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Potential synergies between matrix proteins and soluble factors on resorption and proteinase activities of rabbit bone cells

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Summary. Human growth hormone (GH) has recently been found to stimulate osteoclastic resorption, cysteine-proteinase and metalloproteinase activities (MMP-2 and MMP-9) *in vitro* via insulin-like growth factor-I (IGF-I) produced by stromal cells. The present study investigated the effects of two extracellular matrix components (vitronectin and type-I collagen) on hGH- and hIGF-1-stimulated osteoclastic resorption and proteinase activities in a rabbit bone cell model. After 4 days of rabbit bone cell culture on dentin slices with vitronectin coating, hGH and hIGF-1 stimulated bone resorption and hIGF-1 upmodulated cysteine-proteinase activities. MMP-2 expression (but not resorption, cathepsin or MMP-9 activities) was upmodulated by hGH and hIGF-1 on dentin slices coated with type I collagen as compared to those without coating. Then, vitronectin was synergistic with hIGF-1 in the regulation of cysteine-proteinase production whereas collagen showed synergy with hGH and hIGF-1 in the regulation of MMP-2 production. Anti-αvß3 totally abolished the effects of hGH and hIGF-1 on metalloproteinase release, but had no influence on cathepsin release. The results suggest that cysteine-proteinase modulation is not mediated by αvß3 integrin (strongly expressed on osteoclastic surface) whereas the resorption process and metalloproteinase modulation are clearly mediated by this integrin. Our finding about the collagen coating also suggests that hGH- and hIGF-1-stimulated MMP-2 activity are mediated, along with αvß3 integrin, by another adhesion molecule.

Key words: Osteoclast, Resorption, Proteinase, Vitronectin, Collagen

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Introduction

Regulation of the longitudinal growth of long bones is influenced by cytokines and growth hormones. Human growth hormone (hGH) acts directly on osteoblasts and exerts anabolic effects on bone formation. Although some studies have indicated that hGH plays an essential role in the maintenance of bone mass in adulthood and contributes to the bone remodeling process, its effects on osteoclasts are not well-documented (Isaksson et al., 1987). hIGF-1, a mediator of hGH action on osteoblastic cells, has been shown to stimulate osteoclastic resorption by means of osteoblastic cells (Hill et al., 1995).The stromal cell-mediated action of hGH on bone resorption may be due to an increase in hIGF-1 production (Guicheux et al., 1998)

Osteoclast adhesion to the bone matrix is necessary for bone resorption. This process involves not only growth factors, cytokines and chemoattractants but also a series of cell-matrix and cell-cell interactions leading to invasion, migration and anchorage of osteoclasts to the bone surface. Osteoclasts interact with the organic matrix on bone surface prior to the creation of the bone resorption compartment. Adhesive interactions of osteoclasts with the organic matrix (mediated by the integrin group of adhesion molecules) occur preferentially on type I collagen, the major protein of the bone matrix, and on RGD (Arg-Gly-Asp) peptide-containing proteins such as vitronectin and osteopontin. Specific receptors on osteoclast plasma membrane, such as αvß3 integrin (VNR), interact with RGD-containing proteins including vitronectin (Helfrich et al.,1992, 1996; Shankar et al., 1995). Several groups have shown that the vitronectin receptor is essential in the attachment of osteoclasts to bone surface and that the suppression of VNR interaction with its natural ligand in the bone matrix induces osteoclast detachment and inhibits bone resorption (Fishert et al., 1993). Although adhesion molecules are likely to be present in the sealing zone, the localization of VNR remains controversial. VNR have been reported within the sealing zone by certain authors

(Väänänen and Horton, 1995) and not observed by others (Lakkakorpi et al., 1991). Lakkakorpi et al. (1991) have demonstrated by immunofluorescence, immunoperoxidase staining and immunoelectron microscopy that VNR is localized only at the ruffled borders and basolateral membranes (expect in the sealing zone areas) in rat and chicken osteoclasts. In addition to VNR, two other integrins, $\alpha 2\beta 1$ and $\alpha v\beta 1$, have been identified on osteoclasts (Nesbitt et al., 1993). Helfrich et al. (1996) have shown that $\alpha 2\beta 1$ acts as a receptor for native type I collagen on osteoclasts, whereas osteoclast adhesion to denatured collagen involves $\alpha v\beta 3$ suggesting different levels of control via adhesion mechanisms during the resorption process. Thus, initial recognition of native collagen utilizes $\alpha 2\beta 1$ integrin. Later, during the resorption phase of osteoclast activity when collagen is digested through a low pH in the subosteoclastic compartment, osteoclast adhesion to digested collagen, and spreading on this substrat, may involved the shift in the protein-receptor interactions and the participation of $\alpha v\beta 3$. Subsequent to these cell matrix interactions, proteases may be involved in the access of osteoclasts to their future resorption sites and in degradation of the extracellular matrix (Sato et al., 1998).

The degradation of bone proteins is largely caused by cysteine-proteinases, which are optimally active at the low pH occurring beneath osteoclasts. Cathepsin B and L have been identified in osteoclasts and shown to be capable of depolymerizing and solubilizing collagen under the conditions likely to occur beneath the osteoclast in the subosteoclastic compartment during resorption (Delaisse et al., 1984). Cathepsin K is abundant and selectively expressed in osteoclasts and seems to play a specialized role in bone resorption (Drake et al., 1996). Matrix metalloproteases (MMPs) are zinc-dependent endopeptidases which catalyse the degradation of the extracellular matrix. These enzymes secreted in latent form are blocked by specific tissue inhibitors (TIMPs). The prevention of bone resorption by MMP inhibitors has provided direct evidence for the participation of MMPs in bone resorption (Hill et al., 1994). The gelatinases, a subgroup of MMPs (also known as type IV collagenases), degrade gelatin (denatured collagen) and type IV, V, VII, IX and X collagens. Some investigations have found that cysteine-proteinases are required for degradation of matrix proteins in resorption lacunae, but not for osteoclast activation. Conversely, collagenases, produced by the osteoblastic lineage, seem essential for the initiation of bone resorption by generating collagen fragments that activate osteoclasts to resorb bone (Holliday et al., 1997). Chambers et al. (1985) found that collagenase can predispose bone surfaces to resorption, and Lorenzo et al. (1992) showed that isolated rabbit osteoclasts express MMP-2 (known also as 72-kDa gelatinase/type IV collagenase transcripts/gelatinase A) and MMP-9 (known also as 92-kDa gelatinase/type IV collagenase transcripts/gelatinase B). Okada et al. (1995) suggested that MMP-9 degrades bone collagens in the subosteoclastic microenvironment, in conjunction with other proteinases such as MMP-1 and cysteine-proteinases. Cysteine-proteinases and MMPs can be modulated by hormones and cytokines. However, the effects of these cytokines on protease production by osteoclasts is not clearly understood.

In this context, a total rabbit bone cell model was used to analyze the effects of organic matrix proteins (type I collagen and vitronectin) on hGH and hIGF-1-stimulated osteoclastic resorption and to study proteinase activities.

Materials and methods

Reagents

Recombinant human GH (hGH, Umatrope, 16 IU) was a generous gift from the Lilly Company (Saint Cloud, France), and 1-α,25-dihydroxyvitamin D3 (VD3) was kindly provided by Drs. E.M. Gutknecht and P. Weber (Hoffmann-La Roche, Basel, Switzerland). Recombinant human IGF-I (hIGF-I) was supplied by R&D Systems (Abington, UK). Mouse monoclonal antibodies against $\alpha v\beta 3$ [(clone 23C6) immunogen used : osteoclasts from osteoclastomas] were obtained from Chemicon International, Inc. (Temecula, CA). Type I collagen (extracted in acid from porcine tendons) and human vitronectin were purchased respectively from Nitta Gelatin Co. (Osaka, Japan) and Becton Dickinson (Le Pont-de-Claix, France). α-Minimal essential medium (α-MEM) and fetal bovine serum (FBS) were supplied by Gibco (Eragny, France). Human parathyroid hormone (hPTH), Z-Phe-Arg-AMC substrate (7-N-benzyloxycarbonyl-Lphenylalanyl-Larginylamide-4-methylcoumarine), E64 (specific inhibitor of cysteine proteinases [L-trans-epoxysuccinyl-leucylamido (4-guanidino) butane] were obtained from Sigma. Recombinant MMP-9 and MMP-2 were purchased from R&D System.

Cell culture

Neonatal rabbit bone cells were isolated using a previously reported method (Yamada et al., 1997). Briefly, 13-day-old rabbits were killed by cervical dislocation, and their long bones isolated and freed from soft tissue, minced with scissors and placed in vortex in α-MEM medium. After sedimentation for 2 min, the cell suspension was harvested (repeated twice) and were washed in α-MEM. The cells were then seeded in α-MEM supplemented with 10% fetal bovine serum into 24-well plates at a density of 10^7 cells/well. The cultures were maintained in a humidified 95% air, 5% CO_2 atmosphere at 37°C for 4 days. Cells were incubated for the last 24 h of culture in serum-free α-MEM with or without hGH (1, 10, 50 ng/ml), hIGF-I (1, 10, 50 ng/ml) or hPTH (50 ng/ml), VD3 (10-8 M), an association of anti-$\alpha v\beta 3$ (10 μg/ml) with hIGF-I (50 ng/ml), or an

association of anti-αvß3 (10 mg/ml) with hGH (50 ng/ml).

Pit resorption assay

The resorption activity of osteoclasts from the total rabbit bone cell preparation was estimated by pit formation on sperm whale dentin slices coated or not with vitronectin or type I collagen, washed three times with phosphate-buffered saline solution (PBS), and placed in 24-well plates. Cells were then plated for the last 24 h of culture in serum-free α-MEM, with or without the different factors, at a density of 10^7 cells per well containing dentin slices. Dentin slices were sonicated for 2 min in distilled water and gold palladium-coated for scanning electron microscopic studies (SEM) on a semiautomatic image analyzer (Q500 Quantimeter, Leica, Cambridge, UK). The resorption activity of cells was analyzed in terms of the percentage of dentin surface area resorbed.

Collagen and vitronectin coating

All experiments were performed on dentin slices with or without a coating of human vitronectin and type I collagen extracted in acid from porcine tendons. The collagen solution (0.3 % Cellmatrix I-A) was mixed ice-cold with x 10 concentrated α-MEM and 0.2M HEPES/NaOH (pH 7.4) containing 2.2% $NaCO_3$ at a ratio of 8:1:1. One milliliter of this mixture was poured into each well and incubated at 37 °C for 10 min according to the manufacturer. The vitronectin solution was prepared in PBS at 10 mg/ml, and 100 ml were incubated on a dentin slice for 1 h at 37 °C according to the manufacturer.

Cathepsin activity measurements and determination of total protein concentrations

Cathepsin K, B and L activities were determined by the action of a non-specific fluorogenic substrate, Z-Phe-Arg-AMC, as previously reported (Rousselle et al., 2000). Cathepsin activities were determined in supernatants. Briefly, 50 μl of cell culture supernatants was incubated in 140 μl of 100mM sodium acetate buffer, pH 5.5 containing 1 mM EDTA and 0.1 mM freshly prepared DTT at 37 °C. The reaction was started by addition of 100 ml substrate (5 mM assay concentration) and stopped by addition of 100 ml of 100 mM sodium iodoacetate in 50 mM Tris/HCl buffer, pH 8.0. For fluorescence, the excitation and emission wavelengths were respectively 365 and 465 nm. Specific cathepsin activities were expressed as nmol of hydrolyzed substrate.h^{-1}.mg $protein^{-1}$.

Measurement of matrix metalloproteinase activity

Matrix metalloproteinase activity was determined by zymography as previously described (Rousselle et al., 2000). MMP activities were detected in cell culture supernatants. Electrophoresis gel was composed of 10% polyacrylamide gel containing 0.1% gelatin. A standard enzyme preparation containing both active MMP-2 and MMP-9 was used as reference of molecular weights. Proteinase activity was apparent as clear (unstained) regions. Stained polyacrylamide gels were observed with a video camera, allowing transfer to software for image processing (Q500 Quantimeter) and isolation of migration bands. Gelatinase activity was expressed as a percentage (shades of grey compared to the control).

Statistical analysis

All experiments were done three times in triplicate. The mean ± SD was calculated for all conditions and compared by ANOVA. Differences relative to a probability of two-tailed $p<0.05$ were considered significant.

Results

Vitronectin but not collagen enhances the resorption capability of rabbit bone cells

As revealed by cytological analysis, osteoclasts, with resorption capability, were differentiated from total rabbit bone cells (Rousselle et al., 2000; data not shown). VD3, hPTH, hGH and hIGF-1 enhanced significantly the area resorbed compared to the control without soluble factor (Fig. 1) (Guicheux et al., 1998). No modulation was observed in presence of type I collagen coating and VD3, hPTH, hGH and hIGF-1 had the same stimulator effect on area resorbed.

Vitronectin coating of dentin slices upmodulated the resorption capability of rabbit bone cells. The total area resorbed was markedly increased (6-fold) in the presence of vitronectin. To determine the potential synergy between vitronectin and the soluble factors studied (VD3, hPTH, hGH or hIGF-I), similar experiments were conducted using all possible combinations. Figure 1 shows that vitronectin, but not collagen, was synergistic with these four factors. Thus, the effects of 10^{-8}M VD3, 50 ng/ml hPTH, 50 ng/ml hGH and 50 ng/ml hIGF-I were increased respectively about 3.8-, 2.6-, 4- and 4.6-fold more in the presence of vitronectin than without matrix protein. Moreover, antibodies against αvß3 integrin reduced the basal resorption activity of total rabbit bone cells significantly (about 53%). These antibodies significantly inhibited the resorption activity induced by 50 ng/ml hPTH, 50 ng/ml hGH and 50 ng/ml hIGF-I (about 86%, 52% and 85% respectively), as compared to basal activity. This inhibition was specific since a mouse isotype antibody (IgG) did not induce the same effect (data not shown).

Vitronectin was synergistic with hIGF-1 in regulating cathepsin production by total rabbit bone cells

VD3 (10^{-8} M) (data not shown), hGH and hIGF-I (50 ng/ml) increased the production of cathepsin

(inhibition by E64) by total rabbit bone cells whereas hPTH (50 ng/ml) had no effect (Fig. 2) (Rousselle et al., 2000). No modulation of cathepsin activities was observed with type I collagen coating of dentin slices and the different factors had the same effect on the production of cathepsins as without matrix protein. Thus, 50 ng/ml hGH and 50 ng/ml hIGF-I upmodulated the cathepsin activity of rabbit bone cells about 1.8-fold as compared to the control without soluble factor (Fig. 2).

Vitronectin coating of dentin slices did not modify the basal production of cathepsins by rabbit bone cells. The effect of hGH on cathepsin activity was not influenced by vitronectin coating, whereas the production of cathepsins induced by 50 ng/ml hPTH and 50 ng/ml hIGF-I was respectively 5- and 2-fold higher in the presence of vitronectin than without matrix protein (Fig. 2). These results indicate that hIGF-I and hPTH were synergistic with vitronectin in regulating cysteine-proteinase production. Moreover, no inhibition of cysteine-proteinase activity was detected in the presence of anti-αvß3 antibodies in any of the conditions tested (data not shown).

Collagen was synergistic with hGH and hIGF-1 only in regulating MMP-2 production by total rabbit bone cells

Two bands, inhibited by EDTA, of about 92

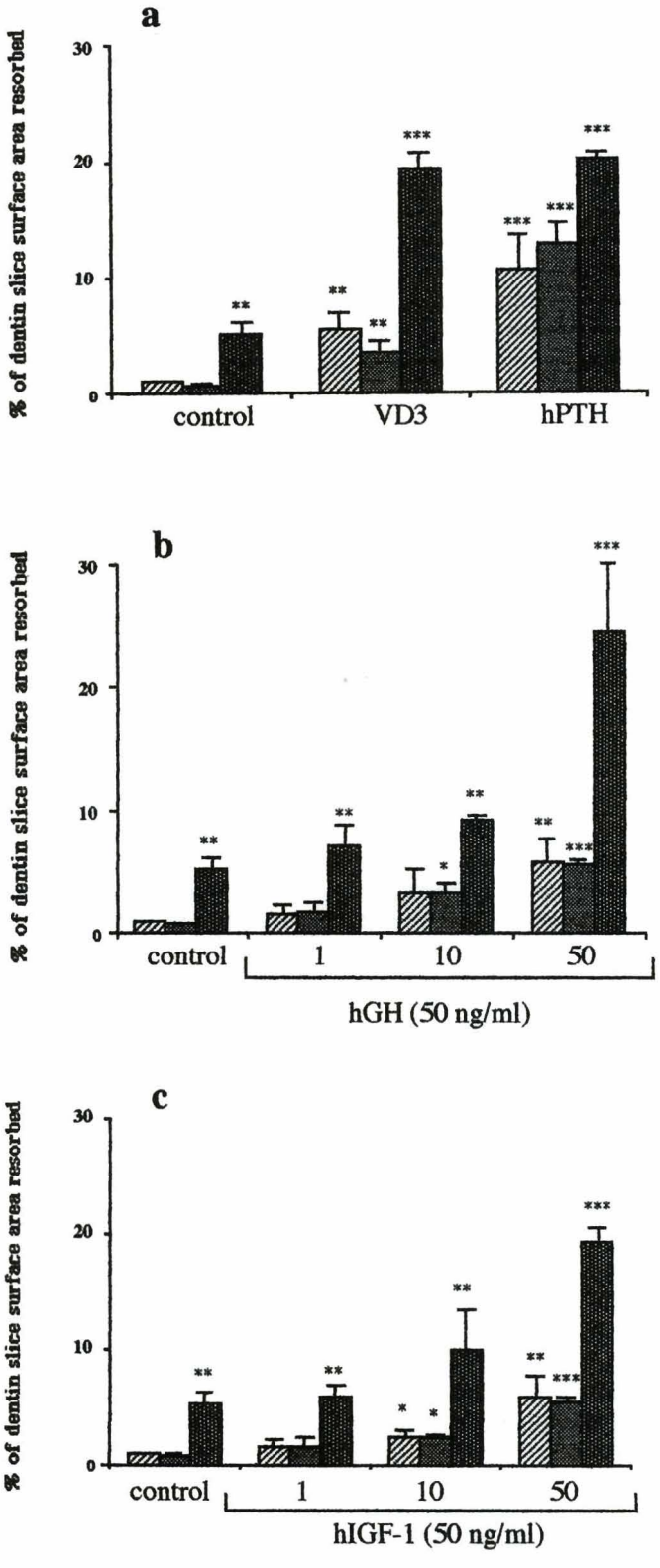

Fig. 1. Effects of bone matrix proteins on the resorption activity of osteoclasts from unfractionated rabbit bone cell culture. Total rabbit bone cells were cultured for 4 days on dentin slices without (hatched histograms) or with type I collagen (grey histograms) or vitronectin coating (dark histograms) in the absence or presence for the last 24 h of culture of hPTH (50 ng/ml) **(a)**, VD3 (10^{-8}M) **(a)**, hGH (1, 10, 50 ng/ml) **(b)**, hIGF-I (1, 10, 50 ng/ml) **(c)** or vehicle (control). Results are the mean ± SD of three separate experiments performed in triplicate. *:p<0.05; **:p<0.01; ***:p<0.001 compared to the control.

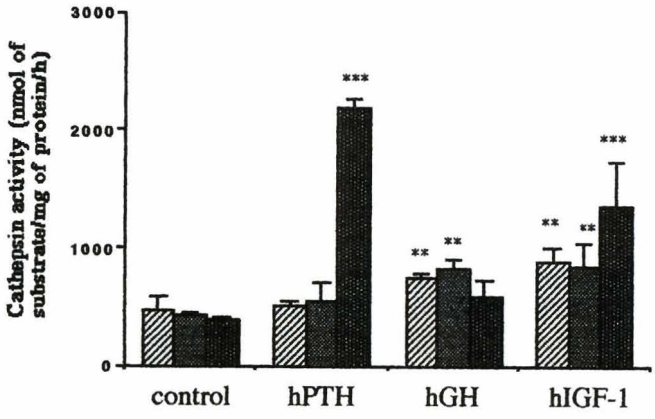

Fig. 2. Effects of bone matrix proteins on cysteine-proteinase activities released by unfractionated rabbit bone cell culture. Total rabbit bone cells were cultured for 4 days on dentin slices without (hatched histograms) or with type I collagen (grey histograms) or vitronectin coating (dark histograms) in the absence or presence for the last 24 h of culture of hPTH (50 ng/ml), hGH (50 ng/ml), hIGF-I (50 ng/ml) or vehicle (control). Results are the mean ± SD of three separate experiments performed in quintuplicate. *:p<0.05; **:p<0.01; ***:p<0.001 compared to the control.

(corresponding to gelatinase B, MMP-9 or type IV collagenase in its latent form) and 62 kDa (known as gelatinase A or MMP-2 in its active form) were observed as previously described with this rabbit bone cell model (Fig. 3) (Rousselle et al., 2000). The accurate molecular weights were determined using molecular weight markers and recombinant MMP-2 and MMP-9. An additional band at 184 kDa was observed in all culture conditions used. The intensity of the last band, was not modulated by addition of hPTH, hGH and hIGF-1 in the culture medium. hPTH, hGH and hIGF-1 (50 ng/ml) upmodulated MMP-2 and MMP-9 activities of rabbit bone cells (Fig. 4). No modulation of metalloproteinase activity was observed in the presence of type I collagen coating as compared to dentin slices without coating. Type I collagen coating did not influence the effect of hGH and hIGF-1 on MMP-9 activity (Fig. 4a), whereas in the presence of collagen, hGH and hIGF-1 increased MMP-2 activity respectively about 3.1-fold and 3.6-fold as compared to the control without collagen (Fig. 4b). These results indicate that hGH and hIGF-1 were synergistic with type I collagen in regulating MMP-2 production.

No modulation of metalloproteinase activities was observed in the presence of vitronectin coating (Fig. 3) compared to the control without coating (Fig. 4, Rousselle et al., 2000) and no synergy occurred between vitronectin and hPTH, hGH or hIGF-1 compared to each condition alone. Anti-αvß3 integrin antibodies significantly strongly inhibited the effects of 50 ng/ml hPTH (data not shown), 50 ng/ml hGH and 50 ng/ml hIGF-I on MMP-2 and MMP-9 production (Fig. 3). This inhibition was specific since a mouse isotype antibody (IgG) did not induce the same effect (data not shown).

Discussion

This study used a total bone cell model to analyze the effects of organic matrix proteins (type I collagen and vitronectin) on hGH and hIGF-I-stimulated resorption and to investigate proteinase activities. This work showed that vitronectin was synergistic with hGH and hIGF-1 in the regulation of bone resorption and with hIGF-1 in the regulation of cysteine-proteinase production. Moreover, collagen only modulated MMP-2 production. The effects of hGH and hIGF-1 on metalloproteinase release were totally abolished when anti-αvß3 was added to the culture medium, but these antibodies had no influence on cathepsin release. This study confirms our recent report that hGH and hIGF-1 stimulates osteoclastic bone resorption *in vitro* (Guicheux et al., 1998) and shows that cysteine-proteinases, MMP-9 (latent form) and MMP-2 (active form) were markedly induced by hIGF-1 and hGH (Rousselle et al., 2000). Indeed, in our previous report, we demonstrated that hGH stimulates osteoclastic resorption and proteinase activities via hIGF-1 produced by stromal cells. Our present study was extended to include the role of bone matrix proteins on these mechanisms and demonstrated a mediation, along with hIGF-1, of bone resorption and proteinase activities by adhesion molecules.

Many cell types [mainly osteoblastic cells (stromal cells)] but also cytokines, growth factors and other molecules are highly involved in the control of these mechanisms (Heymann et al., 1998). Adhesion

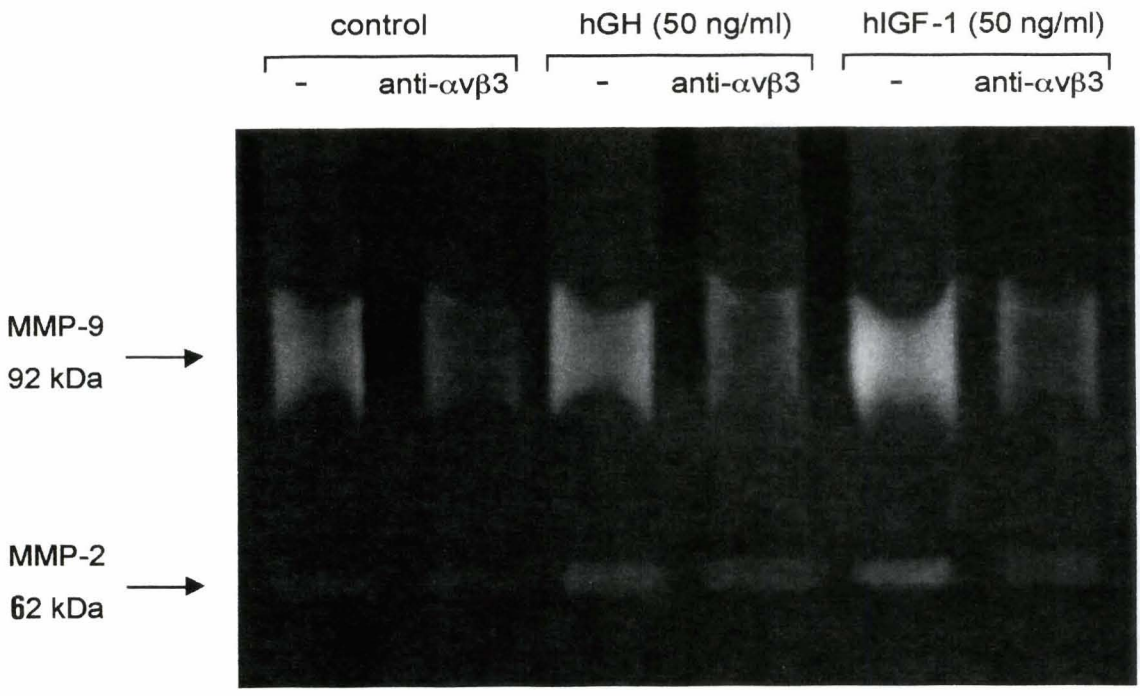

Fig. 3. Metalloproteinase activities released by unfractionated rabbit bone cells. Total rabbit bone cells were cultured on dentin slices coated with vitronectin for the last 24 h with or without hGH (50 ng/ml) or hIGF-I (50 ng/ml) in the absence or presence for the last 24 h of culture of anti-αvß3 integrin (10 μg/ml).

molecules (mainly integrins) are also implicated in processes of osteoclast attachment to organic substrates (Suda et al., 1995). Osteoclasts express an αvß3 integrin receptor, which recognizes an RGD (arg-gly-asp) sequence contained in vitronectin, fibronectin and osteopontin, and an α2ß1 integrin receptor which recognizes type I collagen (Nesbitt et al., 1993; Helfrich et al., 1996). Thus, osteoclast adhesion to the bone surface plays a key role during bone resorption. Väänänen and Horton (1995) found specific interactions (cell-extracellular matrix) in the clear zone of osteoclasts which could induce the activation of these cells.

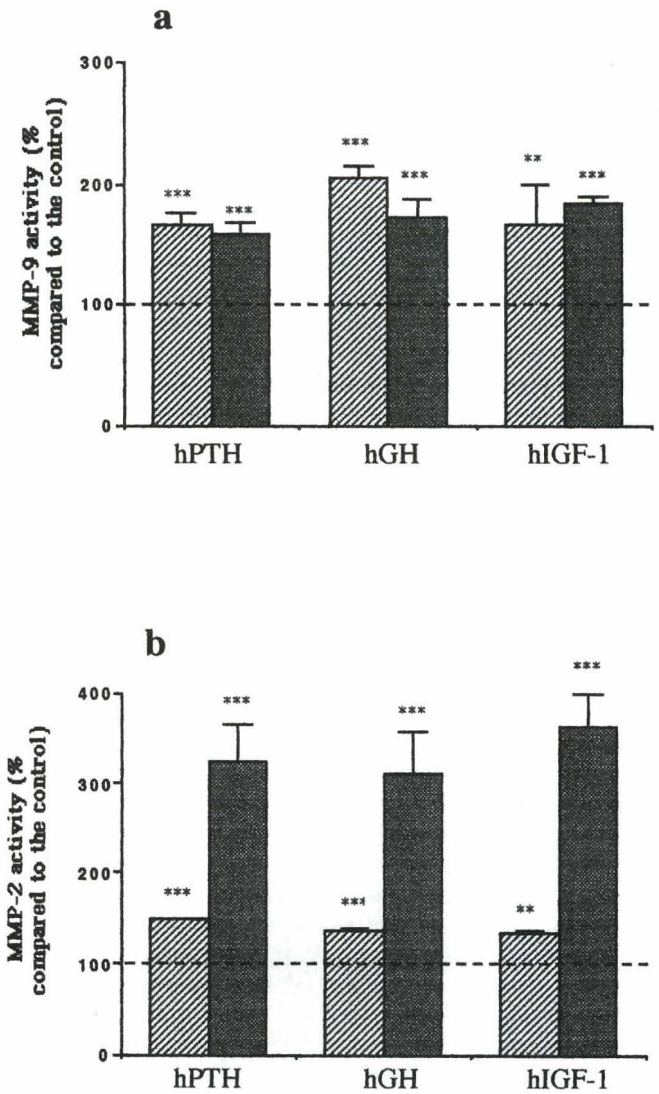

Fig. 4. Effect of type I collagen on metalloproteinase activities released by unfractionated rabbit bone cells. Total rabbit bone cells were cultured for 4 days on dentin slices without (hatched histograms) or with type I collagen (grey histograms) coating in the absence or presence for the last 24 h of culture of hPTH (50 ng/ml), hGH (50 ng/ml) or hIGF-1 (50 ng/ml). MMP-9 **(a)** and MMP-2 **(b)** activities are expressed as percentages (shades of grey compared to the control). Results are the mean ± SD of three separate cultures tested. **: p<0.01, ***: p<0.001 compared to the control.

Although the cellular mechanisms involved in the formation of the clear zone are poorly understood, this step is essential in the process of bone resorption since the clear zone confines acidic solubilization of bone mineral to the area directly beneath the ruffled border. In the present work, osteoclastic resorption was not modulated by bone cell interactions with type I collagen, but was upmodulated by vitronectin coating on dentin slices. Bone cell interactions with vitronectin influenced the resorption capability of rabbit bone cells and were synergistic with hGH and hIGF-1. These results suggest that binding to vitronectin was more efficient than to type I collagen for the resorption capability of rabbit bone cells. The modulation of integrin expression in the presence of various growth factors could account for these events (Ruoslahti et al., 1994). These data are consistent with those of Horton et al. (1985), who showed that antibodies against vitronectin receptor inhibited osteoclastic resorption by rat osteoclasts and our present study confirms that the addition of anti-αvß3 to the culture medium totally inhibited the resorption activity induced or not by hGH and hIGF-1. It has been reported that echistatin, a naturally-occurring protein containing RGD-sequences, blocks osteoclast attachment to bone and inhibits bone resorption *in vitro* (Fisher et al., 1993). Recently, Villanova et al. (1999) have also shown that αv antisense oligodeoxynucleotide (ODN) caused a dose-dependent, substrate-specific reduction of osteoclast adhesion. ODN also induced osteoclast retraction, apoptosis and inhibited bone resorption. These results strengthen the fact that αvß3 integrin-induced adhesion of osteoclasts to bone surface plays an important role in the osteoclast resorption function.

Although extracellular matrix components markedly influenced proteinase activities, they seemed to act differently with cysteine-proteinase and metallo-proteinase. Vitronectin showed no synergy with hGH and hIGF-1 in the regulation of MMP production. Only hIGF-1 was synergistic with vitronectin in the regulation of cysteine-proteinase production and vitronectin prevented the hGH effect which in turn could stimulate proteinase activities via IGF-1 production (Rousselle et al., 2000). The effects of hGH and hIGF-I on vitronectin-coated dentin slices differed from those on dentin slices coated with type-I collagen. In the latter case, hGH and hIGF-I did not influence the cysteine-proteinases released by total rabbit bone cells and only showed synergy with collagen in the regulation of MMP-2 production. These results are in agreement with those recently reported by Sato et al. (1998), who found that cysteine-proteinases, as compared to MMPs, played only a minor role during osteoclast invasion by type-I collagen. In their model, MMP-2 activity seemed to play a key role in the migration of osteoclasts and in access to their future resorption. As cysteine-proteinase activities may be necessary for collagenolytic activity near the osteoclast resorption zone (Foged et al., 1996), migration and resorption functions could be mediated by various proteolytic activities.

Addition of anti-αvß3 to the culture medium totally abolished GH- and IGF-I-stimulated bone resorption and their effects on matrix metalloproteinase release, but had no influence on cathepsin release. The αvß3 antibody used (clone 23C6) was prepared from osteoclasts of giant cell tumors. Immunocytochemical studies have shown that this antibody preferentially binds osteoclasts strongly expressing these antigens (Horton et al., 1985). This suggests that cysteine-proteinase modulation was not mediated by αvß3 integrin whereas the resorption process and the metalloproteinase modulation were clearly mediated by αvß3. Osteoclast adhesion to the extracellular matrix by αvß3 integrin seems to be essential for the hGH- and hIGF-1-stimulated resorption and metalloproteinase activity but not for the cysteine-proteinase activity. These data are consistent with those of Brooks et al. (1996) who found that αvß3 integrin on the surface of tumor cells binds to MMP-2. Moreover, the deletion of αv integrin led to a decreased ability to spread on vitronectin and suppressed MMP-2 expression (Koistinen et al., 1999). However, in presence of collagen, hGH and hIGF-1 only stimulated MMP-2 expression. These findings also suggest that hGH- and hIGF-1-stimulated MMP-2 activity were mediated, along with αvß3, by another adhesion molecule.

In a recent study, experiments realized on purified rabbit osteoclasts have revealed that MMP expression by osteoclasts was directly regulated by hIGF-1 while hIGF-1 had no effect on cysteine-proteinase activities released by osteoclasts (Rousselle et al., 2000). The present paper found that αvß3 abolished hGH- and hIGF-1-stimulated bone resorption and their effect on MMP release but had no influence on cathepsin release. Moreover, αvß3 integrin is strongly expressed on osteoclastic surface. These support the previous hypothesis indicating that non-osteoclastic bone cells may have been the source of cathepsin production in our culture model while MMPs were mainly expressed by osteoclastic cells (MMP-2 and MMP-9 basal production involving non-osteoclastic bone cells).

In summury, the synergy of vitronectin, hGH and hIGF-1 increased the resorption activity of total rabbit bone cells. This work showed that vitronectin was also synergistic with hIGF-1 in the regulation of cysteine-proteinase production and type I collagen showed only synergy with hGH and hIGF-1 in the regulation of MMP-2 expression. This study suggests that the resorption process and the metalloproteinase modulation are mediated by αvß3 whereas cysteine-proteinases seem to have another regulation pathway. In presence of collagen, hGH- and hIGF-1-stimulated MMP-2 activity is also mediated by another adhesion molecule.

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