0021-972X/99/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 84, No. 10
Printed in U.S.A.

Serum Parathyroid Hormone-Related Protein Levels and Response to Bisphosphonate Treatment in Hypercalcemia of Malignancy

R. RIZZOLI, D. THIÉBAUD, N. BUNDRED, M. PECHERSTORFER, Z. HERRMANN, H. J. HUSS, F. RÜCKERT, C. MANEGOLD, M. TUBIANA-HULIN, E. U. STEINHAUER, M. DEGARDIN, B. THÜRLIMANN, M. R. CLEMENS, H. EGHBALI, AND J. J. BODY

Division of Bone Diseases, World Health Organization Collaborating Center for Osteoporosis and Bone Diseases, Department of Internal Medicine, University Hospital (R.R.), CH-1211 Geneva 14, Switzerland; the Department of Internal Medicine, University Hospital (D.T.), CH-1011 Lausanne, Switzerland; the Department of Surgery, University Hospital (N.B.), Manchester M209BX, United Kingdom; the Department of Medicine and Medical Oncology, Wilhelminenspital (M.P.), A-1160 Vienna, Austria; Roche Diagnostics GmbH (Z.H., H.J.H., F.R.), D-68305 Mannheim, Germany; Hospital Rohrbach (C.M.), D-69126 Heidelberg, Germany; Centre R. Huguenin (M.T.-H.), F-92210 Saint-Cloud, France; City Hospital (E.U.S.), D-34125 Kassel, Germany; Centre O. Lambret (M.D.), F-59020 Lille, France; Canton Hospital (B.T.), CH-9007 St. Gallen, Switzerland; University Hospital (M.R.C.), D-54219 Tuebingen, Germany; Institut Bergonié (H.E.), F-33076 Bordeaux, France; and Institut J. Bordet (J.J.B.), B-1000 Brussels, Belgium

ABSTRACT

The pathogenesis of hypercalcemia of malignancy comprises increased net bone resorption and enhanced renal tubular reabsorption of calcium (Ca). To evaluate the prevalence of an increased renal tubular reabsorption of Ca index [tubular reabsorption of calcium index (TRCaI)] in cancer patients with hypercalcemia and of elevated circulating levels of PTH-related protein (PTHrP), which is recognized as a major mediator of this syndrome, we investigated 315 well rehydrated patients, aged 58.1 ± 0.7 yr (mean ± SEM), with hypercalcemia [albumin-corrected plasma Ca (pCa), >2.7 mmol/L] secondary to histologically proven malignancy. Changes in pCa and, therefore, various Ca filtered loads were obtained by different degrees of bone resorption inhibition achieved with a single infusion of the bisphosphonate ibandronate, given at various doses on a randomized, double blind basis. PTHrP was determined at baseline in 147 of the patients and 7 days after bisphosphonate therapy in 73. Before ibandronate therapy, pCa was 3.36 ± 0.02 mmol/L, mean TRCaI was increased at 3.09 ± 0.03 mmol/L glomerular filtration rate (GFR; normal, 2.40–2.90), and 65% of patients had TRCaI above 2.90 mmol/L GFR. Mean serum PTHrP levels were 4.9 ± 0.5 pmol/L (normal, <2.5) and values above the normal range were found in 53% of the patients (76% in lung and upper respiratory tract malignancies). By 7 days after the infusion of ibandronate, a decrease in pCa of 0.69 ± 0.03 mmol/L (20.0 ± 0.7%; $P < 0.001$) and in bone resorption [mean change in fasting urinary Ca, 0.09 ± 0.04 mmol/L GFR (47.6 ± 8.6%; $P < 0.001$) and 14.4 ± 1.7 nmol/mmol (27.6 ± 10.6%; $P < 0.01$) in deoxypyridinoline] was observed. TRCaI was slightly lowered by 0.30 ± 0.09 mmol/L GFR. Mean changes in PTHrP, 1,25-dihydroxyvitamin D_3, and PTH were +0.7 ± 0.4 (P = NS), +27.6 ± 3.0 ($P < 0.001$), and +2.9 ± 0.8 ($P < 0.005$) pmol/L, respectively. After ibandronate treatment, the relative risk of relapsing hypercalcemia was particularly increased (3.43-fold) in lung and upper respiratory tract malignancies. These results obtained in a large cohort of patients indicate a significant prevalence of an increased renal tubular reabsorption of calcium index in hypercalcemia of malignancy and a substantial proportion of patients with detectable PTHrP. (*J Clin Endocrinol Metab* **84:** 3545–3550, 1999)

HYPERCALCEMIA is a frequent metabolic complication of malignant disease (1–4). The pathogenesis of hypercalcemia of malignancy can comprise increased net bone resorption as well as augmented renal tubular reabsorption of calcium (3–5). Both processes can lead to the release into the extracellular compartment of calcium at a rate that exceeds the kidney excretion capacity (1–4). Depending on the tumor type and/or the stage of cancer disease, either augmented net bone resorption or increased renal tubular reabsorption mechanisms can prevail (5–7). The latter mechanism, which may occur in around 50% of the cases (5), is mainly attributed to the action of PTH-related protein (PTHrP), which is recognized as a major mediator of the hypercalcemia of malignancy (2, 4, 8–10). However, other factors could be implicated in this process as well. For instance, interleukin-1 has been shown to stimulate renal tubular reabsorption of calcium in thyroparathyroidectomized rats (11). Increased osteoclastic bone resorption provides the rationale for the treatment with inhibitors of osteoclastic bone resorption such as the bisphosphonates, which are now considered the first choice in the treatment of hypercalcemia of malignancy (4, 12, 13). However, the prevalence of elevated PTHrP levels according to tumor type in hypercalcemia of malignancy and/or the relationship between determinants of hypercalcemia and PTHrP concentrations are not clearly established yet.

Received August 5, 1998. Revision received January 25, 1999. Rerevision received April 9, 1999. Accepted June 21, 1999.

Address all correspondence and requests for reprints to: Dr. R. Rizzoli, M.D., Division of Bone Diseases, World Health Organization Collaborating Center for Osteoporosis and Bone Diseases, Department of Internal Medicine, University Hospital, 1211 Geneva 14, Switzerland. E-mail: rizzoli@cmu.unige.ch.

To study the prevalence of elevated PTHrP values and increased renal tubular reabsorption of calcium index (TRCaI), we analyzed a large cohort of hypercalcemic cancer patients before and after treatment of hypercalcemia by administration of the new bisphosphonate ibandronate (14, 15). This allowed the determination of PTHrP and/or of a renal TRCaI at various plasma calcium levels and various calcium filtered loads.

Subjects and Methods

Patients

This study combines the results of two multicenter randomized trials evaluating the effects of various doses of the new bisphosphonate ibandronate in patients with cancer-associated hypercalcemia as defined by an albumin-adjusted calcemia above 2.70 mmol/L in the first study (n = 174) and above 3.0 mmol/L in the second study (n = 147). The details of the protocol were extensively presented in previous reports (14, 15). The study protocol was approved by all participating local ethics committees, and the patients gave written informed consent. The exclusion criteria were primary hyperparathyroidism, as indicated by unsuppressed or elevated PTH levels, renal impairment (plasma creatinine, ≥260 μmol/L), and therapy with a bisphosphonate during the preceding 3 months, with mithramycin during the 4 preceding weeks, or with cytostatic drugs or calcitonin during the week before enrollment in the study. The beginning of hormonal treatment within 4 weeks or of corticosteroids within 1 week before the study was also taken as exclusion criteria. In the 2 combined studies, 321 patients were first enrolled, but 15 dropped out during the run-in period, because of clinical deterioration, death, insufficient fluid repletion, or recent changes in chemotherapy program. For our analysis, we excluded 6 additional patients because of insufficient rehydration (n = 3), no tumor at baseline (n = 2), and death (n = 1) before study drug administration. After rehydration with at least 2 L normal saline/24 h for a median duration of more than 48 h, patients with histologically or cytologically proven malignancy and with elevated albumin-corrected plasma calcium [according to the formula: corrected calcium (pCa; mmol/L) = plasma calcium (mmol/L) − ([0.02 × albumin (g/L)] + 0.8] above 2.70 mmol/L were randomly assigned to 0.6 (n = 53), 1.1 (n = 55), 2.0 (n = 105), 4.0 (n = 45), or 6.0 (n = 42) mg ibandronate given in a 2-h iv infusion on day 0. The hydration procedure was maintained throughout the first 7-day study period. During the observation period of 28 days, no new cytostatic treatment should have been administered. The administration of additional bisphosphonate or calcitonin resulted in the exclusion of these patients from this time on. The dosage of concurrent corticosteroids remained basically unchanged. The use of loop diuretics was allowed in case of clinically detected fluid overload or cardiac failure. The diagnosis of bone metastases was based on bone scintigram and/or x-ray examinations.

Laboratory investigation

Plasma levels of calcium, albumin, creatinine, phosphate, sodium, potassium, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were determined before ibandronate therapy and on day 3, 7, 14, 21, and 28 of treatment using standard automatic methods. In addition, calcium, phosphate, creatinine, and deoxypyridinoline were measured in the second morning urine spot after an overnight fast (16). Serum intact PTH and PTHrP concentrations were measured by immunoradiometric assays from Nichols Institute Diagnostics (San Juan Capistrano, CA) (17). In the PTHrP assay, the antibodies were directed against the amino-terminal part and a midportion of the molecule (17). Care was taken to prevent degradation of the hormone, by a cocktail of various antiproteases, which was immediately added after blood drawing. Samples were separated within 30 min, and plasma was kept frozen at −20 C. The presence of biologically active amino-terminal fragments, not detected by this assay (18), and/or an accelerated degradation of PTHrP by tumor-derived products, such as a proteolytic activity for PTHrP analogous to prostate-specific antigen (19), cannot be ruled out. The intraassay coefficients of variation were 9.5% and 2.9% at 1.1 and 9.4 pmol/L, respectively, whereas the interassay coefficients of variation were 5.6% and 5.3% at 7.3 and 31.5 pmol/L, respectively. Calcitriol was determined by RIA after purification by HPLC (20). Urinary deoxypyridinoline was measured by fluorometry after hydrolysis and separation by HPLC (21), and was expressed relative to urinary creatinine concentrations. The calcium to creatinine ratio corrected for GFR was taken as a reflection of net bone balance. A renal TRCaI was calculated from a nomogram relating fasting urinary calcium excretion per U glomerular filtration rate (GFR) and albumin-corrected plasma calcium (6). An increase in this index corresponds to a shift toward the right of the relationship between urinary calcium and plasma calcium (6).

Statistical analysis

The values are presented as the mean ± SEM. Differences at baseline were analyzed using a Wilcoxon, a Kruskal-Wallis, or a χ^2 test, depending on the scale of the variables. Changes between baseline and values on day 7 were evaluated with the Wilcoxon rank-sum test. Response rates were compared with the χ^2 test. The relationship between continuous variables or variables on an ordinal scale was analyzed with the Spearman rank correlation coefficient. The relative risk of relapsing hypercalcemia, as defined by an increase in albumin-corrected plasma calcium above 3.0 mmol/L was estimated in patients with prior response using a Cox proportional hazards model. The *P* values given result from the application of two-sided tests. $P < 0.05$ is considered to indicate significant differences in an exploratory sense.

Results

Baseline characteristics

Among the 315 patients first enrolled, breast cancer accounted for one of two cases of hypercalcemia of malignancy in females, whereas lung, upper respiratory, kidney, urinary, or digestive tracts constituted the vast majority of the histological types in males (Table 1). Overt bone metastases were detectable in at least 57% of the cases, with a higher prevalence in females.

TABLE 1. Patient baseline characteristics

	All	Male	Female
No. of patients	315 (147)	166	149
Age (yr; mean ± SEM)	58.1 ± 0.7	60.1 ± 0.8	55.9 ± 1.0[a]
Tumor type (no. of patients)			
Lung and upper respiratory tract	76 (33)	66	10
Breast	84 (43)	2	82
Hemopoietic system	36 (14)	19	17
Kidney, urinary, or digestive tracts, unspecified	119 (57)	79	40
Evidence of bone metastases			
No. of patients	180 (80)	81	99
%	57	49	66[a]

The number of patients with determination of plasma PTHrP concentration is in *parentheses*.
[a] $P < 0.01$ compared with males.

In the 300 patients analyzed at baseline after a median rehydration time of 48 h, the elevated pCa was associated with markedly increased fasting urinary calcium or deoxypyridinoline excretion, indicating a major contribution of elevated bone resorption to the imbalance of extracellular calcium homeostasis (Table 2). The renal TRCaI was above the upper limit of normal range in approximately two thirds of the patients. pCa was positively correlated to fasting urinary calcium and TRCaI ($r = 0.531$; $P < 0.0001$ and $r = 0.604$; $P < 0.0001$, respectively). PTH and calcitriol were at the lower limit of the normal range, whereas mean PTHrP levels were increased, with 53% (79 of 147 patients) having elevated values (>2.5 pmol/L). Patients with increased or normal PTHrP had similar plasma albumin concentrations, suggesting a comparable state of rehydration. The correlations between PTHrP levels and pCa were $r = 0.147$; $P = 0.0753$ and $r = 0.367$; $P = 0.001$ when only PTHrP values above 2.5 pmol/L were analyzed. These two groups displayed no differences in pCa, indexes of bone resorption, TRCaI, or the prevalence of increased levels of TRCaI (Table 2). However, patients with elevated PTHrP had lower plasma phosphate and creatinine and slightly higher calcitriol. Fasting urinary calcium excretion, taken as a reflection of net bone resorption, and TRCaI were weakly, but not significantly, correlated to PTHrP levels ($r = -0.138$; $P = 0.16$ and $r = 0.129$; $P = 0.2$, respectively).

As shown in Table 3, pCa was significantly different among the various tumor types ($P < 0.05$) and was higher in cancer of breast or hemopoietic system. Bone resorption appeared to be the highest in these two tumoral types, whereas mean TRCaI was above the upper limit of the normal range with a similar prevalence of elevated values in all groups. Mean levels of PTHrP, the prevalence of increased PTHrP, as well as calcitriol concentrations were significantly higher in cancer of the lung, upper respiratory, kidney, urinary, and digestive tracts. Patients with these histological types also displayed lower plasma phosphate levels.

TABLE 2. Baseline characteristics according to PTHrP levels

	Normal range	ALL	PTHrP <2.5 pmol/L	PTHrP >2.5 pmol/L
No. of patients		300	68	79
Albumin (g/L)	35–45	31.0 ± 0.3	30.7 ± 0.7	30.4 ± 0.6
Albumin-corrected calcium (mmol/L)	2.2–2.7	3.36 ± 0.02	3.44 ± 0.04	3.46 ± 0.04
Inorganic phosphate (mmol/L)	0.8–1.4	1.00 ± 0.02	1.08 ± 0.04	0.91 ± 0.04[a]
Creatinine (mmol/L)	0.05–0.12	0.12 ± 0.00	0.14 ± 0.01	0.10 ± 0.01[b]
PTHrP (pmol/L)	<2.5	4.9 ± 0.5	1.2 ± 0.1	8.0 ± 0.7[a]
Prevalence PTHrP >2.5 pmol/L (%)		53 (79/147)		
Calcitriol (pmol/L)	70–180	72.1 ± 3.1	66.3 ± 4.3	86.7 ± 6.6[c]
PTH (pmol/L)	1.0–6.0	1.7 ± 0.2	1.5 ± 0.3	0.9 ± 0.1
Fasting urinary calcium excretion (mmol/L GFR)	<0.045	0.20 ± 0.01	0.24 ± 0.02	0.18 ± 0.02
Urinary deoxypyridinoline/creatinine (nmol/mmol)	<18	53.7 ± 2.7	45.2 ± 4.6	58.7 ± 5.7
Tubular reabsorption of calcium index (mmol/L GFR)	2.40–2.90	3.09 ± 0.03	3.09 ± 0.05	3.20 ± 0.07
Prevalence tubular reabsorption of calcium index >2.90 (%)		65	68	73

Baseline serum values were obtained after rehydration. Values are the mean ± SEM. Significance is indicated compared with values in the subgroup with PTHrP below 2.5 pmol/L.

[a] $P < 0.01$.

[b] $P < 0.001$.

[c] $P < 0.05$.

TABLE 3. Baseline characteristics according to tumor type

	Normal range	Lung and upper respiratory tract	Breast	Hemopoietic system	Kidney, urinary, and digestive tracts and unspecified	P^a
No. of patients		61	76	34	129	
Albumin (g/L)	35–45	28.9 ± 0.6	32.9 ± 0.6	31.1 ± 1.2	30.8 ± 0.6	0.001
Albumin-corrected calcium (mmol/L)	2.2–2.7	3.26 ± 0.04	3.43 ± 0.05	3.42 ± 0.06	3.35 ± 0.04	
Inorganic phosphate (mmol/L)	0.8–1.4	0.88 ± 0.03	1.06 ± 0.04	1.40 ± 0.12	0.90 ± 0.03	0.001
Creatinine (mmol/L)	0.05–0.12	0.10 ± 0.00	0.12 ± 0.01	0.19 ± 0.02	0.11 ± 0.01	0.001
PTHrP (pmol/L)	<2.5	5.7 ± 0.6	4.0 ± 1.0	1.5 ± 0.3	5.9 ± 0.9	0.001
Prevalence PTHrP >2.5 pmol/L (%)		76	33	21	63	0.001
Calcitriol (pmol/L)	70–180	69.6 ± 5.9	62.1 ± 5.2	54.4 ± 5.9	87.3 ± 5.7	0.001
PTH (pmol/L)	1.0–6.0	1.2 ± 0.2	1.1 ± 0.2	1.7 ± 0.3	2.3 ± 0.6	
Fasting urinary calcium excretion (mmol/L GFR)	<0.045	0.14 ± 0.01	0.26 ± 0.02	0.24 ± 0.04	0.18 ± 0.02	0.001
Urinary deoxypyridinoline/creatinine (nmol/mmol)	<18	44.9 ± 4.0	68.6 ± 7.8	56.1 ± 8.8	49.0 ± 3.1	0.05
Tubular reabsorption of calcium index (mmol/L GFR)	2.40–2.90	2.97 ± 0.04	3.11 ± 0.05	3.11 ± 0.07	3.14 ± 0.05	
Prevalence tubular reabsorption of calcium index >2.90 (%)		57	66	57	72	

Values are the mean ± SEM.

[a] Analysis using a Kruskal-Wallis test for differences between the various tumor types or a χ^2 test.

Evolution after ibandronate therapy

Among the 264 patients who could be evaluated 7 days after the administration of the bone resorption inhibitor ibandronate, pCa decreased by 0.69 ± 0.03 mmol/L (20.0 ± 0.7%; $P < 0.001$). This was accompanied by a reduction in fasting urinary calcium excretion. TRCaI was slightly reduced by 0.30 ± 0.09 mmol/GFR. There was a 2.9 ± 0.8-fold increase in PTH. The 68 and 79 patients with initial PTHrP levels lower or higher than 2.5 pmol/L had similar responses, except for PTH, for which the increase was of significantly smaller magnitude in patients with elevated PTHrP (Table 4). A significantly greater response in terms of pCa was observed in cancers of breast or the hemopoietic system. The overall mean change in PTHrP levels was +0.7 ± 0.4 pmol/L (P = NS). An apparent, but not significant, decrease in PTHrP levels was found in 33% of the patients (26% in lung and upper respiratory tract and 36% in the other types; P = NS; Fig. 1).

The decrease in pCa was positively correlated with the reduction in fasting urinary calcium and deoxypyridinoline excretions (r = 0.423; $P < 0.001$ and r = 0.478; $P < 0.001$, respectively), and with the slight change in TRCaI (r = 0.555; $P < 0.001$). Variations in the initially elevated PTHrP values were positively correlated to changes in pCa (r = 0.535; P = 0.003). Variations in PTHrP were also correlated to changes in calcitriol (r = 0.665; P = 0.007) and in PTH (r = −0.489; P = 0.008). All of these correlations were adjusted for sex, age, tumor type, and absence or presence of bone metastases.

Recurrence of hypercalcemia

Factors predicting the relapse of hypercalcemia were assessed using a Cox proportional hazards model. The median time to relapse in tumors of the hemopoietic system was superior to the 28 days of follow-up, and this tumor type was thus considered as having a risk ratio of 1.0 (Table 5). Breast cancer had a slightly higher risk of relapsing, whereas the other tumor types clearly showed a shorter time before relapse and thereby a considerably higher risk ratio. Females and patients less than 50 yr old regardless of gender had a prolonged duration of remission and half the risk of relapsing. The association with other variables, including biochemical values such as PTHrP, albumin, calcitriol, PTH, severity of hypercalcemia at presentation, or indexes of bone resorption and tubular reabsorption of calcium, failed to reach a level of statistical significance.

Discussion

This large cohort of patients with hypercalcemia of malignancy allowed the evaluation of determinants of plasma calcium disturbances, with a wide range of calcium filtered loads, achieved by various doses of the bone resorption inhibitor ibandronate. An index of renal tubular reabsorption of calcium was elevated in 65% of the patients. This prevalence was slightly different from previous reports (5, 9, 22–24). An increased index was particularly prevalent in certain tumor types, such as cancers of lung and upper respiratory, urinary, or digestive tracts, in agreement with other studies performed in smaller numbers of patients (5–7, 25, 26). However, the use of historical control data derived from patients with primary hyperparathyroidism or from normal individuals for the calculation of TRCaI and creatinine clearance in severely ill patients for the evaluation of GFR should indicate the need for caution in the interpretation of TRCaI results. PTHrP, which is considered a major mediator of this syndrome, is known to stimulate renal tubular calcium reabsorption (8, 27, 28). Plasma levels of PTHrP could be measured in 147 patients at baseline. Elevated values were detectable in two thirds of the cases and were particularly associated with the same tumor types as those in which an elevated TRCaI was found. The large size of the cohort and the multicenter design allowed a relatively accurate estimate of the overall prevalence of PTHrP involvement in this syndrome.

Bone metastases were detectable in at least 50% of the cases, as shown by bone scintigram and/or conventional radiographs, which are techniques that certainly underestimate the true occurrence of skeletal lesions. In contrast, biochemical indexes of bone resorption were increased in the majority of the patients, suggesting the presence in the circulation of bone-resorbing substances acting upon bone

TABLE 4. Evolution on day 7 after ibandronate administration

	% of baseline values						
			Tumor type				
	PTHrP <2.5 pmol/L	PTHrP >2.5 pmol/L	Lung and upper respiratory tract	Breast	Hemopoietic system	Kidney, urinary, and digestive tracts and unspecified	P^a
No. of patients	24	21	54	70	33	106	
Albumin-corrected calcium	−24.3 ± 2.0	−19.6 ± 2.2	−17.2 ± 1.3	−21.6 ± 1.4	−24.8 ± 2.2	−18.8 ± 1.1	0.001
Inorganic phosphate	−24.1 ± 4.7	−20.3 ± 4.4	−13.4 ± 3.8	−15.7 ± 3.2	−24.7 ± 5.7	−10.0 ± 3.2	
Creatinine	−15.8 ± 4.2	−9.1 ± 2.5	−9.21 ± 2.4	−17.1 ± 2.3	−12.4 ± 4.6	−6.4 ± 2.2	0.01
PTHrP	26.6 ± 12.9	17.5 ± 8.9	16.8 ± 10.9	5.4 ± 11.3	30.6 ± 22.2	34.2 ± 15.8	
Calcitriol	33.7 ± 14.8	47.2 ± 9.1	63.5 ± 27.4	34.6 ± 14.5	39.6 ± 15.7	21.4 ± 11.3	
PTH	412.7 ± 54.2	114.9 ± 75.2[b]	107.0 ± 48.3	443.8 ± 57.7	469.8 ± 18.6	122.7 ± 38.0	
Fasting urinary calcium	−63.6 ± 16.8	−60.8 ± 7.6	−55.0 ± 7.1	−66.3 ± 6.5	−54.1 ± 11.0	−24.3 ± 23.3	
Tubular reabsorption of calcium index	−11.7 ± 3.4	−12.1 ± 3.1	−8.1 ± 1.9	−12.8 ± 1.9	−14.9 ± 2.7	−4.6 ± 6.4	

Values are the mean ± SEM and represent the percent change compared with baseline values.
[a] Analysis using a Kruskal-Wallis test for differences between the various tumor types.
[b] $P < 0.05$ compared with the subgroups with PTHrP values below 2.5 pmol/L.

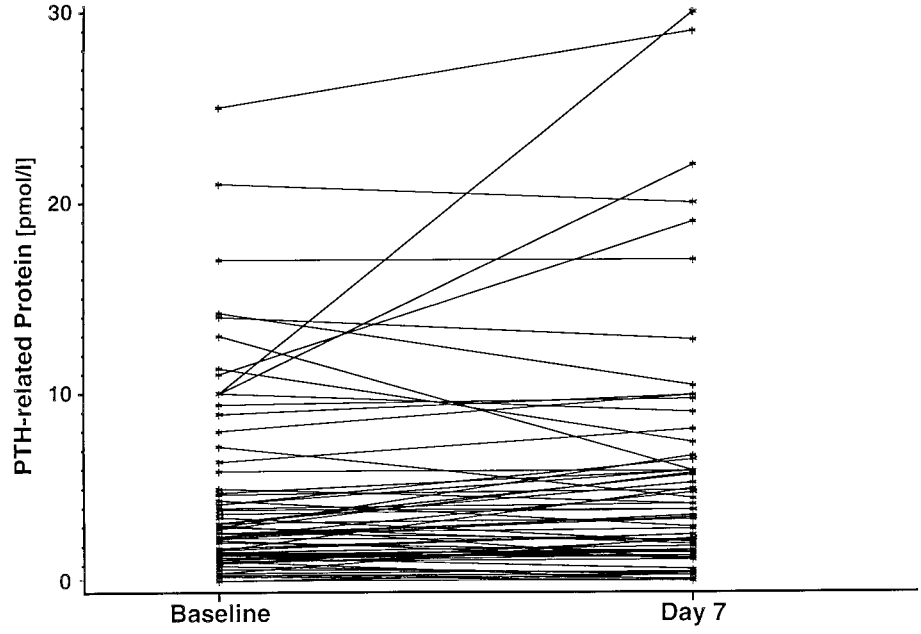

FIG. 1. PTHrP levels at baseline and 7 days after ibandronate therapy. The mean changes are presented in Table 4.

TABLE 5. Time to relapse and relative risk

	Time to relapse median (days)	Relative risk
Tumor type		
Hemopoietic system	>28	1.00
Breast	26	1.36
Kidney, urinary, and digestive tracts, and unspecified	15	2.23
Lung and upper respiratory tract	11	3.43
Sex		
Male	15	1.00
Female	25	0.55
Age		
<50 yr	26	1.00
>50 yr	16	1.52

Relapse was defined as an increase in albumin-adjusted plasma calcium above 3.0 mmol/L. Factors determining the risk of relapsing hypercalcemia were analyzed using a Cox hazards model.

through humoral mechanisms (1–4, 16). PTHrP and a variety of tumor-produced cytokines can be implicated. Under these conditions, we expected to find some correlation among pCa, indexes of bone resorption, and TRCaI, on the one hand, and circulating PTHrP levels, on the other. Despite the relatively large number of patients studied, these correlations showed only weak significant differences from zero.

To account for the poor correlation between function variables of extracellular calcium homeostasis and PTHrP concentrations, one should also remember that tumors are secreting a large variety of cytokines and growth factors capable of directly influencing bone remodeling and renal tubular transports (1–4, 11, 29). Among them, tumor necrosis factors or interleukins are implicated in cancer-mediated osteolysis, and interleukin-1 has been shown to stimulate the renal tubular reabsorption of calcium through a specific mechanism independent of PTH (11). The same cytokines and growth factors could also modulate the responsiveness to PTHrP in bone and kidney (29, 30). Indeed, the expression of the PTH/PTHrP receptor can be modified not only by cytokines and growth factors, known to be secreted by tumors, but also by extracellular matrix constituents (31). Interestingly, all of these factors have also been shown to be strong modulators of PTHrP production by tumor cells (32). Therefore, it is not surprising that PTHrP secreted locally in bone metastases could markedly stimulate bone resorption and not contribute to the levels of circulating PTHrP.

Ibandronate therapy led to a dose-dependent decrease in bone resorption and pCa. Calcemia was normalized in 44–78% of the patients, depending on the dose (14, 15). Although the various ibandronate regimens were equally distributed among the different tumor types, the variability of the response because of the different doses administered precluded the demonstration of a predictive value in terms of clinical or biochemical outcome, according to PTHrP levels, in contrast to previous reports (33–38). Our study performed on a large cohort of patients allowed us to estimate the risk of relapsing hypercalcemia according to tumor type. The risk of relapsing hypercalcemia was clearly related to the histological type. The tumors with the shortest time to relapse were precisely those with a high prevalence of detectable PTHrP and of elevated TRCaI. From a pathophysiological point of view, a faster relapse of hypercalcemia can easily be explained in the context of increased TRCaI. Indeed, under these conditions, any recurrent increase in bone resorption will be rapidly associated with a change in calcemia.

It has been consistently demonstrated that bisphosphonates, particularly ibandronate, are devoid of any direct effect on calcium renal tubular transport (27, 28). In the present study, a small, but significant, decrease in TRCaI was observed 7 days after ibandronate administration, accompanying the correction of pCa. There was a significant and positive correlation between changes in pCa or TRCaI and changes in PTHrP concentrations. Using different assays for PTHrP, several studies have addressed the issue of changes in PTHrP levels in relation to changes in calcemia (22, 33, 36–39). Indeed, *in vitro* experiments have demonstrated that

an increase in extracellular calcium stimulated PTHrP production in Leydig tumor cells (40), a phenomenon that is not consistently found in all tumor types (32). Except in one study in which the decrease in calcemia was accompanied by a statistically significant drop in PTHrP levels (38), mean PTHrP levels barely changed with the normalization of calcemia induced by bisphosphonate therapy when analyzed in whole groups (22, 33, 36, 38, 39). However, looking at individual responses, the PTHrP response appeared to be heterogeneous, with approximately one third of the cases displaying an apparent decrease in circulating levels (22, 39). In our study, a trend toward a decrease could be detected in 40% of breast cancers and in 26% of lung and upper respiratory tract malignancies (P = NS). However, the individual changes were close to the variation expectable from the assay coefficient of variation.

Considering these observations, the following hypothesis regarding association, but not cause, can be proposed. Decreasing bone resorption with ibandronate therapy lowered pCa. A decrease in PTHrP secretion in certain calcium-sensitive tumor types could be reflected by a change in the renal tubular reabsorption of calcium. This hypothesis, however, deserves further study in selected and homogenous cases of hypercalcemia of malignancy and also with accurate PTHrP assays.

Acknowledgments

We are indebted to Dr. J.-P. Bonjour, M.D., for fruitful discussion; we thank Dr. E. White, M.D., for reading the manuscript, and Mrs. M. Perez for secretarial assistance.

References

1. **Mundy GR.** 1987 The hypercalcemia of malignancy. Kidney Int. 31:142–155.
2. **Martin TJ, Grill V.** 1992 Hypercalcemia in cancer. J Steroid Biochem Mol Biol. 43:123–129.
3. **Bonjour JP, Rizzoli R.** 1989 Pathophysiological aspects and therapeutic approaches of tumoral osteolysis and hypercalcemia. Recent Results Cancer Res. 116:29–39.
4. **Ralston SH.** 1994 Pathogenesis and management of cancer associated hypercalcaemia. Cancer Surv. 21:179–196.
5. **Buchs B, Rizzoli R, Bonjour JP.** 1991 Evaluation of bone resorption and renal tubular reabsorption of calcium and phosphate in malignant and nonmalignant hypercalcemia. Bone. 12:47–56.
6. **Bonjour JP, Philippe J, Guelpa G, et al.** 1988 Bone and renal components in hypercalcemia of malignancy and responses to a single infusion of clodronate. Bone. 9:123–130.
7. **Thiébaud D, Jaeger P, Burckhardt P.** 1990 Response to retreatment of malignant hypercalcemia with the bisphosphonate AHPrBP (APD): respective role of kidney and bone. J Bone Miner Res. 5:221–226.
8. **Wysolmerski JJ, Broadus AE.** 1994 Hypercalcemia of malignancy: the central role of parathyroid hormone-related protein. Annu Rev Med. 45:189–200.
9. **Walls J, Ratcliffe WA, Howell A, Bundred NJ.** 1994 Parathyroid hormone and parathyroid hormone-related protein in the investigation of hypercalcaemia in two hospital populations. Clin Endocrinol (Oxf). 41:407–413.
10. **Bilezikian JP.** 1992 Clinical utility of assays for parathyroid hormone-related protein. Clin Chem. 38:179–181.
11. **Caverzasio J, Rizzoli R, Vallotton MB, Dayer JM, Bonjour JP.** 1993 Stimulation by Interleukin-1 of renal calcium reabsorption in thyroparathyroidectomized rats. J Bone Miner Res. 8:1219–1225.
12. **Fleisch H.** 1991 Bisphosphonates. Pharmacology and use in the treatment of tumour-induced hypercalcaemic and metastatic bone disease. Drugs. 42:919–944.
13. **Bonjour JP, Rizzoli R.** 1992 Antiosteolytic agents in the management of hypercalcemia. Ann Oncol. 3:589–590.
14. **Pecherstorfer M, Herrmann Z, Body JJ, et al.** 1996 Randomized phase II trial comparing different doses of the bisphosphonate ibandronate in the treatment of hypercalcemia of malignancy. J Clin Oncol. 14:268–276.
15. **Ralston SH, Thiébaud D, Herrmann Z, et al.** 1997 Dose-response study of ibandronate in the treatment of cancer-associated hypercalcaemia. Br J Cancer. 75:295–300.
16. **Body JJ, Delmas PD.** 1992 Urinary pyridinium cross-links as markers of bone resorption in tumor-associated hypercalcemia. J Clin Endocrinol Metab. 74:471–475.
17. **Pandian MR, Morgan CH, Carlton E, Segre GV.** 1992 Modified immunoradiometric assay of parathyroid hormone-related protein: clinical application in the differential diagnosis of hypercalcemia. Clin Chem. 38:282–288.
18. **Ratcliffe WA, Hutchesson ACJ, Bundred NJ, Ratcliffe JG.** 1992 Role of assays for parathyroid-hormone-related protein in investigation of hypercalcaemia. Lancet. 339:164–167.
19. **Cramer SD, Chen ZX, Peehl DM.** 1996 Prostate specific antigen cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. J Urol. 156:526–531.
20. **Scharla S, Schmidt-Gayk H, Reichel H, Mayer E.** 1984 A sensitive and simplified radioimmunoassay for 1,25-dihydroxyvitamin D3. Clin Chim Acta. 142:325–338.
21. **Black D, Duncan A, Robins SP.** 1988 Quantitative analysis of the pyridinium crosslinks of collagen in urine using ion-paired reversed-phase high-performance liquid chromatography. Ann Biochem. 169:197–203.
22. **Budayr AA, Zysset E, Jenzer A, et al.** 1994 Effects of treatment of malignancy-associated hypercalcemia on serum parathyroid hormone-related protein. J Bone Miner Res. 9:521–526.
23. **Bundred NJ, Ratcliffe WA, Walker RA, Coley S, Morrison JM, Ratcliffe JG.** 1991 Parathyroid hormone related protein and hypercalcaemia in breast cancer. Br Med J. 303:1506–1509.
24. **Grill V, Ho P, Body JJ., et al.** 1991 Parathyroid hormone-related protein: elevated levels in both humoral hypercalcemia of malignancy and hypercalcemia complicating metastatic breast cancer. J Clin Endocrinol Metab. 73:1309–1315.
25. **Body JJ, Dumon JC.** 1994 Treatment of tumour-induced hypercalcaemia with the bisphosphonate pamidronate: dose-response relationship and influence of tumour type. Ann Oncol. 5:359–363.
26. **Rizzoli R, Caverzasio J, Bauss F, Bonjour JP.** 1992 Inhibition of bone resorption by the bisphosphonate BM 21.0955 is not associated with an alteration of the renal handling of calcium in rats infused with parathyroid hormone-related protein. Bone. 13:321–325.
27. **Rizzoli R, Caverzasio J, Chapuy MC, Martin TJ, Bonjour JP.** 1989 Role of bone and kidney in parathyroid hormone-related peptide-induced hypercalcemia in rats. J Bone Miner Res. 4:759–765.
28. **Rizzoli R, Buchs B, Bonjour JP.** 1992 Effect of a single infusion of alendronate in malignant hypercalcaemia: dose dependency and comparison with clodronate. Int J Cancer. 50:706–712.
29. **Law F, Rizzoli R, Bonjour JP.** 1993 Transforming growth factor-β modulates the parathyroid hormone-related protein-induced responses in renal epithelial cells. Endocrinology. 133:145–151.
30. **Pizurki L, Rizzoli R, Caverzasio J, Bonjour JP.** 1991 Stimulation by parathyroid hormone-related protein and transforming growth factor of phosphate transport in osteoblast-like cells. J Bone Miner Res. 6:1235–1241.
31. **Hausmann S, Law FMK, Bonjour JP, Feyen J, Rizzoli R.** 1995 Regulation of parathyroid hormone/parathyroid hormone-related protein receptor expression by osteoblast-deposited extracellular matrix in a human osteoblast-like cell line. J Cell Physiol. 165:164–171.
32. **Rizzoli R, Feyen JHM, Grau G, Wohlwend A, Sappino AP, Bonjour JP.** 1994 Regulation of parathyroid hormone-related protein production in a human lung squamous cell carcinoma line. J Endocrinol. 143:333–341.
33. **Body JJ, Dumon JC, Thirion M, Cleeren A.** 1993 Circulating PTHrP concentrations in tumor-induced hypercalcemia: influence on the response to bisphosphonate and changes after therapy. J Bone Miner Res. 8:701–706.
34. **Dodwell DJ, Abbas SK, Morton AR, Howell A.** 1991 Parathyroid hormone-related protein **(50–69)** and response to pamidronate therapy for tumour-induced hypercalcaemia. Eur J Cancer. 27:1629–1633.
35. **Gurney H, Grill V, Martin TJ.** 1993 Parathyroid hormone-related protein and response to pamidronate in tumour-induced hypercalcaemia. Lancet. 341:1611–1613.
36. **Pecherstorfer M, Schilling T, Blind E, et al.** 1994 Parathyroid hormone-related protein and life expectancy in hypercalcemic cancer patients. J Clin Endocrinol Metab. 78:1268–1270.
37. **Walls J, Ratcliffe WA., Howell A, Bundred NJ.** 1994 Response to intravenous bisphosphonate therapy in hypercalcaemic patients with and without bone metastases: the role of parathyroid hormone-related protein. Br J Cancer. 70:169–172.
38. **Wimalawansa SJ.** 1994 Significance of plasma PTH-rp in patients with hypercalcemia of malignancy treated with bisphosphonate. Cancer. 73:2223–2230.
39. **Grill V, Murray RML, Ho PWM, et al.** 1992 Circulating PTH and PTHrP levels before and after treatment of tumor induced hypercalcemia with pamidronate disodium (APD). J Clin Endocrinol Metab. 74:1468–1470.
40. **Rizzoli R, Bonjour JP.** 1989 High extracellular calcium increases the production of a parathyroid hormone-like activity by cultured Leydig tumor cells associated with humoral hypercalcemia. J Bone Miner Res. 4:839–844.