

Role of Prostaglandins in the Production of Hypercalcemia by Tumors¹

Armen H. Tashjian, Jr.²

Laboratory of Pharmacology, Harvard School of Dental Medicine, and Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

Abstract

This report summarizes the data from two animal and cell culture systems that serve as models that show how certain malignant tumors produce hypercalcemia by means of a humoral mechanism. Studies with the HSDM₁ murine fibrosarcoma and the VX₂ carcinoma in the rabbit have led to the conclusion that these two tumors produce hypercalcemia in the host by means of a mechanism that utilizes prostaglandin E₂ as the mediator between the neoplasm and bone. Analogous or identical mechanisms may operate in a small number of human tumors.

Introduction

Hypercalcemia is a common complication in patients with cancer. It occurs most frequently when there are overt metastatic deposits of tumor in bone. In this situation, localized bone resorption produced by tumor cells themselves or by products of neoplastic cells which stimulate osteoclastic resorption results in the excessive liberation of osseous calcium into extracellular fluid and, hence, hypercalcemia. However, in a significant fraction of patients with cancer and hypercalcemia, no overt or detectable bone metastases are found, and the cause of the hypercalcemia appears to be humoral. In such cases removal of the bulk of tumor leads to remission of the hypercalcemia and recurrence of tumor to the reappearance of hypercalcemia. Several mediators of such humoral hypercalcemic syndromes have been postulated, but few have been documented by experimental or clinical studies. Among those for which current evidence is most compelling are tumors of nonendocrine glands, which produce inappropriately or ectopically parathyroid hormone (11), and tumors of lymphatic or myeloma cell origin, which produce a substance(s) called osteoclast activating factor (8, 10). Other possible mediators, such as vitamin D metabolites and osteolytic sterols, have been proposed as possible humoral mediators, but their occurrence has not been rigorously demonstrated. Clinical observation and laboratory studies indicate that tumors which produce parathyroid hormone or osteoclast activating factor constitute only a fraction of cancers associated with the humoral hypercalcemic syndrome. Therefore, in order to understand the pathophysiology of this disorder, we have been studying 2 animal models which bear a number of relevant similarities to the human syndrome. Results of a series of studies on the cause of the hypercalcemia that occurs in mice bearing

the HSDM₁ fibrosarcoma and in rabbits carrying the VX₂ carcinoma have led to the conclusion that these 2 tumors synthesize and secrete into plasma large amounts of PGE₂.³ This prostaglandin is a potent bone resorption-stimulating agent which, when secreted in sufficient quantity, can act on the skeleton systemically to enhance bone resorption and inhibit bone formation, thus leading to hypercalcemia. It is the purpose of this report to summarize briefly the evidence concerning a role for prostaglandins in the pathogenesis of certain of the humoral hypercalcemias associated with malignant disease. Emphasis is given to studies on the HSDM₁ and VX₂ animal model systems, and no attempt has been made to review the broader subject of the general interrelationships between prostaglandins and calcium metabolism.

HSDM₁ Fibrosarcoma

The transplantable mouse fibrosarcoma HSDM₁ produces an elevation of the plasma calcium concentration within about 2 weeks of tumor implantation at a s.c. or i.m. site (23). The tumor neither metastasizes to bone, nor appears to invade bone locally. The hypercalcemia remits when the tumor is excised surgically.

The tumor produces a potent bone resorption-stimulating factor which can be measured quantitatively in an *in vitro* bone organ culture assay (14, 23). The factor can be extracted from the tumor tissue and harvested from the medium of clonal strains of HSDM₁ cells grown in dispersed cell culture (7, 20). With the use of serological methods, it has been shown that HSDM₁ cells synthesize and secrete large quantities of PGE₂ (7, 23). All of the bone resorption-stimulating activity present in aqueous extracts of HSDM₁ tumors or secreted by HSDM₁ cells in culture can be transferred into diethyl ether at low pH, and the biological activity can be accounted for quantitatively by the PGE₂ content of the tumor extracts or harvested cell culture medium (14, 20, 23). Indomethacin, 5,8,11,14-eicosatetraenoic acid, and hydrocortisone, potent inhibitors of PGE₂ synthesis in HSDM₁ cells (19, 20, 23, 24), also inhibit production of the bone resorption-stimulating factor by the cells (19, 20, 21, 23).

In addition to hypercalcemia, mice bearing the HSDM₁ tumor have elevated concentrations in plasma of PGE₂ and markedly raised concentrations of the longer-lived metabolite of PGE₂, 13,14-dihydro-15-keto-PGE₂ (19, 21, 23). Administration of indomethacin (19) to tumor-bearing mice lowered plasma concentrations of calcium, PGE₂, and 13,14-dihydro-15-keto-PGE₂. Furthermore, indomethacin reduced in parallel tumor bone resorption-stimulating activity and PGE₂ content (19). The plasma concentration of 13,14-dihydro-15-keto-PGE₂ was elevated before the devel-

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² To whom requests for reprints should be addressed.

³ The abbreviation used is: PGE₂, prostaglandin E₂.

opment of hypercalcemia, and the magnitude of the rise was greater than that of PGE₂ (21). When hydrocortisone was administered to tumor-bearing mice, the steroid hormone prevented the rises in plasma PGE₂ metabolite and calcium concentrations (21). At the dose levels used, hydrocortisone did not inhibit the calcium-mobilizing action of parathyroid hormone *in vivo* or the bone resorption-stimulating activity of PGE₂ in organ culture (21). The inhibitory effects of indomethacin and hydrocortisone on tumor prostaglandin production both *in vivo* and in cultured cells and on hypercalcemia required continuous administration of the drugs and were reversible when the agents were stopped or withdrawn.

VX₂ Carcinoma

Because the hypercalcemias that occur in patients are more often associated with carcinomas than with sarcomas and because certain experimental procedures were difficult to perform in an animal as small as the mouse, we extended our experimental approach to rabbits bearing the transplantable VX₂ carcinoma.

The VX₂ carcinoma produces marked hypercalcemia in the rabbit about 3 to 4 weeks after transplantation. Bone resorption-stimulating activity, assayed *in vitro* with mouse calvaria in organ culture, was extracted into diethyl ether from aqueous extracts of tumor tissue and from the medium of a clonal strain of VX₂ cells in monolayer culture (26). The tumors contained large amounts (250 to 500 ng/g fresh weight) of PGE₂ and VX₂ cells in culture secreted into medium 0.5 to 3.0 μg PGE₂ per mg cell protein per 24 hr (26). The production of bone resorption-stimulating activity and PGE₂ by VX₂ cells in culture were both inhibited by indomethacin. Tumors from indomethacin-treated, normocalcemic rabbits contained little or no bone resorption-stimulating activity or PGE₂. Tumor-bearing rabbits receiving indomethacin continuously from the time of tumor implantation did not develop hypercalcemia; however, following cessation of indomethacin administration, hypercalcemia developed rapidly and was again reversed by indomethacin. In untreated hypercalcemic tumor-bearing rabbits, initiation of indomethacin treatment was followed by a rapid return of the plasma calcium to the normal range (26).

Systemic venous plasma from hypercalcemic tumor-bearing rabbits contained small but significant elevations of PGE₂ at the peak of the hypercalcemia (26); however, a clear rise in plasma PGE₂ before the onset of hypercalcemia was not seen (16, 26). On the other hand, the plasma concentration of 13,14-dihydro-15-keto-PGE₂ was elevated within 1 week after tumor implantation and preceded the development of hypercalcemia (22). Both the rate of rise and the magnitude of the increase were greater for the metabolite than for PGE₂; at the time of peak hypercalcemia (about 4 to 5 weeks after tumor implantation), the increase over basal in plasma 13,14-dihydro-15-keto-PGE₂ was about 75-fold, whereas it was less than 2-fold for PGE₂ (16, 22). Hydrocortisone, like indomethacin, prevented the development of hypercalcemia when given at the time of tumor implantation and reversed the elevated plasma calcium in previously untreated animals; the steroid hormone also lowered plasma concentrations of 13,14-dihydro-15-keto-

PGE₂ (22). Venous drainage of the tumor contained higher concentrations of PGE₂ than systemic venous plasma, indicating that the tumor was the source of the PGE₂ (26). In contrast, the venous drainage of the tumor contained low concentrations of 13,14-dihydro-15-keto-PGE₂ in comparison with systemic venous plasma, indicating that the tumor was not the source of the high circulating concentrations of metabolites. This observation is consistent with the findings that the VX₂ tumor does not metabolize PGE₂ extensively (16).

For examination of the systemic effects of the VX₂ carcinoma on the osseous skeleton, bones of rabbits bearing the tumor and bones from comparable control rabbits were studied by a combination of radiographic and histomorphometric techniques (28). No evidence was found of local invasion of bone by the VX₂ tumor or of osseous metastases. There was radiographic evidence of generalized osteopenia. Morphometric analysis of trabecular bone at multiple sites distant from tumor revealed a reduced volume density of bone matrix, osteoid, and osteoid seam thickness, as well as a reduced surface density of osteoblastic layers and osteoid, and an increase in the extent of resorptive surfaces (28). Analogous findings, including an increase in the numbers of osteoclasts at sites remote from the tumor, have been reported by Hough *et al.* (5).

Two additional findings in rabbits bearing the VX₂ tumor are of possible pathophysiological interest. First, the plasma concentrations of 2 acute-phase reactants, ceruloplasmin and haptoglobin, rise rapidly following implantation of the VX₂ tumor, and the increase is related to arachidonic acid metabolism in these animals (25). Plasma concentrations of ceruloplasmin rise in parallel with 13,14-dihydro-15-keto-PGE₂, and both precede the rise in plasma calcium. Indomethacin prevented the rise in ceruloplasmin and reduced the elevation once it had occurred. No changes in plasma albumin concentration were noted. It is possible that arachidonic acid metabolites may thus play a role in the elevation of these acute-phase proteins in certain patients with malignant tumors, as well as in patients with certain chronic inflammatory diseases.

Secondly, it has been noted that rabbits bearing the VX₂ carcinoma have hyperplasia of the gastrin-producing cells in the antropyloric region of the stomach (2). Whether the increase in G-cell mass is secondary to the hypercalcemia, an effect of the hyperprostaglandinemia, or to some other consequence of the tumor has not been determined.

Comments

Our conclusions from studies of the HSDM, fibrosarcoma and VX₂ carcinoma are that both tumors synthesize and secrete excessive amounts of PGE₂. This prostaglandin then acts on the skeleton at sites distant from the tumor to stimulate bone resorption and inhibit bone formation, leading to the hypercalcemia observed in tumor-bearing animals. If this conclusion were valid, it should be possible to produce an increase in plasma calcium concentration in experimental animals by the appropriate exogenous administration of PGE₂. We have accomplished this in the unanesthetized, intact rat by constant *i.v.* infusion (4). Results of other investigations have shown hypercalcemia in thyropar-

athyroidectomized, but not intact, rats by means of intraarterial PGE₂ infusion (12), and histopathological evidence of enhanced bone resorption, but not hypercalcemia, has been reported in mice given injections i.p with 16,16-dimethyl-PGE₂-methyl ester, a long-acting synthetic analog of PGE₂ (15). These results indicate that additional experiments need to be performed with exogenous PGE₂ in normal mice, rats, and rabbits to duplicate more nearly the long-term constant synthesis and release of PGE₂ that occurs in tumor-bearing animals. Nevertheless, the results already in hand indicate that exogenous PGE₂ can affect skeletal composition and morphology as well as plasma calcium concentrations under certain specific experimental circumstances, none of which yet duplicates precisely those produced by a prostaglandin-producing tumor.

Finally, it may be argued that PGE₂ secreted by a tumor is acting on the skeleton by an indirect mechanism, such as by stimulating the release of endogenous parathyroid hormone or by the action on bone of one or more of the accumulated metabolites of PGE₂, rather than PGE₂ itself. The finding that the VX₂ carcinoma produces hypercalcemia in parathyroidectomized rabbits (27) argues strongly that the effect of the tumor is not dependent on endogenous parathyroid hormone. The question as to whether metabolites of arachidonic acid other than PGE₂ are the stimulators of resorption at the osseous target cell requires additional investigation. Of the natural metabolites of arachidonic acid tested for bone resorption-stimulating activity, PGE₂ is the most potent (9, 18). On the other hand, since certain metabolites such as 13,14-dihydro-PGE₂ have significant biological activity on bone resorption *in vitro*, their concentrations in the plasma of tumor-bearing animals and patients need to be determined. Measurements of 13,14-dihydro-PGE₂ in plasma have not yet been reported. Even though 13,14-dihydro-15-keto-PGE₂ accumulates to high levels in plasma, its low level of biological activity on bone (9, 18) makes it unlikely that it is the mediator of resorption. Finally, the possibility that PGE₂ is transformed by bone cells into a metabolite more active on bone than is PGE₂ itself needs to be examined critically. Nevertheless, even if it were to be found that an arachidonic acid metabolite other than PGE₂ was the mediator of bone resorption and hypercalcemia in tumor-bearing animals, the essential tenets of our proposed mechanism will have proven useful in leading to the identification of such a mediator. On the basis of preliminary data in hand, it seems unlikely that metabolites of arachidonic acid along the prostacyclin (PGI₂) or thromboxane pathways will be responsible for enhanced bone resorption and hypercalcemia in tumor-bearing animals. Thus, PGE₂ or a metabolite(s) of it is the most likely mediator.

On the basis of a limited number of clinical investigations reported to date, it is possible that a pathophysiological mechanism involving metabolites of arachidonic acid will explain the hypercalcemia that occurs in certain patients with cancer (1, 3, 13, 17). It must be emphasized, however, that in addition to parathyroid hormone and osteoclast activating factor, prostaglandins are only one additional class of humoral mediators of the hypercalcemic syndrome. It is likely that only a small fraction (probably less than 10%) of such tumor syndromes will be attributable to prostaglan-

din excess; other undiscovered mediators must exist to explain the pathophysiology in many cases. Nevertheless, because the prostaglandin-mediated mechanism is amenable to control by pharmacological means (19, 21-23, 26), vigorous attempts to identify these cases should be undertaken. At present, the most appropriate methods to make such diagnoses are measurements of PGE₂ metabolites in plasma (6) or urine (17), rather than by measurement of the much lower and often insignificantly elevated concentrations of PGE₂ itself (16, 21-23).

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