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Levels of parathyroid hormone-related protein in hypercalcemia of malignancy: comparison of midregional radioimmunoassay and two-site immunoradiometric assay

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Summary. Overproduction of parathyroid hormone-related protein (PTHrP) is a major cause of hypercalcemia of malignancy in patients with solid tumors. We measured plasma levels of the protein by a radioimmunoassay (RIA) against PTHrP(53–84) and by an immunoradiometric assay (IRMA) against PTHrP(1–86). Of 16 affected patients 7 had elevated PTHrP levels in both assays and 4 had elevated levels in the RIA only. Median levels were about tenfold higher in these patients when measured by RIA (median of 34 versus 2.2 pmol/l). Measurements from both assays were, however, highly correlated with each other in this patient group ($P<0.01$). PTHrP was not elevated in 10 normocalcemic patients with lung carcinoma. During long-term follow-up of a patient with a mesothelioma of the pleura, PTHrP levels measured with both assays decreased during chemotherapy in parallel with a normalization of serum calcium. In another hypercalcemic patient suffering from renal carcinoma, PTHrP measured by IRMA decreased by 40% within 12 h after nephrectomy, whereas PTHrP measured by RIA did not show a significant decline. Direct comparison of the assay results thus pointed to the existence of heterogeneity of circulating forms of PTHrP in plasma. In conclusion, both immunoassays detected elevated levels of PTHrP in a fraction of patients with hypercalcemia of malignancy and thus may be a tumor marker during treatment of malignancies.

Key words: Hypercalcemia of malignancy – Parathyroid hormone-related protein – Serum calcium – Parathyroid hormones – Immunoassay

Parathyroid hormone-related protein (PTHrP) is now known to play a major role in the pathogenesis of the syndrome of humoral hypercalcemia of malignancy [1, 2, 6, 7, 9, 14, 24]. PTHrP and parathyroid hormone (PTH) have a similar amino acid sequence in their biologically active N-terminal ends, with comparable bioactivities on bone and kidney [8, 13, 17]. PTHrP mRNA, isolated from a variety of tumors, encoded PTHrPs with an identical common sequence but with lengths ranging between 139 and 173 amino acids [8, 15, 25]. Very little is known about the circulating forms of PTHrP in blood, their half-lives, and the influence of therapy on the plasma levels of these proteins. Since the known PTH-like biological activity of PTHrP is located within the first 34 amino acids, radioimmunoassay (RIAs) have been directed against the N-terminal sequence of PTHrP [7, 11, 12, 14, 19]. Subsequently, the more sensitive two-site immunoradiometric assay (IRMA) has been used to detect PTHrPs consisting approximately amino acid residues 1–74 and 1–86 [9, 18, 20]. These immunoassays for PTHrP were able to detect elevated levels of PTHrP in hypercalcemia of malignancy in up to 80–90% of patients [3, 22].

We have now evaluated the usefulness of PTHrP measurements in the detection and follow-up of patients with hypercalcemia of malignancy by using an established in-house RIA for the mid-regional sequence of PTHrP [4] and a recently introduced commercially available two-site IRMA.

Materials and methods

Measurements

PTHrP levels were determined by two different methods, RIA and IRMA. These assays detect different parts of the PTHrP molecule and do not

Abbreviations: PTHrP = parathyroid hormone-related protein; PTH = parathyroid hormone; RIA = radioimmunoassay; IRMA = immunoradiometric assay

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Table 1. Serum calcium, PTHrP, and PTH levels in 17 patients with hypercalcemia of malignancy and 11 normocalcemic patients with malignancy

Malignancy	Calcium (mmol/l)	PTHrP RIA (pmol/l)	PTHrP IRMA (pmol/l)	PTH intact (ng/l)
Normal range	2.10–2.65	<5–21	<0.5–2.6	10–65
Hypercalcemic				
Angiosarcoma	2.72[a]	63[a]	2.4	1.0[a]
Lung carcinoma (adeno)	2.83[a]	36[a]	2.1	11.3
Lung carcinoma (squamous)	3.40[a]	52[a]	8.4[a]	12.0
Lung carcinoma (squamous)	2.76[a]	55[a]	5.0[a]	2.7[a]
Lung carcinoma (squamous)	3.00[a]	77[a]	2.1	1.0[a]
Lung carcinoma (squamous)	2.81[a]	31[a]	3.2[a]	4.2[a]
Non-Hodgkin's lymphoma	2.74[a]	14	0.5	18.9
Bladder carcinoma	3.13[a]	58[a]	10.3[a]	–
Hepatocellular carcinoma	2.83[a]	16	1.6	17.6
Breast carcinoma	2.98[a]	40[a]	2.2	25.2
Breast carcinoma	2.90[a]	14	0.5	35.3
Breast carcinoma	3.75[a]	17	0.5	18.3
Mesothelioma	2.78[a]	31	3.1[a]	3.5[a]
Renal carcinoma	3.78[a]	333[a]	25.4[a]	18.9
Renal carcinoma	3.14[a]	34[a]	5.0[a]	14.0
Esophagus carcinoma	3.53[a]	14	1.1	3.3[a]
Pancreatic carcinoma	2.75[a]	1	0.5	4.4[a]
Normocalcemic				
Lung carcinoma	2.60	9	0.5	
Lung carcinoma (adeno)	2.48	6	1.4	
Lung carcinoma (small cell)	2.54	3	0.5	
Lung carcinoma (squamous)	2.55	3	0.5	
Lung carcinoma (squamous)	2.19	3	1.2	
Lung carcinoma (squamous)	2.22	12	1.7	
Lung carcinoma (squamous)	2.14	3	2.1	
Lung carcinoma (squamous)	2.37	19	1.6	
Lung carcinoma (squamous)	2.30	3	0.5	
Lung carcinoma	2.55	12	0.5	
Thymus carcinoma	2.40	20	0.5	

[a] Out of normal range

cross-react with PTH or with the N-terminal sequence 1–34 of PTHrP.

The RIA detects the midregional part of the protein, as described elsewhere [4]. The assay cross-reacts with PTHrP(1–86) and PTHrP(53–84) but not with the shorter synthetic peptides PTHrP(38–64) and PTHrP(67–86). In brief, 100 µl goat antiserum (1:8000) raised against amino acid residues 53–84 and 100 µl sample were incubated under nonqeuilibrium conditions. Synthetic human PTHrP(1–86) was used as a standard and for tracer preparation. Separation was performed by a second antibody against goat immunoglobulin. The assay has a detection limit of 5 pmol/l with a normal range of less than 5 to 21 pmol/l. The intraassay variation ranged from 4.3 to 12.6% (at 59 and 18 pmol/l), the interassay variation was between 4.2 and 20.3% (at 76 and 9.7 pmol/l). The assay was found to be useful in identifying patients with hypercalcemia caused by solid tumors [4].

The PTHrP two-site IRMA (Nichols Institute, San Juan Capistrano, CA) employs two polyclonal antisera, from goat and sheep, which have been purified by affinity chromatography and which are directed against the different epitopes PTHrP(1–40) and PTHrP(60–72). The N-terminal antibody is labeled with radioiodine and the other antibody is coupled to biotin, which allows capture of the formed complex by avidin-coated beads. The suggested upper limit of the normal range is 2.6 pmol/l; the limit of detection is 0.3 pmol/l; the intraassay variation ranged from 2.9 to 9.5% (at 9.4 and 1.1 pmol/l), and the interassay variation was between 5.3 and 5.6% (at 31.5 and 7.3 pmol/l; supplier's information). All samples measured with this assay had been obtained by using the provided

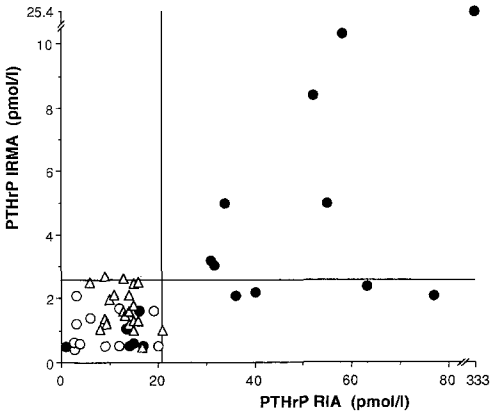

Fig. 1. Concentrations of PTHrP in serum or plasma of 21 normal subjects (△), 11 patients with malignancy and normal serum calcium (○), and 17 patients with hypercalcemia of malignancy (●). PTHrP was measured by RIA for the midregional sequence of the peptide (*x-axis*) and by two-site IRMA (*y-axis*). *Solid lines*, upper limits of the normal ranges

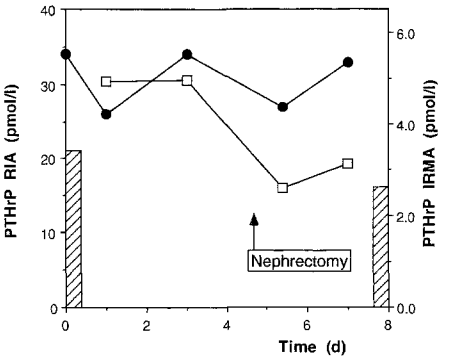

Fig. 2. Concentrations of PTHrP in serum or plasma of a 54-year-old patient with renal carcinoma without evidence of metastases or hypercalcemia of malignancy. PTHrP was measured before and after nephrectomy by RIA (●) and by two-site IRMA (□). *Hatched areas*, normal ranges

collecting tubes and a proteinase inhibitor mixture and by freezing separated plasma within 1 h. In our series all detected values below 1.0 pmol/l with this assay were assigned to 0.5 pmol/l for further evaluation.

Intact PTH was determined by an immunochemoluminometric two-site assay (normal range 10–65 ng/l; Ciba Corning, Fernwald, FRG).

Total serum calcium was determined by flame photometry (normal range 2.10–2.65 mmol/l).

Subjects

A total of 17 patients with hypercalcemia of malignancy were studied (11 men and 6 women, ages 35–73 years; serum calcium 3.05 ± 0.36 mmol/l). A total of 16 patients had solid tumors, and one suffered from a non-Hodgkin's lymphoma; the tumor types are summarized in Table 1. Underlying primary hyperparathyroidism as another cause for hypercalcemia was excluded by simultaneously measuring intact PTH in all except one patient, from whom no PTH value was available. PTHrP was also measured in 11 normocalcemic patients with malignancy, 10 of them suffering from lung carcinoma (7 men and 4 women; ages 47–71 years; Table 1). The normal ranges of both assays were confirmed by measuring PTHrP in 21 control subjects (14 women and 7 men, ages 29–74 years) without any evidence of calcium metabolism disorders (Fig. 1).

Results

The biochemical profiles of the 28 patients with malignancy are shown in Table 1. Of the 17 patients with hypercalcemia of malignancy, 16 had solid malignancies, 11 of the 16 (69%) having elevated PTHrP levels in the RIA and 7 (44%) having elevated levels in the IRMA (Fig. 1). These 7 patients also had elevated RIA values, whereas in 4 patients only RIA PTHrP levels were elevated. The patient with a hematologic malignancy (non-Hodgkin's lymphoma) had normal PTHrP levels in both assays. The median of the PTHrP RIA values was 34 pmol/l (1.6-fold above the upper limit of the normal range), whereas the median of the PTHrP IRMA values was 2.2 pmol/l (about 0.8-fold above the upper limit of the normal range). The PTHrP levels obtained with these two assays were highly correlated ($n=17$; $P=0.0011$, Kendall rank correlation coefficient). The values of the two assays, however, did not correlate significantly with the serum calcium values.

By contrast, PTHrP levels were still within the normal ranges of both assays in all normocalcemic patients with malignancy (Fig. 1). The PTHrP levels of 21 normal subjects were also within the expected normal ranges of both assays (Fig. 1). Six patients with primary hyperparathyroidism all had normal PTHrP levels as determined with both assays (data not shown).

In one patient with hypercalcemia of malignancy, it was possible to measure PTHrP before and after removel of the tumor (Fig. 2). The patient suffered from a renal carcinoma without evidence of metastases. Preoperatively, he had serum calcium values between 2.9 and 3.2 mmol/l, which be-

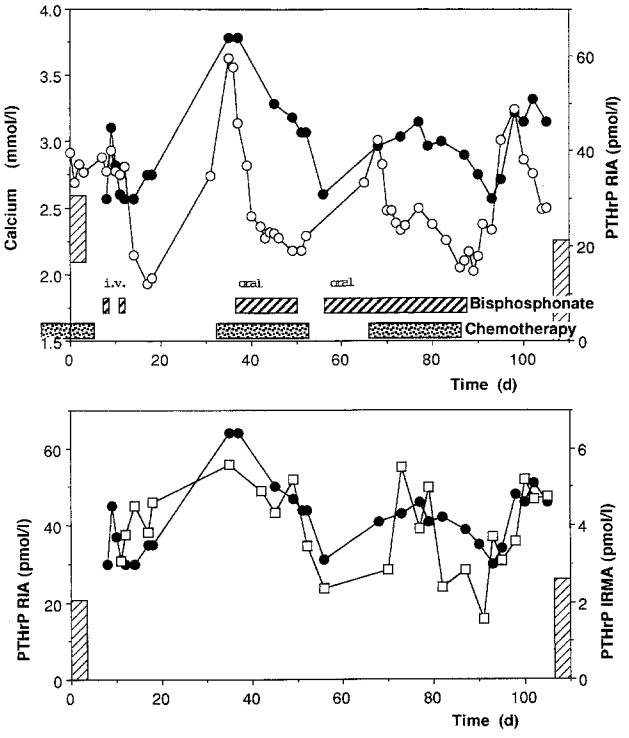

Fig. 3. Clinical course of a 63-year-old patient with pleuramesothelioma and hypercalcemia of malignancy. *Filled blocks* (*upper panel*), time periods of therapeutic intervention to lower serum calcium either by chemotherapy or by therapy with a bisphosphonate. Total serum calcium concentrations (○) are shown (*upper panel*). PTHrP was measured by RIA (●, identical curve in *upper*, *lower panels*) and by two-site IRMA (□, *lower panel*). *Hatched areas* normal ranges

came normal after nephrectomy. Midregional PTHrP, as measured by RIA, was elevated and remained high up to 60 h after the operation (the last time point from which a sample was available); the levels fluctuated between 26 and 34 pmol/l. By contrast, PTHrP as measured with the IRMA fell significantly from a preoperative level of 5.0 pmol/l to 2.6–3.1 pmol/l 12–60 h postoperatively (Fig. 2).

In another patient with hypercalcemia of malignancy, we were able to study PTHrP levels during chemotherapy over 3 months (Fig. 3). This 63-year-old patient had a mesothelioma of the pleura and received several cycles of chemotherapy with etoposide and a calcium-lowering therapy with various bisphosphonates administered intravenously. As was the case with other patients [5], the bisphophonate given on days 8 and 11 normalized serum calcium but had no sustained influence on PTHrP levels. Bisphosphonates later combined with chemotherapy, however, resulted in a temporary substantial decline in PTHrP levels accompanied by a fall in serum calcium (Fig. 3, upper panel) while tumor growth showed no change. PTHrP RIA values remained elevated throughout the entire follow-up period, whereas PTHrP IRMA values fluctuated and even became normalized on some occasions (Fig. 3, lower panel).

Discussion

Both PTHrP immunoassays were clinically useful in identifying patients with hypercalcemia associated with solid tumors. With the RIA, 69% of these patients had elevated PTHrP levels, whereas the IRMA showed elevated PTHrP in only 44% of patients. This is comparable with the results of other assays directed against different sequences of PTHrP in which 48% [14] to 69% [7] of patients with solid tumors and hypercalcemia were identified. Recently published results obtained with two-site assays were superior, however, in detecting elevated levels of PTHrP(1–74) in approximately 80% [9] or elevated PTHrP(1–86) in 90–95% of similarly affected patients [18, 20].

A practical advantage of the midregional PTHrP RIA is the stability (several hours) of this fragment in serum and plasma after collection. By contrast, PTHrP, as measured by two-site assays, degrades rapidly in collected samples with a half-life of less than 4 h [20] and must therefore be collected in tubes containing proteinase inhibitors [18].

Although the two-site assay used in our study was about one order of magnitude more sensitive than the RIA, the upper limits of the normal range were similar concerning their clinical relevance (2.6 versus 21 pmol/l), which might have contributed to the similar performance of the two assays. Neither of the two assays detected elevated PTHrP levels in normocalcemic patients with malignancies. PTHrP levels that were found to be elevated by only one of the two assays might be a result of different secretion patterns from the tumors. Circulating forms of PTHrP, especially those not containing the N-terminal sequence, have been insufficiently characterized by immunoassays so far [23]. Immunoassayable PTHrP(56–86) was found to circulate in levels up to 35 ng/l [16], whereas an RIA against PTHrP(37–67) recognizing residues 52–61 detected much higher concentrations (320 pmol/l) in normals [21]. Therefore, several different fragments of PTHrP seem to circulate in the blood.

In this study the influence of tumor removal and chemotherapy as two different means to reduce tumor mass and therefore serum PTHrP levels were investigated. In a patient with a renal carcinoma. PTHrP levels, as measured by IRMA, fell

by 40–50% within 12 h after nephrectomy whereas midregional PTHrP remained elevated for at least 2 days. This might indicate longer biological half-lives of PTHrPs detected by the midregional RIA. No data about half-lives of circulating PTHrPs are available from the literature, in part due to the fact that patients with hypercalcemia of malignancy usually have advanced disease and are seldom candidates for surgery. Chemotherapy, however, was also able to lower circulating levels of PTHrP and subsequently serum calcium, although no change was seen in the observation period. Similar results have also been shown by others [16]. The decline in PTHrP levels during our combined chemotherapy and bisphosphonate treatment must be attributed to the chemotherapy, since bisphosphonates have been shown to be unable to lower PTHrP levels [5]. The levels of PTHrP, as measured by both assays, changed in parallel, with greater fluctuation in the IRMA results. Again, this might point to shorter half-lives of PTHrPs detected by this assay. The role of PTHrP as a tumor marker in this setting remains to be established. One possible use of measuring PTHrP, which has already been demonstrated, is the ability to predict the therapeutic effectiveness of bisphosphonates in individual patients with hypercalcemia of malignance [5, 10].

In conclusion, both PTHrP immunoassays were able to detect elevated levels in a fraction of patients with hypercalcemia of malignancy. It appears that different patterns of circulating forms of PTHrP may exist in individual patients. Using PTHrP as tumor marker, follow-up of patients undergoing surgery or chemotherapy may be improved.

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