.J.-J.H.G. extracts in the mouse calvarial bone cells.

Treatment of mouse calvarial bone cells with IL-1β resulted in a dose dependent stimulatin of bone resorption. The dose response for stimulating bone resorption differed significantly between the fetal mouse long bone organ tissue culture (unpublished data) and this culture system of mouse calvarial bone

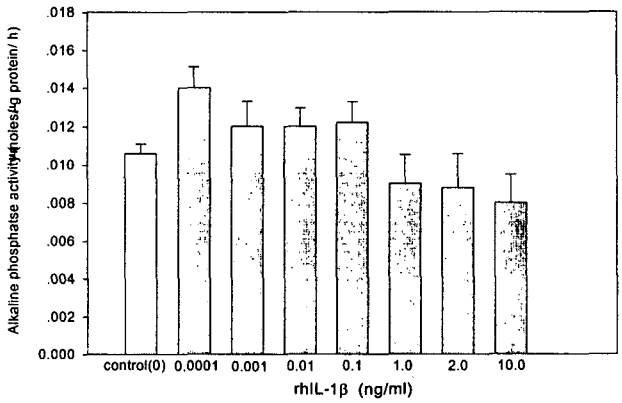

Fig. 4. Effect of IL-1β on alkaline phosphatase activity in the mouse osteoblast cells

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.M (n=5). Significant difference from control, *<0.05, *** P<0.001.

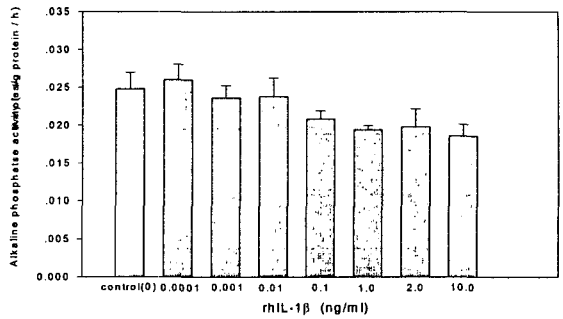

Fig. 5. Effect of IL-1β on alkaline phosphatase activity stimulated by vitamin D in the mouse osteoblast cells

Vitamin D (5μg/ml) was treated to cells and a series of concentration of IL-1β was added to the vitamin D-treated cells. 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.M (n=5). Significant difference from vitamin D-treated cells, * P<0.05, *** P<0.001

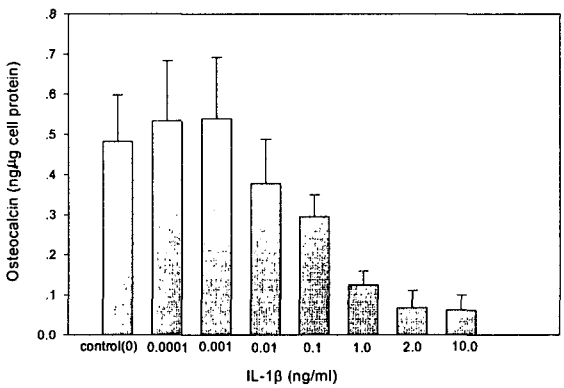

Fig. 6. Effect of IL-1β on osteocalcin production stimulated by vitamin D in the mouse osteoblast cells

Vitamin D (5μg/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.M (n=5). Significant difference from vitamin D-treated cells, * P<0.05, *** P<0.001

cells. As shown in Fig. 7. human 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. 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.

Interestingly, T.Y.J.-J.H.G. extracts were shown to have the inhibiting effects against IL-1β-stimulated bone resorption in the mouse calvarial bone cells having both of the osteoblast and osteoclast cells. When we examined the effect of the pretreatment with a various concentrations of the T.Y.J.-J.H.G. extracts then treated the agents, pretreatment of the T.Y.J.-J.H.G. extracts for 1 h, which by itself had little effect on cell survival, did not enh -ance the bone resorption, nor significantly reduced the bone resorption by pretreatment. These results are similar to the results from calcitonin treatment (Table 1) and the T.Y.J. -J.H.G. extracts play key role in inhibition of the osteoclast-mediated bone resorption induced by IL-1β.

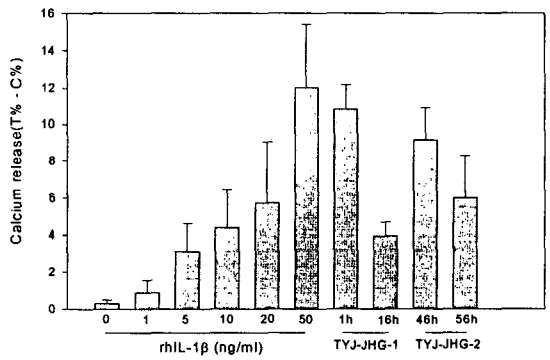

Fig. 7. Dose-dependent bone resorption of IL-1β in mouse calvarial bone cells and inhibitory effect of T.Y.J.-J.H.G. extracts on IL-1β-induced bone resorption

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, *** P<0.001

T.Y.J-J.H.G.-1 , The cells were treated with IL-1β(2,0ng/ml), and then after 56 hrs, T.Y.J.-J.H.G. extracts were further treated to the cells, then IL-1β(2,0ng/ml) induced collagenolysis were assayed with time courses of 1 and 16 hrs.

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Table 1. Inhibition of IL-1β-mediated bone resorption by calcitonin and T.Y.J.-J.H.G. treatment. 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.

Addition to bone culture	Bone resorbing activity[a] (Calcium release (T% - C%)
None	9.9
Calcitonin (0.5 U/ml)	4.2
Calcitonin (0.5 U/ml) + T.Y.J.-J.H.G. extract	4.0
IL-1β (100 ng/ml)	15.1
Calcitonin + IL-1β	8.4*
Calcitonin + IL-1β + T.Y.J.-J.H.G. extract	5.6**
Devitalized bone	1.9

a Data shown are means ± S.E.M for quadruplicate determinations. Bone were devitalized by three cyclyes of freeze-thawing.

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

IV. Discussion

In bone resorption reaction, it has been known that rhIL-1β is reactive to progessive degradation of bone by activating osteoblast cells and by causing the progeniror cells to mature cells[1]. rhIL-1β stimulated the plasminogen activator activity of the mouse osteoblast cells in a dose-dependent manner. The stimulation of plasminogen activator activity by rhIL-1β has been observed in several connective tissue cell types, indicating human osteoblast-like cells[3]. IL-1β is a potent stimulator of bone resorption both in vitro[1] and in vivo through an action which may be mediated primarily via the osteoblast [2,3]. The observation that rhIL-1β stimulates the plasminogen activator activity of the mouse osteoblast cells may indicate a potential mechanism for the osteoblast-mediation of bone resorption, as shown: Plasminogen ➡ plasmin ➡collagenase activation ➡bone matrice degration. Plasminogen activator has been associated in several processes governing connective tissue degradation. The existence of a plamin-dependent proteolytic system in mineralized materices has been known and so may contribute to the breakdown of the constitutents of the bone matrix[3]. One mechanism whereby this may be achieved is the activation of latent collagenase via action of plasmin, following its generation from plasminogen[1,3]. Rodent osteoblast cells produce latent collagenase in response to several bone resorbing agents, including IL-1β[3], but this has not been observed with the mouse osteoblasts. Mouse osteoblast cells, however, produce high levels of a metalloproteinase inhibitor which may mask any collagenase activity[19].

On the other hand, the stimulation of prostaglandin E_2 production by IL-1β allows b one breakdown by bone resorption and by st imulating the plasminogen activator activity of osteoblast-cell like cells[3]. 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 indicating that the prostaglandin E_2 synthesis by osteoblast-like cells in response to IL-1β may contribute to this effect. Elevated production of IL-1β has been implicated in the pathogenesis od osteoporosis and with humoral hypercalcemia associated with squamous cell carcinomas. The present study therefore provides further support for the role of IL-1β in the bone metabolism process. In addition, the demonstration of the production of IL-1β-like factors by osteoblasts may indicate a potential paracrine/autocrine regulatory mechanism affecting both osteoblasts and osteoclasts and hence the cellular processes governing the regulation of bone metabolism.

It is well known that osteocalcin and alkaline phosphatase are widely accepted phenotype markers of the osteoblast cells[3] and the induction of these two factors in response to vitamine D was strongly antagonized by rhIL-1β in a dose-dependent manner. The ability of rhIL-1β to suppress these markers of the mature osteoblast in association with the stimulation of cell proliferation is interesting. Elevated production of IL-1β has been implicated in the pathogenesis od osteoporosis and with humoral hypercalcemia associated with some calcinomas. Thus, the present study provides further support for the role of IL-1β in the processes governing bone metabolism.

Taeyoungjon(T.Y.J.) is a name of traditional chinese medicine. Chang Kyong-Ak(張景岳), one of Myung dynasty doctors have made it, that effect is enriching of blood and alleviation of pain in back, knees etc. Placenta Hominis(紫河車, J.H.G.) is a name of traditional korean drug, the human placenta. Its effects are tonifying vital essence, enriching blood and supplementing qi[20].

When the medicinal extracts of T.Y.J.-J.H.G. was tested for whether they could inhibit IL-1β-induced PGE2 production (Fig. 2), cell viability was not significantly affected by tre atment with the indicated concentration and the medicinal extracts were shown to have the inhibitory effects against the synthesis of PGE_2, indicating that the T.Y.J.-J.H.G. 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. However, their effects were not stringent to protect the synthesis of PGE 2. Pretreatment of the T.Y.J.-J.H.G. extracts fo r 1 h, which by itself had little effect on cell survival, did not enhance the synthesis of P GE_2. nor significantly reduced the synthesis of PGE_2 by pretreatment (Fig. 2).

Next, the T.Y.J.-J.H.G. extracts were tested for whether they could protect against IL-1β (1 ng/ml)-induced plasminogen dependent fibrinolysis in the mouse calvarial cells (Fig. 3). Cell viability was not significantly affected by treatment with the indicated concentration of the extracts. Also, the T.Y.J.-J.H.G. extracts were shown to have the protective effects against plasminogen dependent fibrinolysis induced by the bone resorption agents of IL-1β. Pretreatment of the T.Y.J.-J.H.G. extracts for 1 h, which by itself had little effect on cell survival, did not enhance the plasminogen dependent fibrinolysis. Interestingly, T.Y.J.-J.H.G. extracts showed the inhibiting effects against IL-1β-stimulated bone resorption in the mouse calvarial bone cells having both

of the osteoblast and osteoclast cells. When we also examined the effect of the pretreatm ent with the T.Y.J.-J.H.G. extracts. the extra cts strongly reduced the bone resorption. The absolutely same result was also observed in case of calcitonin treatment (Table 1). Thus, these results suggested that the T.Y.J.-J.H. G. extracts inhibit the bone resorption and osteoporosis by inhibiting the osteoclast-mediated bone resorption reaction, which is usually induced by IL-1β.

V. References

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