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A Case of Leiomyosarcoma Associated with Humoral Hypercalcemia of Malignancy: Demonstration of Biological and Immunological Activities of Parathyroid Hormone-related Protein in the Tumor Extract

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Hypercalcemia occurred in a patient with leiomyosarcoma when multiple lung metastases developed. Despite normal plasma parathyroid hormone (PTH) levels and low 1,25-dihydroxyvitamin D, this hypercalcemic patient had a marked hypercalciuria and phosphaturia associated with an increased excretion of nephrogenous cyclic AMP (NcAMP). Administration of cisplatin ameliorated both the hypercalcemia and hypercalciuria without any reduction in tumor size or NcAMP excretion. Terminally, acute pancreatitis occurred producing a profound hypocalcemia. In the extract of tumor tissue obtained post mortem, bioactivity stimulating the generation of cyclic AMP in osteogenic cells was demonstrated along with the immunoreactive PTH-related protein (PTH-rP). This is the first report of a solid non-epithelial malignancy producing PTH-rP and associated with humoral hypercalcemia of malignancy. The hypercalcemia in this case caused acute pancreatitis, which led to a profound hypocalcemia.

Key words: Leiomyosarcoma — Humoral hypercalcemia of malignancy — Parathyroid hormone-related protein — Cisplatin — Acute pancreatitis

About 75% of cases of malignancy-associated hypercalcemia (MAHC) seem to be caused by humoral hypercalcemia of malignancy (HHM).[1)] HHM is a pathological condition in which a tumor-generated hormone-like factor, or factors, is responsible for the hypercalcemia. A peptide closely related to parathyroid hormone (PTH), PTH-related protein (PTH-rP), is a causative factor of abnormal calcium (Ca) metabolism in most HHM cases.[2)] Based upon increased excretion of nephrogenous cyclic AMP (NcAMP), Godsall *et al.*[1)] reported that squamous cell and urothelial carcinomas were the most frequent malignancies causing HHM in their series of MAHC. Except for hematological malignancies and a mesothelioma, the tumors included in their HHM group were exclusively of epithelial origin.

We report here the first case of leiomyosarcoma, a solid non-epithelial malignancy, producing immunologically detectable PTH-rP with biological activity. Furthermore, the effects of cisplatin on Ca metabolism and acute pancreatitis, as a complication of HHM, are also discussed.

MATERIALS AND METHODS

All serum Ca values were corrected based on the respective serum albumin level according to Payne *et al.*[3)] Cisplatin (100 mg) was infused over a period of 2 h along with 300 ml of 20% mannitol. The administration of cisplatin was preceded by 1,500 ml of saline and 5 mg of betamethasone, and followed by 1,000 ml of half saline. Several days before and after the cisplatin treatment, fasting 2 h urine was collected and venous blood was obtained at the midpoint of each urine sampling. Tumor tissue obtained at autopsy was extracted with acid/urea followed by ethanol/NaCl fractionation according to Burtis *et al.*[4)] PTH-like adenylate cyclase-stimulating activity was examined by measuring cyclic AMP production of the osteogenic cell line MC3T3E1.[5)] Cyclic AMP was measured by radioimmunoassay (Yamasa Shouyu Co., Chiba). PTH-rP in the urea extract of the tumor tissue was measured by radioimmunoassay employing a specific antibody raised against human (h) PTH-rP (1-34).[6)] Synthetic hPTH-rP(1-34) was purchased from Peninsula Laboratories (Belmont, CA) and employed as a tracer and assay reference. Radioimmunoassay of PTH, using an antibody raised against the mid-region of PTH, and measurement of vitamin D metabolites were kindly performed by Mitsubishi Yuka BCL Co., Tokyo.

CASE REPORT

A 62-year-old man was admitted to the hospital on the 10th of October, 1987, because of exertional dyspnea and paresthesia on the right leg. In March, 1977, he had felt

pain and swelling of the right leg, but the symptoms remitted spontaneously. They recurred in June, 1980, and he visited another hospital. A 10×9 cm tumor on the right lower leg was found and resected; the diagnosis was leiomyosarcoma. The serum Ca was 8.8 mg/dl and phosphorus was 2.4 mg/dl just prior to the operation. He was well until December, 1986, when an abnormal shadow was detected on routine chest X-ray examination. He revisited the hospital where multiple metastatic pulmonary coin lesions were detected. He also had hypercalcemia (12.4–14.6 mg/dl) and hypophosphatemia (1.7–1.8 mg/dl). After several courses of anti-cancer chemotherapy, he was referred to our hospital.

Physical examination was unremarkable except for moderate anemia and paresthesia at the operation scar on the right leg. The total serum protein was 6.3 g/dl (albumin 3.5 g/dl), serum aspartate aminotransferase 17 U/liter (normal 8 to 40), lactic dehydrogenase 192 U/liter (normal 100 to 225), and alkaline phosphatase 91 U/liter (normal 30 to 110). The urea nitrogen was 32 mg/dl, creatinine 1.2 mg/dl, Na 138 meq/liter, K 4.3 meq/liter, Cl 102 meq/liter, Ca 13.7 mg/dl and P 2.3 mg/dl. The arterial blood gas analysis revealed PaO_2 89.1 Torr, $PaCO_2$ 30.7 Torr, HCO_3^- 19.9 meq/liter and pH 7.424. The urinalysis was normal, but the glomerular filtration rate (GFR) was 42 ml/min. The chest X-ray film showed multiple nodular lesions and the pyelogram demonstrated calcification of the pelvis. The bone scan showed a generalized increase in the uptake of ^{99m}Tc methylene diphosphonate.

Oral hydration was recommended and the 24 h urine volume was maintained between 1,900 and 2,500 ml. On the ninth hospital day, a course of cisplatin therapy was undertaken aiming at reduction of the tumor mass and serum Ca level. The plasma Ca was successfully decreased to less than 12 mg/dl for more than three weeks with no obvious reduction of the tumor size.

After another course of cisplatin therapy, he was discharged at the end of December; however, he returned to the hospital four months later because of dyspnea resulting from acute epiglossial edema due to neck phlegmon. Granulocytosis (white blood cell count of 19,500/mm^3) and hypercalcemia of 16.9 mg/dl were measured. *Staphylococcus aureus* was cultured from the neck lesion. Respiration was supported by an intratracheal tube. Antibiotics and synthetic eel calcitonin (Elcitonin, Toyo Jozo Co., Tokyo) were administered along with sufficient amounts of fluid. Pulmonary edema and acute pancreatitis developed and he died on the 16th of April, 1988. A dramatic fall of plasma Ca associated with the onset of pancreatitis, identified by an increase in serum amylase level, is depicted in Fig. 1.

Autopsy revealed a wide area of hemorrhagic necrosis and abscess formation at the bilateral larynx extending to the right thyroid. Pulmonary edema, bilateral pleural effusion and multiple metastatic pulmonary lesions were observed. The necrotic peritoneal and peripancreatic adipose tissue was extensively calcified and an acute pancreatitis was evident. None of the three examined para-

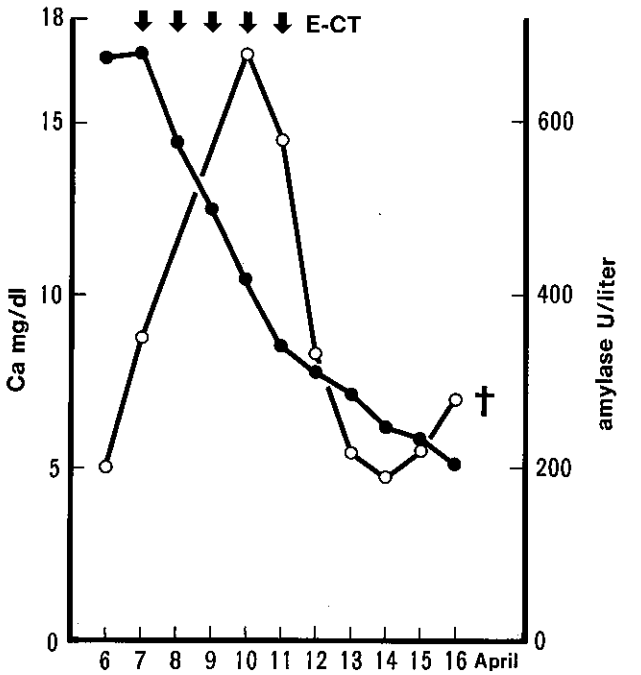

Fig. 1. Changes in serum Ca (●) and amylase (○) observed terminally in association with acute pancreatitis. E-CT denotes synthetic eel calcitonin administered to treat hypercalcemia.

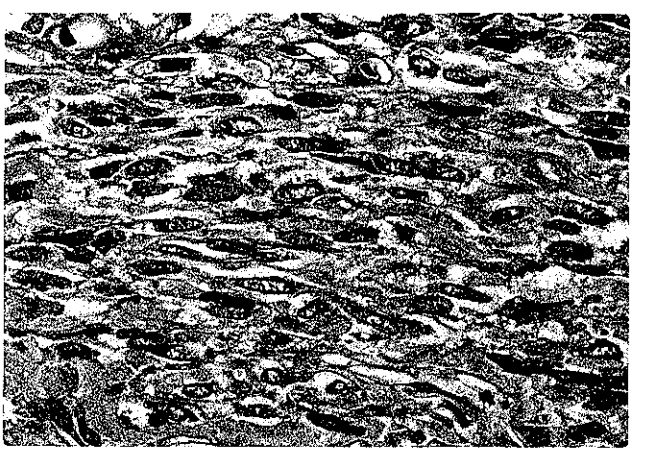

Fig. 2. Photomicrograph of the pulmonary metastatic tumor showing an array of spindle-shaped malignant cells with blunt-ended nuclei. (H-E staining, ×400)

thyroid glands was enlarged. Histopathologically, the lung tumor consisted of spindle-shaped malignant cells with blunt-ended nuclei (Fig. 2). Silver staining demonstrated a fine basement membrane network typical of leiomyosarcoma. The histology was entirely consistent with that of an operated specimen resected in 1980 at another hospital.

RESULTS

The laboratory data related to Ca metabolism are summarized in Table I. Although the plasma level of PTH was not increased and the 1,25-dihydroxyvitamin D ($1.25(OH)_2D$) level was decreased, we noted hypercalcemia, fasting hypercalciuria, phosphaturia and an increased excretion of NcAMP.

Changes in chemical data during a course of cisplatin therapy are presented in Fig. 3. Although the hypercalcemia and fasting hypercalciuria were ameliorated after cisplatin administration, the phosphaturia and NcAMP excretion remained elevated.

The tumor extract increased cyclic AMP in MC3T3E1 cells dose-dependently and the dose-response relationship was parallel to that of hPTH-rP (1-34) (Fig. 4A).

A radioimmunoassay employing specific antibody raised against hPTH-rP(1-34) detected 23 ng of hPTH-rP in 1 g of wet tissue. The dilution curve of the sample was parallel to the standard curve of synthetic hPTH-rP (1-34) (Fig. 4B). The immunological activity of PTH measured by a mid-region assay was less than 1 ng per g wet tissue.

DISCUSSION

This case presents three important clinical observations. First, HHM was observed in a patient with a solid tumor of non-epithelial origin, and PTH-rP was detected in the tumor tissue. Second, cisplatin administration decreased the serum Ca level without a decrease in NcAMP. And third, acute pancreatitis was provoked by hypercalcemia and led him to be hypocalcemic.

Development of hypercalcemia in patients with non-hematological non-epithelial malignancy is very rare. None were included in a review by Blomqvist[7] and only one mesothelioma was reported in a series by Godsall *et al.*[1] In a few pediatric cases, rhabdomyosarcoma is known to induce diffuse trabecular bone destruction due to massive metastatic bone marrow involvement, resulting in hypercalcemia.[8] These cases can hardly be classified as HHM. Of these hypercalcemic cases three had elevated serum immunoreactive PTH levels[9, 10] and one showed the increased prostaglandin E_2 production by tumor cells,[11] but neither NcAMP nor bioactive or immunoreactive PTH-rP in their tumor extracts has been examined.

Table I. Laboratory Data Concerning Hypercalcemia

Serum Ca	13.7 mg/dl	(8.5–10.2)
Fasting Ca excretion	0.5 mg/dlGF	(<0.12)
$TmPO_4/GFR$	0.9 mg/dl	(2.3–4.5)
NcAMP	8.8 nmol/dlGF	(0.8–2.8)
mid-region PTH	280, 450 pg/ml	(300–1000)
25(OH)D	7.6 ng/ml	(12–62)
$1,25(OH)_2D$	17.8 pg/ml	(20–50)

The normal ranges are shown in parenthesis.
Abbreviations: $TmPO_4/GFR$, transport maximum of phosphate reabsorption; GFR, glomerular filtration rate; NcAMP, nephrogenous cyclic adenosine monophosphate; PTH, parathyroid hormone; D, vitamin D; GF, glomerular filtrate.

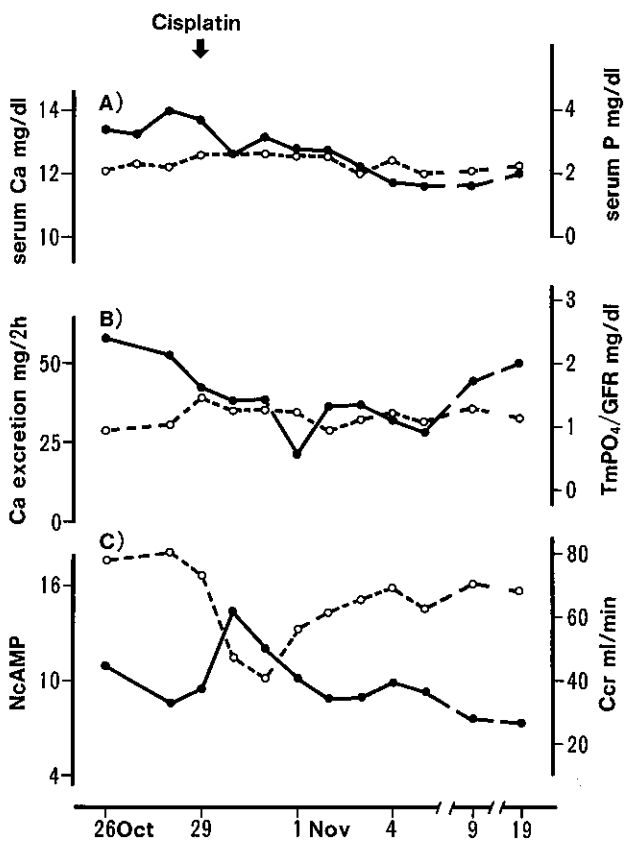

Fig. 3. Effect of cisplatin administration on the metabolism of Ca, P and NcAMP. Chemical analyses were performed on the fasting 2 h urine and venous blood sampled at the mid-point of each urine collection. A) serum Ca (●) and P (○), B) Ca excretion (●) and $TmPO_4/GFR$ (○), C) NcAMP (●) and creatinine clearance (○).

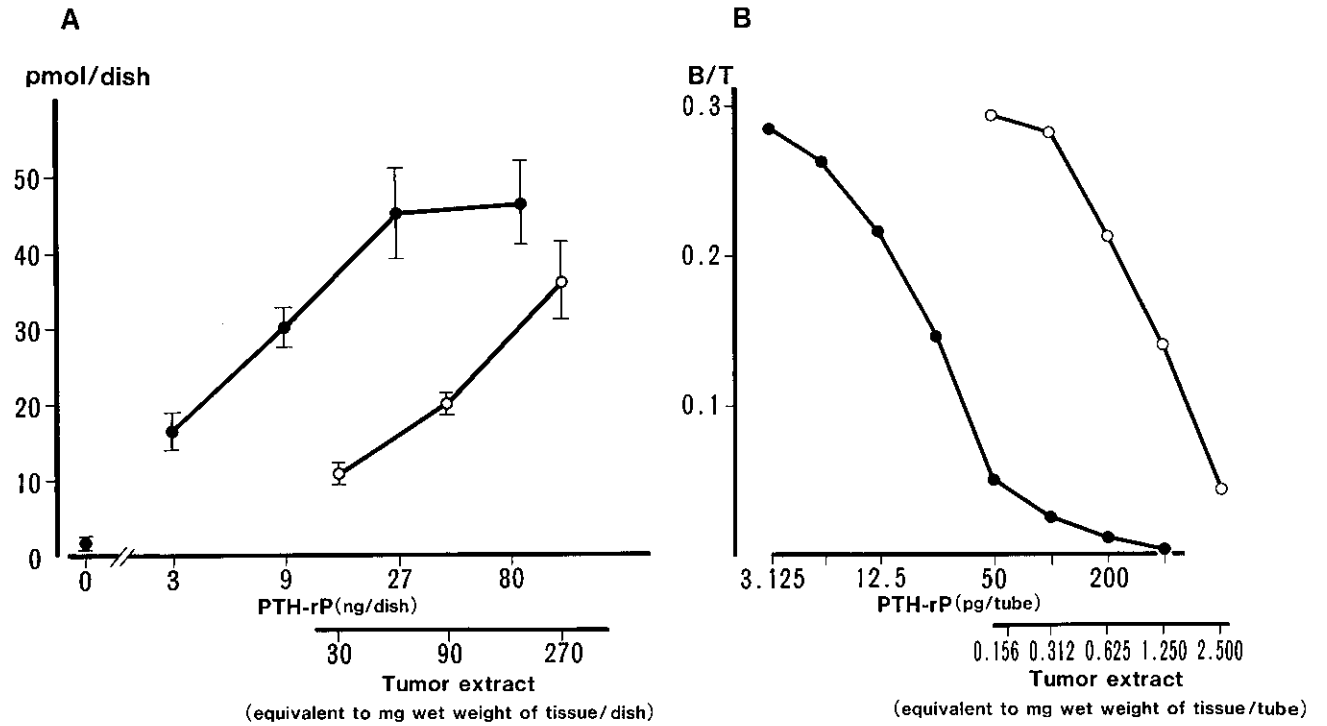

Fig. 4. Biological and immunological activities of the tumor extract (○) as compared with synthetic hPTH-rP (●). A) Dose-response curve in the production of cyclic AMP in MC3T3E$_1$ cells. Each mean±SD is of 3 incubations. B) Dose-response curve in radioimmunoassay of hPTH-rP(1-34).

Thus, to our knowledge, this is the first well defined case of HHM associated with leiomyosarcoma or even with a solid non-epithelial malignancy. The patient was normocalcemic when the tumor was limited to his leg and hypercalcemia developed with multiple pulmonary metastases. The increased production of NcAMP and decreased tubular threshold of phosphate reabsorption suggested an increased PTH action on the kidney, but the plasma immunoreactive PTH level was not increased and the plasma concentration of 1,25(OH)$_2$D was decreased. These clinical chemistry data along with the unremarkable bone scintigram indicate that the hypercalcemia was caused through the mechanism of HHM. Actually a bone cell adenylate cyclase-stimulating activity was detected in the tumor extract, which was found to contain a marked immunoreactive hPTH-rP activity with a negligible immunoreactive PTH activity. It is most likely that PTH-rP produced by the tumor tissue caused hypercalcemia in this patient, but the possibility that other factor(s) contributed to the hypercalcemia can not be excluded completely. These findings suggest that PTH-rP is produced not only in tumors of epithelial origin but also in those of non-epithelial origin, even though the latter situation is very rare. Recently, PTH-rP was shown to be pathogenetic in the hypercalcemia of adult T-cell leukemia/lymphoma[6,12] and probably of a canine lymphosarcoma.[13]

Cisplatin has been shown to have an antihypercalcemic effect in MAHC,[14] and decreased plasma Ca in the present study. The hypocalcemic effect was not accompanied by a decrease in NcAMP, indicating that the effect of lessening hypercalcemia was not through the reduced tumor production, or release, of PTH-like adenylate cyclase-stimulating factor, namely PTH-rP. Since the fasting hypercalciuria was also suppressed by cisplatin, the hypocalcemic effect was probably exerted through a direct inhibition of bone resorption. Other cytotoxic agents such as mithramycin[15] and actinomycin D[16] possess hypocalcemic activities not mediated via tumor destruction. It is probable that osteoclasts, especially those having been stimulated by a bone resorbing factor such as PTH-rP, are sensitive to the cytotoxic effects of anti-cancer drugs.[17]

Acute pancreatitis is listed as one of the complications of primary hyperparathyroidism with an incidence of 1.5 to 19%.[18] Although not conclusive, the association is not due to PTH excess *per se*, but is rather due to hypercalcemia resulting in activation of digestive en-

zymes. The hypercalcemia during parenteral nutrition,[19] or attributable to Ca infusion,[20] vitamin D intoxication,[21] myeloma,[22] disseminated breast cancer[23] or severe hyperthyroidism,[24] has sometimes been associated with pancreatitis. However, we are not aware of any report on the association between acute pancreatitis and HHM. Whether PTH-rP itself had any role in developing pancreatitis in addition to hypercalcemia remains to be elucidated.

Acute pancreatitis is a well known cause of acute hypocalcemia; it develops in about one-third of the patients.[18] Although hypocalcemia associated with acute pancreatitis appears to have multiple etiologies, it is generally agreed that saponification of Ca in the pancreatic bed is insufficient to explain the hypocalcemia.[25] However, the hypocalcemia developed in the present case may have occurred through that mechanism because of the massive Ca-soap formation found in the necrotic fat tissue at autopsy. It is at least clear that insufficient secretion of PTH associated with pancreatitis, which was proposed as a mechanism of hypocalcemia,[26] does not apply to the present situation, where the high serum Ca level had been maintained by PTH-rP produced by the tumor.

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