Kidney International, Vol. 31 (1987), pp. 736–743

Aluminum action on mouse bone cell metabolism and response to PTH and $1,25(OH)_2D_3$

M. Lieberherr, B. Grosse, G. Cournot–Witmer, M.P.M. Hermann–Erlee, and S. Balsan

Laboratoire des Tissus Calcifiés (CNRS UA.583-INSERM U.30), Hôpital des Enfants–Malades, 149, rue de Sèvres, 75743 Paris Cédex 15, France, and Laboratory for Cell Biology and Histology, University of Leiden, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands

Aluminum action on mouse bone cell metabolism and response to PTH and $1,25(OH)_2D_3$. Aluminum (Al) accumulation in bone is associated with low bone formation and mineralization rates; resorption may also be reduced. The mechanism of these Al-induced changes was investigated using cultured mouse osteoblast–like (OB) and osteoclast–like (OC) cells. The Al effect on bone resorption was measured by the in vitro release of ^{45}Ca and β-glucuronidase from mouse fetal limb–bones. Al had a biphasic effect. High concentrations ($>1.5 \times 10^{-6}$ M) of Al inhibited collagen and DNA synthesis, ornithine decarboxylase and alkaline phosphatase activity in OB, and depressed tartrate-resistant acid phosphatase activity in OC. Lower Al concentrations stimulated these cellular activities and ^{45}Ca and β-glucuronidase release from fetal bones. Al had no effect on basal cAMP levels in OB but inhibited the stimulating effect of bPTH on cAMP content. Al also altered the $1,25(OH)_2D_3$ effects on the ornithine decarboxylase activity of OB cells. These data suggest that: (i) the low bone formation observed in vivo during Al intoxication may be due to the inhibition of collagen synthesis and to depressed cell proliferation; and (ii) Al may indirectly influence bone remodeling by interfering with the actions of bPTH and $1,25(OH)_2D_3$ on bone cells.

Aluminum (Al) accumulation is an important toxic factor in patients with renal failure [1]. The main sources of Al are phosphate binders and/or Al-contaminated dialysate solutions [2–5].

Skeletal accumulation of Al induces vitamin D-resistant osteomalacia, a disorder characterized by reduced numbers of osteoblasts and osteoclasts associated with normal or low plasma, alkaline phosphatase activity and relative parathyroid hormone (PTH) deficiency [5, 6]. The mechanism by which Al induces these bone lesions is not fully understood. Several studies have shown that Al may be toxic to osteoblasts [7–9] and that the abnormal function of these cells may be responsible for the reduced bone–matrix formation and mineralization. Concerning bone resorption, aluminum intoxicated osteomalacic patients do not usually have histologic evidence of osteitis fibrosa. This could be secondary to an inhibition of PTH release by Al which has been shown to occur in severely Al-intoxicated patients [10] and in parathyroid cells or slices incubated in vitro with high doses of Al [11, 12]. However, the possibility of a direct Al effect on the sensitivity of osteoclasts to PTH cannot be excluded.

We have recently shown that, in organ culture, Al interferes directly not only with the synthesis of bone alkaline phosphatase, which reflects osteoblast activity, but also with the synthesis of acid phosphatase, which is abundant in osteoclasts [13]. In the present study we tested the effect of Al on osteoblast function by measuring alkaline phosphatase activity, ornithine decarboxylase activity, DNA and collagen synthesis in cultured osteoblast–like cells derived from neonatal mouse parietal–bones. We also investigated a possible direct effect of Al on bone resorption using tartrate–resistant acid phosphatase activity in cultured osteoclast–like cells and ^{45}Ca and β glucuronidase release from fetal mouse limb–bones as indications of osteoclastic function. Finally, we explored the possibility of an interaction between Al and bPTH or $1,25\text{-}(OH)_2D_3$ influencing bone cell activities.

Methods

Cell isolation and culture

Parietal bones without sutures were aseptically removed from neonatal mouse calvaria, care being taken not to damage the periosteum. The dissected bones were then transferred to a sterile 25 ml Erlenmeyer flask, containing solution A (137 mM NaCl, 2.7 mM KCl, 3 mM NaH_2PO_4) plus 4 mM EDTA, pH 7.2. After a five minute digestion, the solution was removed and fresh solution A plus EDTA was added for 20 minutes. The released cells constituted the osteoclast–like (OC) cell population. Solution A containing 180 U/ml crude collagenase (CLS type I) was added to the remaining bones. After four sequential 20 minute digestions, the cells obtained from the last three digestions were pooled and constituted the osteoblast–like (OB) population. Aliquots from both OB and OC populations were counted in a hemocytometer. Cells were seeded in multiwell plates (Costar cluster 12) and cultured at 37°C in an atmosphere of 95% air/5% CO_2 until confluent. OC were seeded at a density of 20×10^3 per cm^2 in 2 ml of α-MEM medium containing 2% fetal calf serum (FCS). OB were seeded at a density of 10×10^3 per cm^2 in 2 ml of modified Ca^{2+}-free BGJ (m BGJ) medium supplemented with 10% FCS, cultured in this medium for 24 hours, and then in BGJ medium with 1 mM Ca and 10% FCS. The media were renewed every 48 hours.

Received for publication September 13, 1985
and in revised forms February 25 and June 23, 1986

Cyclic AMP assay

Cells were incubated for 5, 15, 30 and 120 mintues in Hanks' balanced salt solution containing 25 mM HEPES, 0.05% bovine serum albumin (BSA), 1 mM Ca, 0.2 mM isobutyl methylxanthine (IBMX), and the substances to be tested or their vehicle. Cells were then extracted by sonication in the presence of 90% propanol. cAMP was measured by the protein binding assay of Lust et al [14] and expressed as pmoles per μg DNA [15].

Alkaline and acid phosphatase assays

Cells were pre-incubated for 16 to 18 hours in serum–free medium before being placed in serum–free medium containing the substance to be tested or its solvent for 24 hours. Following incubation, the cells were washed three times with 0.25 M sucrose, removed from the dish with a rubber policeman, resuspended in 0.25 M sucrose, 0.1% Triton X100 and 0.3 M KCl and disrupted by sonication (2 × 20 sec at 40 KHz). Particulate matter was removed by centrifugation at 600 ×g for 10 minutes and the supernatants were stored frozen until used for enzyme assays. The incubation solutions for acid phosphatase contained 100 mM acetate buffer, pH 5, 5 mM ascorbic acid, 0.1% Triton X100, 10 mM sodium tartrate and 8 mM p-nitrophenylphosphate. The incubation solutions for alkaline phosphatase contained 100 mM triethanolamine buffer pH 9.8, 25 mM $MgCl_2$ and 15 mM p-nitrophenylphosphate. Incubations at 37°C were stopped after 30 minutes by the addition of 1.5 ml of 100 mM NaOH plus 10 mM EDTA to prevent precipitation of divalent metal ions. Acid or alkaline phosphatase was assayed by measuring the absorbance of the liberated p-nitrophenol at 405 nm. All reactions were linear with respect to the amount of enzyme and the incubation time.

Activities are expressed as nanomole of substrate liberated per minute per μg of DNA.

Tartrate–resistant acid phosphatase activity was measured in cell extracts and in culture media. Alkaline phosphatase activity (ALP) was only measured in cytoplasmic extracts because of the low level found in media (5 ± 2 nmol/min/μg DNA).

Collagen analysis

Cells were cultured for 24 hours in modified BGJ medium (mBGJ) containing 1 mM Ca, 3 mM phosphate, 1 mM proline, 100 μg/ml ascorbic acid and 0.05 mg/ml BSA. They were then incubated for 24 hours in fresh medium containing the appropriate test substance. Five μCi 2,3-^{3}H proline (SA:30 Ci/mmole) were added to the medium for the final four hours of the incubation period. The cells were washed five times with phosphate–buffered saline, pH 7.4 at 4°C, to terminate incubation, scraped off and homogenized in 0.5 M acetic acid using an all–glass homogenizer. The incorporation of ^{3}H proline into collagenase–digestible (CDP) and non-collagen (NCP) proteins was determined using purified bacterial collagenase according to the method of Peterkofsky and Dielgelmann [16]. Blanks without enzyme were included for each sample; counting efficiencies were determined by internal standardization. The relative rate of collagen synthesis was corrected for the relative abundance of proline residues in collagen compared to non-collagen protein [17].

An aliquot of the homogenate was treated with 100 μg/ml of pepsin for 15 hours at 5°C to analyze the types of synthesized collagen. The pepsin digest was brought to pH 8 with NaOH and submitted to salt precipitations using 15 and 5% NaCl. Collagen types were separated by SDS-PAGE on slab gels according to the method of Laemmli [18]. Reduction of disulfide bonds were carried out on some samples [19].

Ornithine–decarboxylase assay

Osteoblasts were inoculated at a density of 10×10^3 cells/cm^2 in 25-T flasks with silicon stoppers and cultured until confluency in mBGJ medium supplemented with 10% FCS. Activation of ODC was achieved by a 16 to 18 hour serum starvation in medium minus serum followed by replacement with mBGJ medium without FCS and supplemented with the substances to be tested. Enzyme assay was done in situ by the method of Patterson and Maxwell [20]. Assay mixtures contained 0.2 ml of 0.5 mM pyridoxal 5′ phosphate (PLP), 0.2 ml of 25 mM dithiothreitol (DTT), 0.4 ml of L-(^{14}C)ornithine prepared by mixing the isotope with 1 mM carrier ornithine to give 250 to 300 dpm/nmol and 1.2 ml of Tris-HCl buffer, pH 7.1. For reagent controls, PLP was replaced by 0.2 ml of 50 mM semicarbazide and DTT by 0.2 ml of 1 mM p-chloromercuriphenylsulfonic acid. All reactions were linear with respect to the number of cells assayed and the incubation time. ODC activity is expressed as nmole of CO_2 per mg of protein per hour. Proteins were assayed by Bradford's method [21].

Measurement of DNA content

Total DNA was measured according to the method of Karsten and Wollenberger [15] using ethidium bromide for the direct fluorometric estimation of DNA.

Bone resorption

The forelimb–rudiment method of Raisz and Niemann [22] was employed. Seventeen–day pregnant mice were injected with 200 μCi of ^{45}Ca (SA 22 Ci/g) and, 24 hours later, the 18-day–old fetuses were removed and the radii and ulnae of the fetuses dissected out. Forelimb rudiments were precultured in mBGJ medium for 24 hours. They were then transferred to mBGJ medium supplemented with 0.1 mg/ml BSA and cultured in the presence or absence of Al. ^{45}Ca and β-glucuronidase activity were measured in media and bones after 48 hours in culture. β glucuronidase was assayed by measuring the hydrolysis of phenolphthaleine glucuronidate in 0.1 M acetate buffer, pH 4.5. Bones were homogenized with an all–glass homogenizer in 0.1% Triton X100. Aliquots were taken for enzyme assay and the remainder was extracted with 0.5 M EDTA for two hours at 37°C for ^{45}Ca analysis. Values are presented as percentage of total ^{45}Ca or β-glucuronidase activity released into the medium.

Test substances

The water used for the preparation of Al solutions and mBGJ or αMEM medium was purified using a Milli-Q Millipore (Bethesda, Maryland, USA) water system and contained no detectable traces of Al.

Aluminum chloride ($AlCl_3$, 6 H_2O; Sigma Chemical Corporation, St. Louis, Missouri, USA) dissolved in water was used at concentrations varying from 10^{-8} M to 10^{-5} M. Aluminum

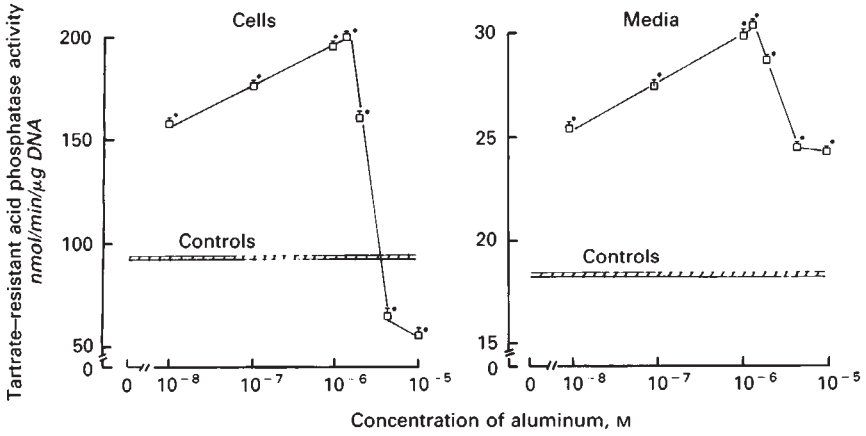

Fig. 1. *Effects of aluminum on tartrate-resistant acid* phosphatase activity measured in osteoclast-like cell extracts (**A**) and in media of osteoclast-like cells (**B**). Values are means ± SEM of five different experiments with four wells for each point. Values significantly different from control values, $^{*}P < 0.001$.

measurements were performed directly in the various media by flameless atomic absorption [23]. Concentrations found in the media compared to the theoretical concentrations were: 9.9 µM/liter versus 10 µM/liter (10^{-5} M); 5.8 µM/liter versus 6 µM/liter (6×10^{-6} M); 2.8 µM/liter versus 3 µM/liter (3×10^{-6} M); 1.6 µM/liter versus 1.5 µM/liter (1.5×10^{-6} M); 1 µM/liter versus 1 µM/liter (10^{-6} M); 0.09 µM/liter versus 0.1 µM/liter (10^{-7} M); and 0.01 µM/liter versus 0.01 µM/liter (10^{-8} M). Control media did not contain any detectable trace of Al. These results represent the mean values of 10 measurements. The addition of 5 µl of Al stock solutions to the medium (2 ml) neither changed the medium pH nor produced cloudiness in the medium. The supplementation of the medium with 0.05 mg/ml BSA did not modify the measured Al concentrations in the media and the biochemical results obtained with or without BSA in media containing Al were comparable in amplitude.

1,25-$(OH)_2D_3$ was used at a concentration of 10^{-9} M. The stock solution of the metabolite was 10^{-4} M in ethanol.

bPTH(1-84) was used at a final concentration of 10^{-9} M.

All experiments on the test substances were done in the absence of fetal calf serum. Preliminary results showed that the addition of ethanol, bPTH solvent, water or ethanol plus bPTH solvent and water did not modify the results as compared to untreated cultures.

Materials

Radiolabeled products were purchased from Amersham (Les Ulis, France). Crude CLS type I collagenase and 5275 CLS Pa bacterial collagenase were purchased from Worthington Biochemical Corporation (Freehold, New Jersey, USA). Sources of other chemicals were: Sigma Chemical Company (St. Louis, Missouri, USA) for BSA, p-nitrophenyl phosphate, proline, EDTA, ascorbic acid, TCA, Hepes, calcium chloride, tanic acid, Trizma buffer and L-ornithine; and PackardBecker BV Chemical Operations (Groningen, The Netherlands) for scintillation solutions.

Statistical analysis

First, the SNEDECOR F test was used to determine whether there was a variation from one experiment to another for each Al concentration. None was found ($P < 0.001$). Data were expressed as the mean ± standard error of the mean (SEM) for the total set of figures (N) at each Al concentration. The statistical significance was determined by Student's t-test for unpaired data.

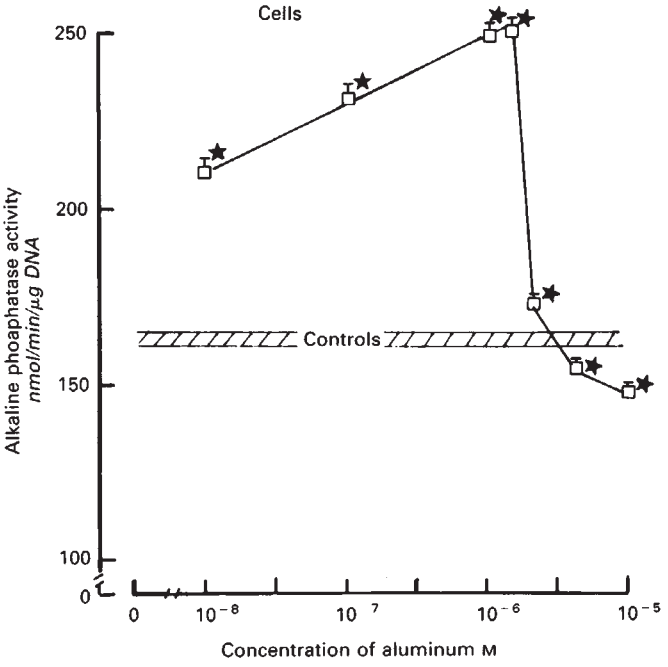

Fig. 2. *Effects of aluminum on alkaline phosphatase activity measured in osteoblast-like cells.* Values are means ± SEM of five different experiments with four wells for each point. Values significantly different from control values, $^{*}P < 0.001$; $^{**}P < 0.05$.

Results

Acid and alkaline phosphatases of osteoblast- and osteoclast-like cells

Tartrate-resistant acid phosphatase activity, which represents 95% of the total acid phosphatase activity in OC cells, was stimulated by Al concentrations ranging from 10^{-8} M to 1.5×10^{-6} M, (Fig. 1A). A decrease of the stimulatory action or even

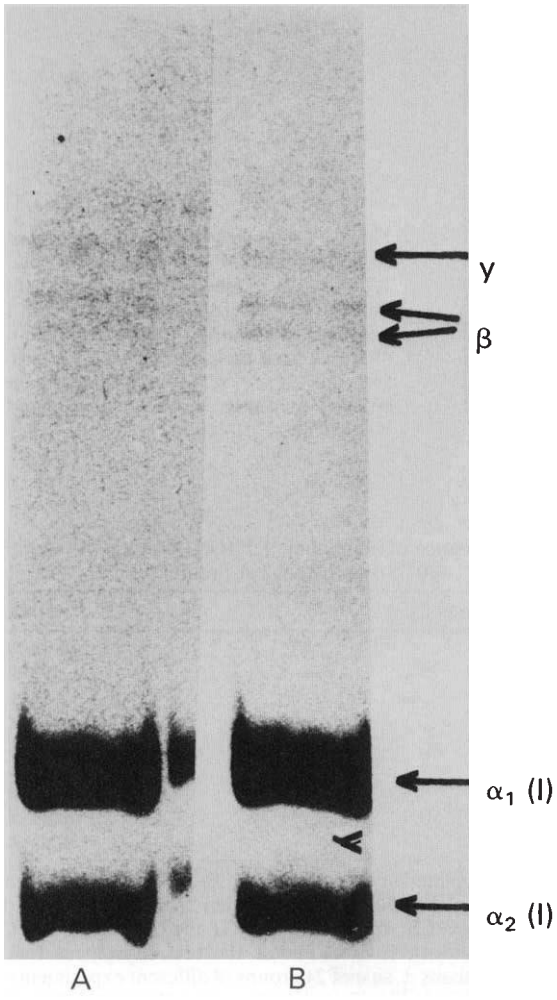

Fig. 3. *SDS polyacrylamide gel electrophoresis.* Collagen preparations were denatured and electrophoresed in 5% polyacrylamide slab gels using the Laemmli method. A and B indicate unreduced and reduced samples, respectively.

an inhibitory effect was observed at higher Al concentrations (Fig. 1A). The release of tartrate–resistant acid phosphatase into the medium was augmented at low Al concentrations and remained somewhat above control levels at high (10^{-6} M) Al concentrations (Fig. 1B).

A dose–dependent biphasic effect of Al was also observed on the alkaline phosphatase activity of OB cells. High doses of Al significantly inhibited ($P < 0.001$) ALP whereas low concentrations (from 10^{-8} M to 1.5×10^{-6} M) increased activity (Fig. 2).

Collagen synthesis of osteoblast–like cells

As shown in Figure 3, OB cells synthesized type I collagen. Al concentrations up to 10^{-7} M did not affect 3H proline incorporation into CDP or NCP (Fig. 4). An increase in the NCP labeling was observed at concentrations, from 10^{-6} M to 1.5×10^{-6} M, which induced a relative diminution of the percentage of collagen synthesized. At higher concentrations, the ^{3}H proline incorporation into CDP was decreased, indicating an inhibition of collagen synthesis. Both $1,25\text{-}(OH)_2D_3$ and bPTH (10^{-9} M) induced an inhibition of the CDP labeling under the same experimental conditions (Fig. 4).

Ornithine–decarboxylase activity in osteoblast–like cells

ODC activity of OB cells was increased in Al concentrations ranging from 10^{-8} M to 1.5×10^{-6} M, whereas higher concentrations inhibited enzyme activity (Fig. 5). In the absence of added aluminum, $1,25\text{-}(OH)_2D_3$ (10^{-9} M) decreased and bPTH (10^{-9} M) increased ODC activity. Addition of $1,25\text{-}(OH)_2D_3$ to the medium of cells treated with Al decreased the stimulating effect of these low Al doses (Fig. 5) while the inhibitory effects of $1,25\text{-}(OH)_2D_3$ and Al were not additive in cells incubated with high doses of Al. No cumulative effect was observed when the cells were treated simultaneously with bPTH and stimulatory concentrations of Al (Fig. 5). However, high doses of Al produced a significant decrease in the stimulatory effect of bPTH (Fig. 5).

Effects of aluminum, $1,25\text{-}(OH)_2D_3$ and bPTH on osteoblast–like cell proliferation

Increasing the concentration of Al from 10^{-8}M to 10^{-6} M resulted in a progressive increase in cell proliferation as measured by DNA synthesis. High doses of Al significantly reduced ($P < 0.001$) DNA content. Under the same experimental conditions, bPTH increased and $1,25\text{-}(OH)_2D_3$ had no effect on the DNA content (Table 1).

cAMP content of osteoclast– and osteoblast–like cells

The cAMP content of OC or OB cells was not sensitive to exposures of 5 to 120 minutes to concentrations of Al from 10^{-8} M to 10^{-5} M. In OC it was found to be 1 ± 0.04 pmol/μg DNA for controls and from 0.9 ± 0.03 to 1.22 ± 0.09 pmol/μg DNA in the presence of Al. In the presence of sCT (0.1 U/ml) cAMP content was increased (7.8 ± 0.09 pmol/μg DNA) after five minutes of incubation. In OB control values (2.1 ± 0.1 pmol/μg DNA) were comparable to those found in the presence of Al (2.05 ± 0.06 to 2.4 ± 0.2 pmol/μg DNA). Prior incubation with Al produced a dose–dependent reduction of the cAMP content of OB cells stimulated by bPTH (Table 2).

Aluminum effects on bone resorption

Al concentrations from 10^{-8} M to 10^{-6} M increased the release of ^{45}Ca and β-glucuronidase in 48 hour cultures (Fig. 6). The effects were much greater for β-glucuronidase. The total activity of the enzyme in bone and medium showed a progressive increase with increasing concentrations of Al. The activity of bone enzyme was inhibited at concentrations above 10^{-6} M and the release into the medium was reduced. High doses of Al had no effect on ^{45}Ca release (Fig. 6).

Discussion

These results show that Al has a direct effect on the metabolism of mouse bone cells in culture and on in vitro calcium release from fetal limb bones. The effect is dose dependent and biphasic. Al exerts an inhibiting action at concentrations above 10^{-6} M, whereas below this concentration, the majority of cellular activities tested are stimulated. This biphasic dose–dependent effect appears to be a general phenomenon since a

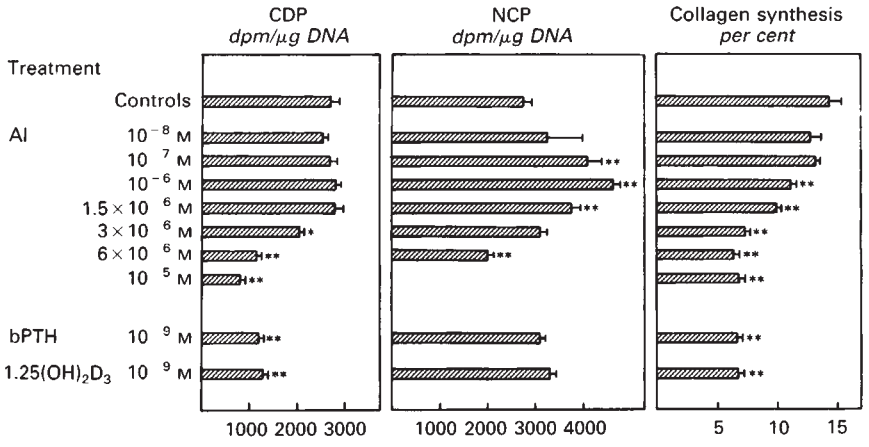

Fig. 4. *Collagen synthesis by osteoblast-like cells.* Influence of aluminum, bPTH or $1,25(OH)_2D_3$. Values are means ± SEM of 20 groups (five different experiments with four wells for each point). Values significantly different from their corresponding controls, $^{**}P < 0.001$; $^{*}P < 0.05$. The percentage of collagen synthesis was calculated from CDP × 100/CDP + 5.4NCP.

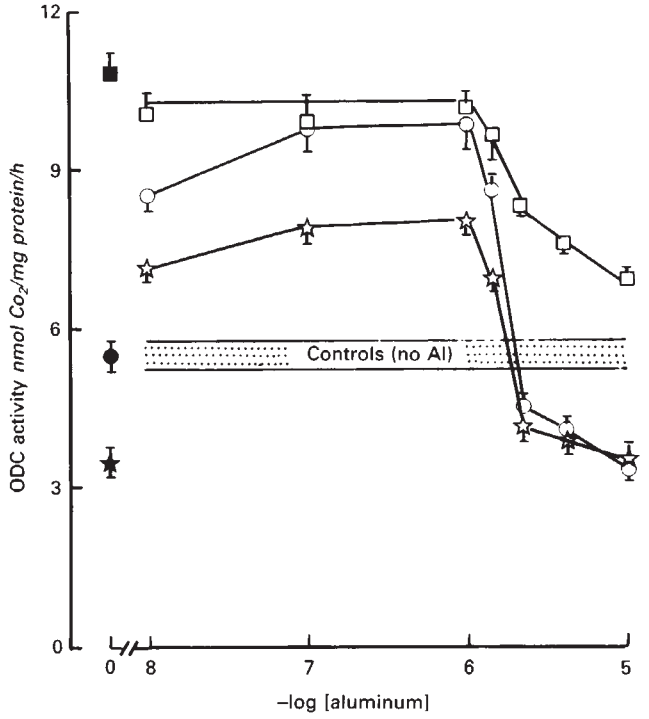

Fig. 5. *Influence of aluminum on ODC activity of osteoblast-like cells.* Symbols represent the cells incubated with: solvent (●); with bPTH (10^{-9} M) (■); with $1,25(OH)_2D_3$ (10^{-9} M) (★); with Al alone (○); simultaneously with Al and bPTH (□); and with Al plus $1,25(OH)_2D_3$ (10^{-9} M) (☆). Values are means ± SEM of eight experiments with three flasks for each point.

Table 1. Influence of aluminum, $1,25(OH)_2D_3$ (10^{-9}M) and bPTH (10^{-9}M) on osteoblast proliferation

Treatment of the cells	DNA μg
Controls	10.10 ± 0.15
Al (10^{-8}M)	11.98 ± 0.11[a]
Al (10^{-7}M)	12.14 ± 0.08[a]
Al (10^{-6}M)	13.11 ± 0.09[a]
Al (3×10^{-6}M)	11.73 ± 0.05[a]
Al (6×10^{-6}M)	8.13 ± 0.09[a]
Al (10^{-5}M)	7.52 ± 0.11[a]
$1,25(OH)_2D_3$ (10^{-9}M)	9.58 ± 0.09
bPTH (10^{-9}M)	13.01 ± 0.04[a]

OB cells were plated at a density of 2×10^5 cells/well and cultured for 24 hr with 10 % FCS. The cells were then washed 3 times with BGJ medium without FCS, incubated in BGJ medium without FCS but supplemented with 0.1% BSA and/or the test substances for 24 hr. Values are the means ± SEM of 24 groups (8 different experiments with 3 wells for each point). Significantly different: [a] $P < 0.001$, [b] $P < 0.05$.

Table 2. Interaction between aluminum and bPTH on cellular cAMP content of osteoblast-like cells

Treatment	cAMP *pmole/μg DNA*
bPTH	295 ±30
Al (10^{-8}M) + bPTH	87 ± 3[a]
Al (10^{-7}M) + bPTH	88 ± 3[a]
Al (10^{-6}M) + bPTH	89 ± 4[a]
Al (3×10^{-6}M) + bPTH	62 ± 1[a]
Al (10^{-5}M) + bPTH	43 ± 1[a]

Cells were preincubated with Al or solvent for 2 hr before the addition of bPTH (10^{-9}M) (5 min). Values are the means ± SEM of 4 experiments in which each point represent 4 wells. When compared to addition of bPTH in the absence of Al: [a] $P < 0.001$.

similar mode of action has been observed in various in vitro systems for several other metals [24] and hormones [25–27].

It is difficult to compare the in vitro Al concentrations employed in serum-free medium, with circulating in vivo Al concentrations, where Al is largely protein-bound [28]. Furthermore, osteoblast and osteoclast phenotypes were mainly defined by the digestion procedure used and their cAMP responses to bPTH and calcitonin [29] in the present study, and not by morphological criteria. However, some of our data are in agreement with several clinical and experimental observations and may help to clarify the mechanisms of the Al effect on bone turnover.

Indeed it has been shown that in both severely intoxicated patients and in intoxicated rats the numbers of osteoblasts and the rates of bone formation are decreased [5, 7, 8]. Moreover treatment with desferroxiamine, which removes Al from bone, is associated with an increase in matrix synthesis [30, 31]. Our data indicate that several mechanisms may be involved in these

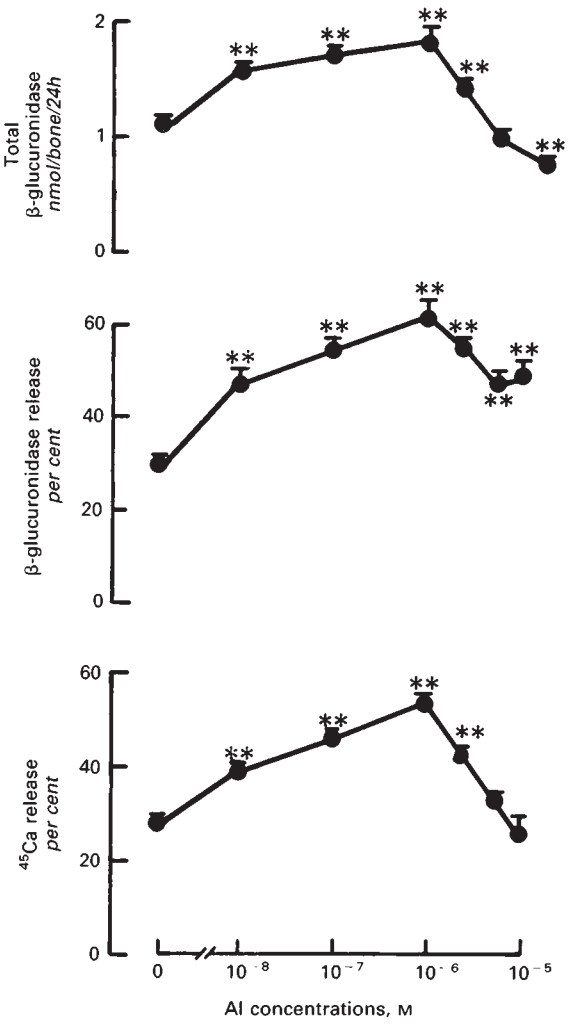

Fig. 6. *Effect of aluminum on ^{45}Ca and β-glucuronidase release from fetal mouse long bones and on bone β-glururonidase activity.* Values are means ± SEM for 10 cultures of radius plus ulna incubated 48 hours. Values significantly different from their corresponding controls, $^{**}P < 0.001$, $^{*}P < 0.05$.

phenomena. First, high concentrations of Al depress the activity of osteoblast–like cells and reduce collagen synthesis. Second, an inhibition of cellular proliferation occurs at these high Al concentrations, that is, DNA synthesis is depressed as is the cell growth marker, ornithine–decarboxylase activity (ODC). In addition, Al depresses the bPTH-mediated stimulation of cell proliferation.

With regard to the observed in vitro stimulating effect of low Al concentrations, in patients such an effect would hardly be detected, because the secondary hyperparathyroidism which occurs in the majority of them also has a stimulatory action on bone cellular activity [32].

The effect of Al on bone mineralization could not be tested by the methods used in this study. However, the inhibition of alkaline phosphatase activity observed with high Al concentrations may indicate a disturbance of the mineralization process, since it is generally accepted that alkaline phosphatase hydrolyses pyrophosphates, which are inhibitors of mineral deposition [33].

Our data show that at low concentrations (10^{-8} to 10^{-6} M) Al increases calcium and β-glucuronidase release from long bones, and tartrate–resistant acid phosphatase from osteoclast–like cells. The effect on calcium release disappears at higher concentrations (6×10^{-6} M to 10^{-5} M) and the intracellular activity of both lysosomal enzymes is inhibited, whereas their release into the medium persists. These results are in agreement with in vivo data obtained by Goodman, Gilligan and Horst showing increased endosteal tibial resorption in Al-intoxicated rats [8]. The rapid hypercalcemia observed in dogs [34] and rats [35] following a single i.v. injection of Al could also reflect a direct action of Al on skeletal calcium mobilization. Moreover, Henry and Norman recently reported that Al administration elevates serum calcium in vitamin D deficient chicks [36]. All these observations suggest that the spontaneous hypercalcemia observed in Al-intoxicated patients [37] may be due not only to reduced calcium entry into the bone, but also to increased calcium release from the bone.

The relationship between Al and parathryoid gland function have been extensively analyzed over the past few years, leading to the conclusion that Al could inhibit PTH release [10–12], thus inducing the relative PTH deficiency observed in severely Al-intoxicated patients. Our results show that Al also interferes with the effect of PTH on bone tissue, not only by derepressing the PTH-mediated stimulation of cell proliferation mentioned above, but also by inhibiting the stimulating effect of bPTH on cAMP content of osteoblast–like cells, while the basal cAMP production is unaffected. These results are in agreement with those reported for perfused tibiae of Al-intoxicated dogs [38]. As in our experiment, Al inhibited the bPTH-induced cAMP release. These effects, which indicate disturbed osteoblast function, appear to be specific to bone cells, because Al intoxication does not alter the renal cAMP response to bPTH [39].

It has been reported that the metabolism of vitamin D is disturbed in Al-intoxicated animals [36, 39, 40]. The present data and those obtained in a previous study [13], show that the action of vitamin D on bone is also disturbed by the presence of Al. Al inhibits the stimulating effect of 1,25-$(OH)_2D_3$ on acid and alkaline phosphatase of bones in organ culture while altering the 1,25-$(OH)_2D_3$-induced changes in ODC activity of cultured bone cells.

In conclusion, the present data show that Al influences several cellular functions in bone tissue and its action is dose dependent. These findings confirm the depressant effect of Al intoxication on bone matrix formation observed in vivo and show that this effect is due not only to the inhibition of collagen synthesis, but also to the inhibition of cell proliferation. Moreover, Al directly influences bone resorption, and interacts at the cellular level with the effects of PTH and 1,25-$(OH)_2D_3$.

Acknowledgments

The authors thank Monsieur le Professeur R. Bourdon, Laboratoire de Biochimie, Hôpital F. Vidal, Paris, France for his appropriate comments, Dr. O. Parkes for his linguistic help and Miss M. Poitou for her secretarial assistance. 1,25-$(OH)_2D_3$ used in this study was a gift from Hoffmann–la Roche, Basel, Switzerland. bPTH(1-84) used in this

study was a gift from the late Dr. J.A. Parsons, National Institute of Biochemical Standards and Controls, Holly Hill, London, UK.

Reprint requests to M. Lieberherr, Laboratoire des Tissues Calcifiés, (CNRS UA.583—INSERM U.30), Hôpital des Enfants-Malades, 149, rue de Sèvres, 75743 Paris Cedex 15, France.

References

1. ALFREY AC: Aluminum intoxication. *N Engl J Med* 310:1113–1115, 1984
2. PLATTS MM, GOODS GC, HISLOP JS: Composition of the domestic water supply and the incidence of fractures and encephalopathy in patients on home dialysis. *Br Med J* 2:657–660, 1977
3. BOUKARI M, ROTTEMBOURG J, JAUDON MC, CLAVEL JP, LEGRAIN M, GALLI A: Influence de la prise prolongée de gels d'alumine sur les taux sériques d'aluminium chez les patients atteints d'insuffisance rénale chronique. *Nouv Presse Méd* 7:85–88, 1977
4. GRISWOLD W, REZNIK RV, MENDOZA SA, TRAUNER D, ALFREY AC: Accumulation of aluminum in a nondialzyed uremic child receiving aluminum hydroxide. (abstract) *Pediatrics* 71:56, 1983
5. COURNOT-WITMER G, ZINGRAFF J, PLACHOT JJ, ESCAIG F, LEFEVRE R, BOUMATI P, BOURDEAU A, GARABEDIAN M, GALLE P, BOURDON R, DRÜEKE T, BALSAN S: Aluminum localization in bone from hemodialyzed patients: Relationship to matrix mineralization. *Kidney Int* 20:375–385, 1981
6. HODSMAN AB, SHERRARD DJ, ALFREY AC, OTT S, BRICKMAN AS, MILLER NL, MALONEY NA, COBURN JW: Bone aluminum and histomorphometric features of renal osteodystrophy. *J Clin Endocr Metab* 54:539–546, 1982
7. OTT SM, MALONEY NA, KLEIN GL, ALFREY AC, AMENT ME, COBURN JW, SHERRARD DJ: Aluminum is associated with low bone formation in patients receiving chronic parenteral nutrition. *Ann Int Med* 98:910–914, 1983
8. GOODMAN WG, GILLIGAN J, HORST R: Short-term aluminum administration in the rat: Effects on bone formation and relationship to renal osteomalacia. *J Clin Invest* 73:171–181, 1984
9. PLACHOT JJ, COURNOT-WITMER G, HALPERN S, MENDES V, BOURDEAU A, FRITSCH J, BOURDON R, DRÜEKE T, GALLE P, BALSAN S: Bone ultrastructure and X-ray microanalysxis of aluminum-intoxicated hemodialysed patients. *Kidney Int* 25: 796–803, 1984
10. ANDRESS D, FELSENFELD A, VOIGTS A, LLACH F: Parathyroid hormone response to hypocalcemia in hemodialysis patients with osteomalacia. *Kidney Int* 24:364–370, 1983
11. MORRISSEY J, ROTHSTEIN M, MAYOR G, SLATOPOLSKY E: Suppression of parathyroid hormone secretion by aluminum. *Kidney Int* 23:699–704, 1983
12. BOURDEAU A, PLACHOT JJ, COURNOT G, POINTILLART A, SACHS C, BALSAN S: In vitro effects of aluminum on parathyroid glands: Correspondence between hormonal secretion and ultrastructural aspects, *Endocrine control of bone and calcium metabolism*, edited by COHN DV, FUJITA T, POTTS JR JT, TALMAGE RV. Amsterdam, Elsevier Science Publishers B.V., 1984, pp. 230–231
13. LIEBERHERR M, GROSSE B, COURNOT-WITMER G, THIL CL, BALSAN S: In vitro effects of aluminum on bone phosphatases: A possible interaction with bPTH and vitamin D_3 metabolites. *Calcif Tissue Int* 34:280–284, 1982
14. LUST WD, DYE E, DEATON AV, PASSONNEAU JV: A modified cyclic AMP binding assay. *Anal Biochem* 72:8–15, 1976
15. KARSTEN U, WOLLENBERGER A: Improvements in the ethidium bromide method for direct fluorometric estimation of DNA and RNA in cells and tissue homogenates. *Anal Biochem* 777:464–470, 1977
16. PETERKOFSKY B, DIELGELMANN R: Use of a mixture of proteinase-free collagenase for the specific assay of radioactive collagen in the presence of other proteins. *Biochemistry* 10: 998–994, 1971
17. PETERKOFSKY B: The effect of ascorbic acid on collagen polypeptide synthesis and proline hydroxylation during the growth of cultured fibroblasts. *Arch Biochem Biophys* 152:318–328, 1972
18. LAEMMLI UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* (London) 227:680–685, 1970
19. SYKES B, PUDDLE B, FRANCIS M, SMITH R: The estimation of two collagens from human dermis by interrupted gel electrophoresis. *Biochem Biophys Res Commun* 72:1472–1480, 1976
20. PATTERSON MK JR, MAXWELL MD: In situ assay for ornithine decarboxylase in tissue culture. *Anal Biochem* 104:452–456, 1980
21. BRADFORD MM: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254, 1976
22. RAISZ LG, NIEMANN I: Effects of phosphate, calcium, magnesium on bone resorption and hormonal responses in tissue culture. *Endocrinology* 85:446–456, 1969
23. RANISTEANO-BOURDON S, PROUILLET F, BOURDON R: Dosage de l'aluminium et du gallium dans les liquides biologiques. *Ann Biol Clin* 36:39–44, 1978
24. STERN PH: Biphasic effect of manganese on hormone-stimulated bone resorption. *Endocrinology* 117:2044–2049, 1985
25. CORVOL MT, DUMONTIER MF, GARABEDIAN M, RAPPAPORT R: Vitamin D and cartilage. II Biological activity of 25-hydroxycholecalciferol and 24,25- and 1,25-dihydroxycholecalciferols on cultured growth plate chondrocytes. *Endocrinology* 102:1269–1274, 1978
26. LIEBERHERR M, GARABEDIAN M, GUILLOZO H, BAILLY DU BOIS M, BALSAN S: Interaction of 24,25-dihydroxyvitamin D_3 and parathyroid hormone on bone enzymes in vitro. *Calcif Tiss Int* 27:47–52, 1979
27. LIEBERHERR M, ACKER M, GROSSE B, PESTY A, BALSAN S: Rat endometrial cells in primary culture: effects and interaction of sex hormones and vitamin D_3 metabolites on alkaline phosphatase. *Endocrinology* 115:824–829, 1984
28. KAEHNY WD, ALFRED AC, HODMAN RE, SHORR WJ: Aluminum transfer during hemodialysis. *Kidney Int* 12:361–365, 1977
29. LUBEN RA, WONG GL, COHN DV: Biochemical characterization with parathormone and calcitonin of isolated bone cells: Provisional identification of osteoclasts and osteoblasts. *Endocrinology* 99:526–534, 1976
30. MALLUCHE HH, SMITH AJ, ABREO K, FAUGERE MC: The use of deferoxamine in the management of aluminum accumulation in bone in patients with renal failure. *N Engl J Med* 311:140–144, 1984
31. OTT SM, NEBEKER HG, ANDRESS DL, MILLINER DS, MALONEY NA, COBURN JW, SHERRARD DJ: Desferroxiamine therapy in patients with aluminum-related osteodystrophy. (abstract) *Kidney Int* 27:148, 1985
32. MALUCHE HH, REITZ R, ENDRES D, FAUGERE MC: Serum parathyroid hormone levels predict preferentially osteoblastic activity in patients with renal failure. (abstract) *Kidney Int* 27:122, 1985
33. POSEN S, CORNISH C, KLEEREKOPER M: Alkaline phosphatase and metabolic bone disorders, *Metabolic bone disease*, edited by AVIOLI LV, KRANE SM. New York-London, Academic Press, 1977, pp. 142–181
34. HENRY DA, GOODMAN WG, NUDELMAN RK, DIDOMENICO NC, ALFREY AC, SLATOPOLSKY E, STANLEY TM, COBURN JW: Parenteral aluminum administration in the dog: I. Plasma kinetics, tissue levels, calcium metabolism, and parathyroid hormone. *Kidney Int* 25:362–369, 1984
35. RODRIGUEZ M, FELSENFELD A, LLACH F: Intravenous (IV) Aluminum (AL) increases total plasma calcium (Ca), decreases ionized calcium (iCa), and suppresses the parathyroid hormone (PTH) response to hypocalcemia (Ca) in the rat. (abstract) *Kidney Int* 27:125, 1985
36. HENRY HL, NORMAN AW: Interactions between aluminum and the actions and metabolism of vitamine D_3 in the chick. VIIth Annual Scientific Meeting of the American Society for Bone and Mineral Research, Washington DC, 1985, abstract 267
37. COCHRAN M, PLATTS MG, MOORHEAD PJ, BUXTON A: Spontane-

ous hypercalcemia in maintenance dialysis patients: An association with atypical osteomalacia and fractures. *Miner Electrol Metab* 5:280–286, 1981

38. GLACERAN T, FINCH J, BERGFELD M, COBURN J, TEITELBAUM S, MARTIN K, SLATOPOLSKY E: Biological effects of aluminum on the isolated perfused bone. (abstract) *Kidney Int* 27:116, 1985
39. SMOTHERS RL, KAWASHIMA H, COBURN JW, KUROKAWA K: Effect of aluminum administration on 25-hydroxyvitamin D_3-1.-hydroxylase in the rat kidney. (abstract) *Calcif Tissue Int* 35:703, 1983
40. KLEIN GL, VACCARO M, JONGEN M, BISHOP JE, KUROKAWA K, COBURN JW, NORMAN AW: Aluminum blocks the PTH-stimulated rise in serum 1,25-$(OH)_2$-vitamin D levels in D-replete rats. (abstract) *Calcif Tissue Int* 36:518, 1984