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In vitro effects of S12911-2 on osteoclast function and bone marrow macrophage differentiation

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Abstract

In order to determine whether 5-[bis(carboxymethyl) amino]-2-carboxy4-cyano-3-thiopheneacetic acid distrontium salt (S12911-2) inhibits bone resorption by acting on the differentiation and/or function of osteoclasts, its effects were assessed on the 1,25-dihydroxyvitamin D_3-induced expression of carbonic anhydrase II and vitronectin receptor in chicken bone marrow cells, and on the resorbing activity of authentic rat osteoclasts cultured on bone slices. S12911-2 dose-dependently inhibited, after a 6-day exposure, the expression of carbonic anhydrase II and vitronectin receptor in stimulated osteoclasts (46% and 40%, respectively, at 10^{-3} M Sr^{2+}, $P<0.05$). A pre-incubation of bone slices with S12911-2 induced a dose-dependent inhibition of bone resorbing activity from 32% at 10^{-4} M Sr^{2+} to 66% at 10^{-3} M Sr^{2+} ($P<0.05$ in each case). A continuous incubation (10^{-3} M Sr^{2+}) induced a greater inhibition of bone resorbing activity (73%, $P<0.05$). The inhibition of bone resorption obtained specifically with S12911-2 is related to an inhibition of the differentiation and resorbing activity of the osteoclasts.

Keywords: S12911-2; Osteoclast; Bone resorption; Differentiation

1. Introduction

Strontium (Sr^{2+}) is a bone-seeking trace element which is incorporated in bone in a similar way to that of Ca^{2+} (Brandi, 1993; Dahl et al., 2001; Marie, 1996). Sr^{2+} stimulates bone formation in vitro (Canalis et al., 1996) and in vivo in normal rats leading to improvement in bone strength (Ammann et al., 2001; Marie et al., 2001). Previous studies, performed in vivo, on the effects of 5-[bis(carboxymethyl) amino]-2-carboxy4-cyano-3-thiopheneacetic acid distrontium salt (S12911-2) (Fig. 1), made up of two atoms of stable Sr^{2+} and an organic moiety (ranelic acid), suggested that this compound prevents trabecular bone loss after ovariectomy in rats with a profound and significant decrease in histomorphometric parameters of bone resorption (Marie et al., 1993). Furthermore, S12911-2 was also shown to reduce excessive bone resorption without reducing bone formation in rats with osteopenia induced by immobilization (Marie et al., 1995). Since this decrease in bone resorption was associated with a decrease in the number of osteoclasts lining the bone surfaces, S12911-2 may interfere with the differentiation and possibly with the activity of these cells.

The multinucleated osteoclasts are derived from progenitor cells of hematopoietic origin and recruited from bone marrow. After differentiation of these progenitor cells in mononuclear preosteoclasts, they fuse to form multinucleated osteoclasts (Suda et al., 1992).

1,25-Dihydroxyvitamin D_3 is an osteotropic hormone that stimulates bone resorption. Cultures of chicken marrow macrophages under osteotropic hormonal regulation constitute an in vitro experimental model for the evaluation of the effects of products on the recruitment and the differentiation of bone marrow cells.

The primary purpose of this study was to determine whether S12911-2, a potential new antiosteoporotic drug, affects the differentiation of osteoclasts using a culture system where chicken marrow macrophages are induced to differentiate into osteoclasts under the influence of 1,25-dihydroxyvitamin D_3 (Billecocq et al., 1990; Davies et al., 1989; Lomri and Baron, 1992; Prallet et al., 1992). These

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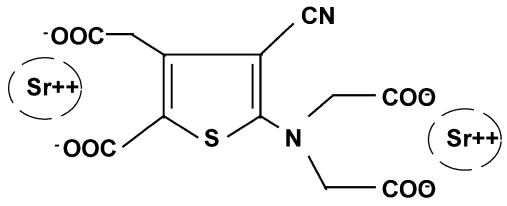

Fig. 1. Chemical structure of S12911-2.

effects were compared with those obtained with the calcium (Ca^{2+}) or sodium (Na^{+}) salts of ranelic acid. This could be assessed by the measurement of osteoclast differentiation markers, such as carbonic anhydrase II, and the vitronectin receptor (Baron et al., 1985; Billecocq et al., 1990; Clark and Brugge, 1995; Davies et al., 1989; Lomri and Baron, 1992; Nijweide et al., 1986; Prallet et al., 1992).

The prevention of trabecular bone loss by S12911-2 could result from an action on the function of osteoclasts (Marie et al., 2001). The second set of studies presented here analyzes the effects of S12911-2 at the cellular level, using freshly isolated authentic rat osteoclasts enriched by adherence to acellular bone matrix formed by bovine bone slices, and determines the number and size of resorption pits formed over a 24-h culture period as a function of the number of adherent osteoclasts. The action of S12911-2 and S12911-0 on the attachment and viability of osteoclasts after a 24-h period of culture on bone slices is deduced after a specific staining of an isoenzyme of the acid phosphatase, the tartrate-resistant acid phosphatase (TRAP), which is expressed at a high level in osteoclasts.

Ca^{2+} ions could exert a regulatory role in bone remodeling. High concentrations (>3 mM) blunt the action of 1,25-dihydroxyvitamin D_3 on the differentiation of cultured human monocytes (Sugimoto et al., 1993). Furthermore, higher concentrations (5 mM) indirectly stimulate the differentiation of osteoclasts via the presence of osteoblasts (Kaji et al., 1996). Therefore, the study includes comparisons between the effects of S12911-2 (5-[bis(carboxymethyl) amino]-2-carboxy4-cyano-3-thiopheneacetic acid distrontium salt, Fig. 1) and two other salts of ranelic acid, S12911-0 (Ca^{2+} salt) and S12911-5 (Na^{+} salt).

2. Materials and methods

2.1. Osteoclast formation

Bone marrow cells were isolated in phosphate-buffered saline (PBS) +0.1% bovine serum albumin from the tibias and femurs of white leghorn chickens (SPAFAS, Norwich, CT) which had been fed a Ca^{2+}-deficient diet (Purina, Ralston, IN) for 1–2 weeks after hatching.

Freshly isolated cells were passed through a 10-μm nylon filter (Small Parts, Boca Raton, FL) to remove mature multinucleated osteoclasts. Mononuclear cells were then fractionated on Ficoll-Paque (Pharmacia, Piscataway, NJ) by sedimentation to the Ficoll-Saline interface. Isolated cells were plated at 5×10^6 cells/ml in α Minimum Essential Medium (αMEM) (Sigma, St Louis, MO) containing 2% heat-inactivated chick serum (Sigma), 8% heat-inactivated fetal calf serum (Sigma), 50 IU/ml penicillin and 50 μg/ml streptomycin. To deplete these preparations of the most mature stromal and myelomonocytic cells, nonadherent cells were recovered after 16 h and replated at the density of 10^{-5} cells/0.1 ml/well in 96-well plates for up to 6 days. Osteoclast formation and differentiation were assessed by enzyme-linked immunosorbent assay (ELISA) of two markers: vitronectin receptor and carbonic anhydrase II (see below).

Control cells were grown in the absence of $1,25(OH)_2D_3$ while experimental groups were grown in presence of 10^{-11}–10^{-7} M $1,25(OH)_2D_3$ (Provided by Dr. Milan Uskokovic, Hoffman-Laroche, Nutley, NJ). S12911-2 was tested at 0.05, 0.1, 0.5 and 1 mM (expressed as actual Sr^{2+} concentration in the culture medium). S12911-0 and S12911-5 were tested to obtain a concentration in the culture medium of 1 mM Ca^{2+} and 2 mM ranelic acid, respectively, in order to match the concentrations obtained with S12911-2.

2.2. ELISA of osteoclast differentiation markers

After 3 or 6 days of culture in 96-well plates, cells were washed with PBS, fixed in 3.7% formaldehyde, treated with 0.01% H_2O_2 to deplete endogenous peroxidase activity, and pre-incubated for 1 h with a 3% bovine serum albumin solution in PBS. Cells were then incubated for 2 h with one of several primary antibodies diluted in PBS+3% bovine serum albumin. Saponin (0.05%) was added to all solutions in order to permeabilize the cells. After extensive washing in PBS+0.1% bovine serum albumin, cells were incubated with peroxidase-labeled goat anti-mouse immunoglobulin antibody (Cappel, Pennsylvania, PA) diluted 1:1000 in PBS+0.3% bovine serum albumin, washed in PBS–bovine serum albumin, then reacted with 2 mg/ml *O*-phenylenediamine (Sigma) and 0.03% H_2O_2. The reaction was halted after 15 min by the addition of H_2SO_4. Absorbance was measured in duplicate on an ELISA reading system (EAR 400, Labinstruments) at 492 nm versus 620 nm. Three monoclonal antibodies (Mab) were used: mouse anti-chicken carbonic anhydrase II as culture supernatant (7C6-1, gift of Dr. Paul Linser, diluted 1:20), anti-human vitronectin receptor (McAb23C6, diluted 1:100) (Davies et al., 1989) and anti-LEP 100 (lysosomal membrane protein of 100 kDa) as ascites fluid (gift of Dr. Douglas Fambrough, diluted 1:1000). The background absorbance, measured in wells without primary antibodies or with an irrelevant primary antibody (anti-dinitrophenyl, gift of Dr. Richard Anderson, diluted 1:200), was subtracted from the absorbance measured after incubation with the specific antibodies. In three experiments, the protein content of each well was

also measured by the Lowry assay after lysis in 0.1% sodium dodecyl sulfate (SDS).

Results were normalized with reference to the LEP 100 expression (Baron et al., 1985).

2.3. Preparation of devitalized bone slices

Bone slices (4 × 4 × 0.2 mm) were prepared according to the methods of Boyde et al. (1984) and Arnett and Dempster (1986). Transverse slices of dense cortical bone were cut from the diaphysis of adult bovine femurs using a low-speed diamond saw. Slices were cleaned by ultrasonication three times for 10 min each time in distilled water and both sides were sterilized overnight under ultraviolet light. Scanning electron microscopy was performed on a number of uncultured bone slices in order to ensure that their surface was smooth and that no cavities were present.

Slices were pre-incubated for 24 h with the medium or with S12911-2, S12911-0 or S12911-5 solution. At the end of the pre-incubation period, the slices were washed and transferred to 96-well tissue culture plates.

2.4. Isolation and culture of rat osteoclasts

The isolation of rat osteoclasts was performed according to the procedures previously described (Ali et al., 1984; Boyde et al., 1984; Chambers and Magnus, 1982). Briefly, the femur, tibia and humerus were removed from 2–4-day-old Wistar rats, dissected free of adherent tissue, placed in αMEM (0.3 ml/rat) and cut into small fragments. The bone fragments were pipetted vigorously to release the osteoclasts, and then allowed to sediment for 30 s. The cell suspension was transferred into 96-well tissue culture plates containing the bone slices. After incubation in αMEM + 10% fetal calf serum at 37 °C for 40 min (100 ml/slice), the slices were vigorously washed twice with αMEM to remove nonadherent cells. The bone slices were then incubated in αMEM + 10% fetal calf serum for 24 h at 37 °C in a 95% humidified atmosphere with 5% CO_2, with the pH of the culture medium at the normal value of 7.4 or lowered to 6.8 in order to increase basal resorptive activity of the osteoclasts (Arnett and Dempster, 1986).

2.5. Identification of resorption pits

At the end of the culture period, the bone slices were removed from the culture dishes, immersed in water containing 1% of 1 M NaOH and ultrasonicated to remove cell debris. The bone slices were then stained with toluidine blue prepared at 1% in distilled water containing 1% Na^+ borate; this led to a characteristic ring-like staining of the edges of resorption pits and staining of the pit itself (Arnett and Dempster, 1986; Boyde et al., 1984). The number of resorption pits, also identified by epi-illumination, was then counted (Walsh et al., 1990).

2.6. Tartrate-resistant acid phosphatase (TRAP) staining

Staining for TRAP was performed according to Evans et al. (1979). The cells adherent to bone slices were fixed using 3% glutaraldehyde in 0.2 M Na^+ cacodylate buffer at 37 °C for 4 min. They were then incubated for 45 min at 37 °C in acetate buffer (0.1 M Na^+ acetate, pH 5) containing naphtol–ASTR–phosphate (Sigma) as substrate and hexazotized pararosaniline (Eastman Kodak, Rochester, NY) as a stain for the reaction product. This procedure was performed in the presence of 50 mM tartratic acid, a concentration which blocks most nonspecific reactions.

2.7. Quantification of bone resorption

The pit area was measured by digitized morphometry and, as previously reported for chicken osteoclast precursor cultures, three types of pits were observed and classified in categories A (single resorption pit of diameter roughly equivalent to a mononuclear cell <10 μm), B (single resorption pit of a larger diameter corresponding approximately to the size of mature multinucleated osteoclasts ≃20 μm) and C (composite pits formed by a variable number of individual lacunae usually of type B) (Prallet et al., 1992). Since the mean area of medium and large pits were 4- and 15-fold larger, respectively, than the mean area of a small pit, a Pit Area Index (PAI) was then calculated by multiplying the number of pits in category A × 1, the number of pits in category B × 4 and the number of pits in category C × 15 (PAI = A + 4B + 15C).

2.8. Statistical analysis

The data was tested statistically by performing a one-way (treatment) analysis of variance (ANOVA). Statistical significance was taken as $P < 0.05$. In case of significant difference, this was followed by inter-group comparisons using Fisher's Test.

3. Results

3.1. S12911-2 inhibits carbonic anhydrase II, vitronectin receptor expression in chicken bone marrow culture

1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) induced a 4.8-fold increase of the expression of carbonic anhydrase II (Fig. 2A). After 6 days of treatment, S12911-2 significantly and dose-dependently inhibited the $1,25(OH)_2D_3$-induced carbonic anhydrase II expression by 30%, 39% and 46% at 0.1, 0.5 and 1 mM, respectively ($P < 0.05$ in each case).

$1,25(OH)_2D_3$ induced an eightfold increase of the expression of the vitronectin receptor (Fig. 2B). After 6 days of treatment, S12911-2 significantly and dose-dependently

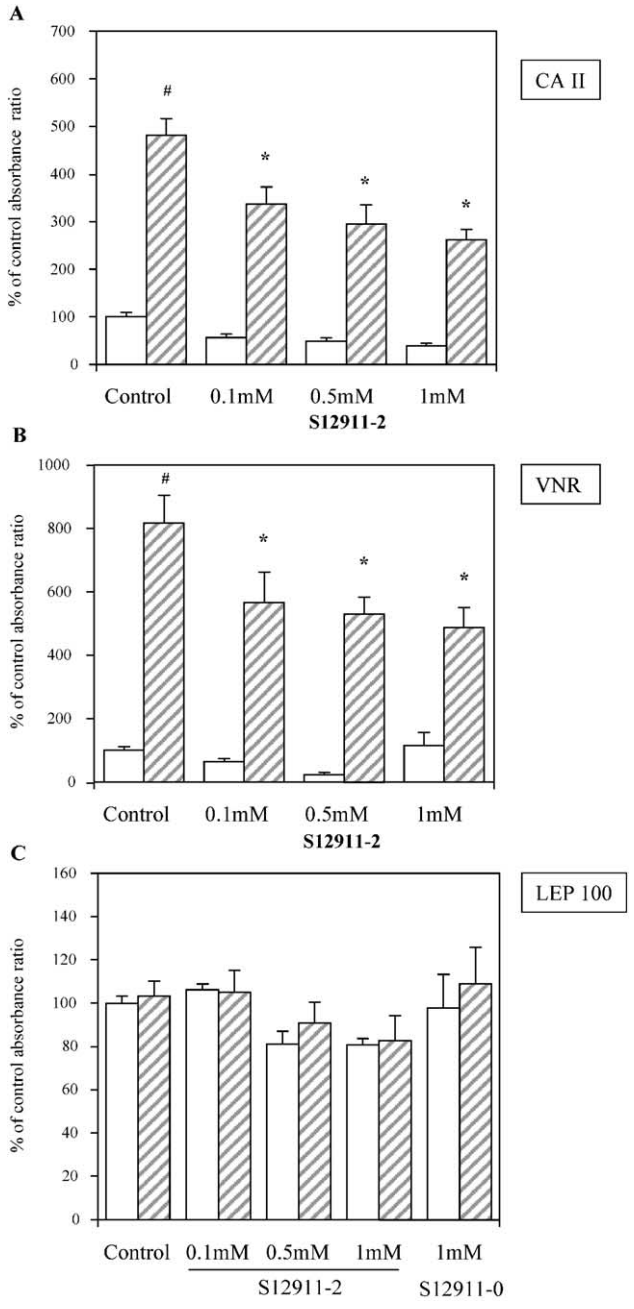

Fig. 2. Dose effects of S12911-2 on carbonic anhydrase II (A), vitronectin receptor (B), or LEP 100 (C) expression in chicken bone marrow culture for 6 days. □ = without 1,25-dihydroxyvitamin D3; ▨ = with 1,25-dihydroxyvitamin D3 *: $P<0.05$ versus control with 1,25-dihydroxyvitamin D3; #: $P<0.05$ versus control without 1,25-dihydroxyvitamin D3; $n=7$ to 23 per group.

inhibited the $1,25(OH)_2D_3$-induced vitronectin receptor expression by 30.7%, 35.1% and 40.6% at 0.1, 0.5 and 1 mM, respectively ($P<0.05$ in each case).

$1,25(OH)_2D_3$ had no effect on the expression of the lysosomal protein LEP 100 used here as control for cells (Fig. 2C). S12911-2, from 0.1 to 1 mM and S12911-0 (1 mM) did not affect the expression of LEP 100 in the presence or absence of $1,25(OH)_2D_3$ hereby indicating that the effects were selective on osteoclast markers.

Table 1
Dose effects of S12911-2 on the attachment of osteoclasts

Experimental condition	Attachment of osteoclasts on bone slice	
	pH 7.4 $TRAP^+$ cell number	pH 6.8 $TRAP^+$ cell number
Untreated control	19.25 ± 5.31	19.25 ± 5.63
S12911-2		
1 μM	25.40 ± 3.20	27.50 ± 4.29
0.01 mM	27.60 ± 2.58	27.80 ± 4.18
0.1 mM	27.80 ± 3.53	30.20 ± 3.44
1 mM	17.75 ± 3.42	22.25 ± 4.13

$n=4-5$. Mean ± S.E.M. One-way ANOVA (treatment) showed no significant effect for pH 7.4 or 6.8. Cells were stained for 45 min before counting.

3.2. Effects of S12911-2, S12911-0 and S12911-5 on carbonic anhydrase II and vitronectin receptor expression in chicken bone marrow culture

In this experiment, $1,25(OH)_2D_3$ induced a five- and eightfold increase of the expression of the carbonic anhydrase II and vitronectin receptor, respectively, as compared with the basal condition. After 6 days of incubation, the $1,25(OH)_2D_3$-induced carbonic anhydrase II expression was significantly inhibited by S12911-2 (1 mM), S12911-0 (1 mM) and S12911-5 (2 mM) by 46%, 31% and 35%, respectively. However, only S12911-2 at 1 mM significantly inhibited the $1,25(OH)_2D_3$-induced vitronectin receptor expression by 40%. On the contrary, S12911-0 (1 mM) significantly stimulated the 1,25-dihydroxyvitamin D_3-induced vitronectin receptor expression by 48%, and S12911-5 had no significant effect.

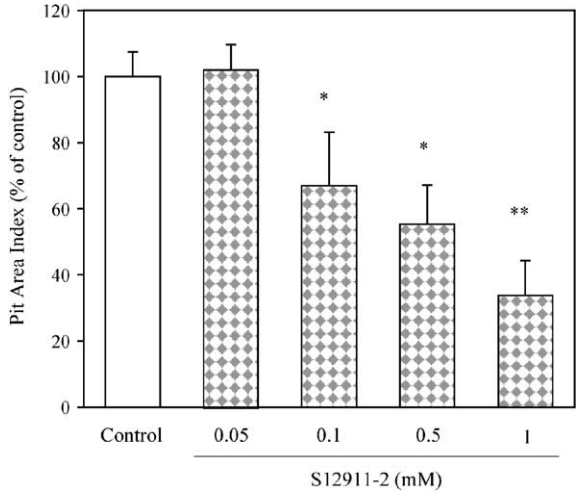

Fig. 3. Dose effects of pre-incubation with S12911-2 on bone resorption at pH 7.4. *: $P<0.05$ versus control; **: $P<0.01$ versus control.

3.3. S12911-2 does not affect the attachment of osteoclasts

At concentrations ranging from 1 μM to 1 mM, and at pH 6.8 or 7.4, S12911-2 did not have any significant effect on the attachment of osteoclasts on bone slices as assessed by the count of TRAP-positive multinucleated cells (Table 1). Whatever the experimental conditions (pretreatment of bone slices, treatment of cultured osteoclasts, or continuous treatment) S12911-2 at 1 mM did not affect the attachment of osteoclasts on bone slices. The pre-incubation of bone slices or continuous incubation of cultured osteoclasts with the S12911-0 at 1 mM did not affect the attachment of osteoclasts on bone slices (data not shown).

3.4. Pretreatment with S12911-2 inhibits osteoclasts activity

Pretreatment of bone slices with S12911-2 at pH 7.4 induced a significant and dose-dependent inhibition of the bone resorbing activity of untreated rat osteoclasts at concentrations equal to or greater than 0.1 mM (from 32% at 0.1 mM, $P<0.05$ to 66% at 1 mM, $P<0.01$) (Fig. 3).

3.5. Effects of pretreatment and continuous treatment with S12911-2, S12911-0 and S12911-5 on bone resorption

Pretreatment of bone slices with S12911-2 at 1 mM significantly inhibited, by 49%, the bone resorbing activity at pH 7.4 (Fig. 4). A weaker effect (33% of inhibition) was observed at pH 6.8 (data not shown). Pretreatment of bone slices with S12911-0 or S12911-5, at the same concentration (1 mM), did not affect the resorbing activity of osteoclasts at either pH level.

The continuous treatment of bone slices and cultured osteoclasts with S12911-2 at 1 mM significantly increased the inhibitory effects up to 73% at pH 7.4 ($P<0.05$) and up to 53% at pH 6.8 (data not shown). S12911-0 (1 mM) and S12911-5 (2 mM) in the same conditions did not exhibit any significant effects on bone resorption.

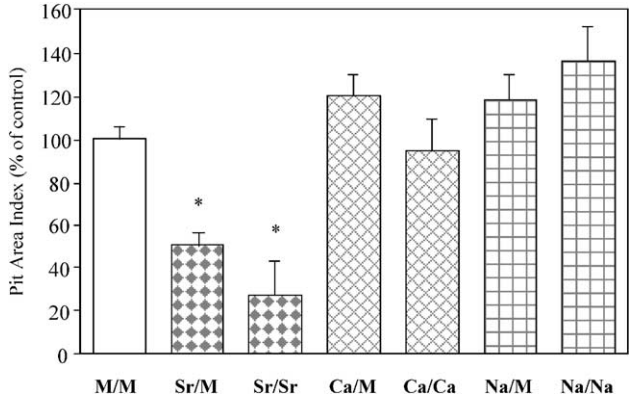

Fig. 4. Effects of pre-incubation and continuous incubation with different salts of the ranelic acid on bone resorption at pH 7.4. M/M = untreated control; Sr/M = pretreatment with S12911-2 (1 mM); Ca/M = pretreatment with S12911-0 (1 mM); Na/M = pretreatment with S12911-5 (2 mM); Sr/Sr = continuous treatment with S12911-2 (1 mM); Ca/Ca = continuous treatment with S12911-0 (1 mM); Na/Na = continuous treatment with S12911-5 (2 mM). *: $P<0.05$ versus control.

4. Discussion

In the present study, it has been shown that S12911-2 (1 mM Sr^{2+}) inhibits osteoclast differentiation (reduction of carbonic anhydrase II and vitronectin receptor expression by 46% and 40%, respectively) and osteoclast activity (reduction by 66%). However, there were no effects on the attachment of the osteoclast. Furthermore, pretreatment of bone slices with S12911-2 is sufficient to obtain a significant inhibition of the osteoclast resorbing activity. Carbonic anhydrase II is a key enzyme for bone resorption and is involved in the acidification process (Billecocq et al., 1990; Lomri and Baron, 1992), while the vitronectin receptor (integrin $a_v\beta_3$) is involved in the formation of podosomes, inducing, during the attachment process, a reorganization of the cell ultrastructure preliminary to the ruffled border formation (Baron et al., 1985; Davies et al., 1989; Juliano and Haskill, 1993; Lakkakorpi et al., 1989; Lakkakorpi and Väänänen, 1996; Prallet et al., 1992; Sastry and Horwitz, 1993; Väänänen and Horton, 1995). Furthermore, vitronectin receptor is implicated in the motility of the osteoclast and in the maintenance of the sealing zone (Nakamura et al., 1999). These markers characterize osteoclast differentiation. The 1,25-dihydroxyvitamin D_3 (active metabolite) may induce the expression of carbonic anhydrase II (Billecocq et al., 1990) by acting directly on myelomonocytic cells mostly in increasing the gene transcription of the carbonic anhydrase II gene (Lomri and Baron, 1992). The monoclonal antibody anti-vitronectin receptor used in these experiments is specific for the α_v subunit of the receptor (Davies et al., 1989). The results obtained with S12911-2 clearly differ from those obtained with S12911-0 or S12911-5. Only S12911-2 inhibited, in a dose-dependent manner, the two osteoclastic markers under the induction of 1,25-dihydroxyvitamin D_3, without acting on a noninducible marker (LEP 100) by 1,25-dihydroxyvitamin D_3. After 6 days of treatment, the inhibition of the inducible expression of carbonic anhydrase II is detected from 0.1 mM (-30%) and reached -46% at 1 mM Sr^{2+}. After the same time of culture, the inhibition of the inducible expression of the α_v subunit of vitronectin receptor is detected from 0.1 mM (30.7%) and reached 40.6% at 1 mM Sr^{2+}. The inhibition is present with the same magnitude for both differentiation markers. The action of S12911-0 or S12911-5 differs depending on the marker.

Considering the inhibition of the inducible expression of carbonic anhydrase II, the S12911-0 and S12911-5, like S12911-2, are active but to a lesser extent. In the case of the vitronectin receptor, after 3 days of treatment, S12911-0 does not significantly affect the inducible expression of the receptor (data not shown) but after a 6-day treatment, it

stimulates the inducible expression of the receptor (48%, $P<0.05$), while the S12911-5 has no effect. The blunt of the response to 1,25-dihydroxyvitamin D_3 observed on human monocytes by high concentrations of extracellular Ca^{2+} (Sugimoto et al., 1993) is confirmed by the results of carbonic anhydrase II expression, which is inhibited by S12911-0 (1 mM Ca^{2+}). In osteoporosis, the two- to threefold increase of the number of osteoclasts may be associated with an increase in the activation frequency of bone remodeling and the number of osteoclasts that differentiate within each resorption site may also increase (Kanis, 1996). In postmenopausal osteoporosis, the ratio between the reversal surface and the active eroded surface is increased (Baron et al., 1981) and may demonstrate an increase in the removal period (Kanis, 1996). The two combined actions of S12911-2 on two markers of the maturation process of differentiated osteoclasts (carbonic anhydrase II and the α_v subunit of vitronectin receptor) may explain, at least in part, its capacity of preventing the bone loss in pathological conditions.

Osteoclasts are giant multinucleated cells, which acidify the bone resorbing compartment which is sealed off by their attachment to the bone matrix and where the dissolution of the organic matrix with proteolytic enzymes occurs (Baron, 1989). A 24-h pretreatment of the bone slices with S12911-2 dose-dependently inhibits the subsequent bone resorption by up to $66 \pm 10.6\%$ at 1 mM Sr^{2+}. This inhibitory effect was even higher ($73 \pm 16\%$, $P<0.05$) when S12911-2 was also present during the 24-h period of cell culture. The other salts of the same organic compound (S12911-0 or S12911-5) have no inhibitory effects on the function of freshly isolated rat osteoclasts in culture. The osteoclasts are rich in TRAP isoenzymes (Minkin, 1982), their function may be involved in the dephosphorylation of bone proteins and provide an efficient marker for the activity of osteoclasts (Kanis, 1996). The pre-incubation and/or incubation with S12911-2 does not affect the number of active osteoclasts present in the bone slices at the end of the culture period and has no effect on their attachment. The fact that pre-incubation of bone slices with S12911-2 significantly affects subsequent bone resorption by isolated osteoclasts without affecting their attachment and viability suggests that the effect of S12911-2 may be at least in part due to a direct and/or matrix-mediated inhibition of the bone resorbing activity of the osteoclast. In addition, it has recently been demonstrated that Sr^{2+} administered to mice induced an inhibition of the formation of the ruffled border and of the clear zone in osteoclasts and chondroclasts (Shibata and Yamashita, 2001). These observations are in agreement with those found in vitro by Takahashi and Suda (unpublished data) on isolated mouse osteoclasts observed by transmission electronic microscopy, showing that osteoclasts treated with strontium ranelate failed to form the ruffled border and clear zone.

The bone resorption reducing activity of S12911-2 is associated with a preserved bone formation potency in intact rats (Ammann et al., 2001) or in rats with estrogen deficiency (Marie et al., 1993). In humans, a clinical study on postmenopausal women demonstrated that after 1 year, S12911-2 (associated with a vitamin D and Ca^{2+}-enriched diet) increased the bone mineral density (Meunier et al., 1996). Therefore, the dose level at which the S12911-2 acts on the physiology of the bone does not have consequences on Ca^{2+} incorporation in the bone and the dynamic of bone remodeling allows this new product to have a powerful action on disorders observed in osteoporosis (Mulligan and Grandjean, 2000; Reginster et al., 1998; Ringe, 2000).

5. Conclusion

In cultured cells from chicken bone marrow, treated for 6 days, only S12911-2 dose-dependently inhibited the 1,25-dihydroxyvitamin D_3-inducible expression of two markers of osteoclastic differentiation: the carbonic anhydrase II and the vitronectin receptor. In response to the active metabolite of 1,25-dihydroxyvitamin D_3, the expression of both carbonic anhydrase II and the α_v subunit of vitronectin receptor were significantly reduced from 0.1 mM (30% and 30.7%, respectively) to 1 mM (46% and 40%, respectively). The pre-incubation of bone slices with S12911-2 significantly inhibits subsequent bone resorption by isolated rat osteoclasts without affecting their attachment and viability. This effect is observed with neither S12911-0 nor S12911-5, and may in part be explained by a direct and/or matrix-mediated inhibition by S12911-2 on the bone resorbing activity of the osteoclasts.

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