Calcitriol: The Major Humoral Mediator of Hypercalcemia in Hodgkin’s Disease and Non-Hodgkin’s Lymphomas

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A DISTINCT SYNDROME of calcitriol (1,25-dihydroxyvitamin D₃)-mediated hypercalcemia is seen in patients with Hodgkin’s disease and non-Hodgkin’s lymphomas (NHL). In addition to hypercalcemia, this syndrome is characterized by intestinal hyperabsorption of calcium, normal serum phosphate levels, the absence of renal phosphate wasting, and increased renal excretion of calcium. Other characteristic features include normal or suppressed serum parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP) concentrations and normal nephrogenous cyclic adenosine monophosphate production.

Dysregulated extra-renal production of calcitriol, the active metabolite of vitamin D, appears to be the underlying defect, although it is unclear whether the lymphoma cells or infiltrating host monocytes and macrophages are the primary source. Applying the pathophysiology of hypercalcemia in sarcoidosis as a model, interferon-γ production by lymphocytes stimulating monocyte and macrophage 1α-hydroxylase activity and enhancing vitamin D activation may be an early initiating event. There is also evidence that other osteolytic factors such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) may act in concert with calcitriol to exacerbate the hypercalcemia. In isolated cases of human T-cell lymphotropic virus type-1 (HTLV-1)-related adult T-cell leukemia/lymphoma (ATLL) and isolated cases of NHL,5,6 most patients with hematologic malignancies complicated by hypercalcemia do not have elevated systemic levels of PTHrP.7-9 Conversely, dysregulated calcitriol production is a very rare occurrence in patients with hypercalcemia associated with solid tumors,10-12 where suppressed serum concentrations of calcitriol are characteristic.

The second major category of malignancy-associated hypercalcemia is local osteolytic hypercalcemia. The predominant cause of the hypercalcemia in these cases is localized bone resorption by activated osteoclasts in the vicinity of tumor cells metastatic to bone. Although not well characterized, a variety of cytokines and local regulatory factors have been implicated in this type of hypercalcemia (Table 1).

The third major category of hypercalcemia is calcitriol-mediated hypercalcemia. This is the most frequent cause of hypercalcemia seen in patients with Hodgkin’s disease and NHL,14,15 where hypercalcemia has been reported to occur in 5% and 15% of patients, respectively.16 There are no data specifically examining the clinical impact of hypercalcemia in these disorders. However, patients with malignancy-associated hypercalcemia are usually symptomatic, and hypercalcemia adds significantly to their morbidity and mortality.17 Further, hypercalcemia is a poor prognostic feature in a number of hematologic malignancies,18-20 and therapies specifically directed toward preventing osteolysis have shown improved outcomes for patients with both metastatic breast cancer21,22 and multiple myeloma.23,24 Finally,
because the mediators of hypercalcemia are often integrally involved in the pathogenesis and progression of the malignancy, clarifying these processes will hopefully provide insights facilitating the development of more specific antitumor therapies.

BIOCHEMICAL PROFILE OF PATIENTS WITH CALCITRIOL-MEDIATED HYPERCALCEMIA

The selection and application of therapy appropriate for calcitriol-mediated hypercalcemia depend on recognition of the disorder and an understanding of the underlying pathophysiologic processes. Characteristic and distinct biochemical profiles are seen in patients within the three categories of malignancy-associated hypercalcemia described, as illustrated in Table 2. In patients with calcitriol-mediated hypercalcemia there is enhanced osteoclastic bone resorption and excessive gastrointestinal calcium absorption. The laboratory findings in this clinical syndrome include a normal or slightly elevated serum phosphate, and normal levels of the inactive precursor 25-hydroxyvitamin D, with an elevated calcitriol level, or a calcitriol level that is inadequately suppressed for the degree of hypercalcemia. If measured, the tubular reabsorption of phosphate is normal or increased and the nephrogenous cyclic adenosine monophosphate (cAMP) level, an indicator of the activation of renal tubular PTH receptors, is low. These findings contrast those seen in PTHrP-mediated humoral hypercalcemia of malignancy (Table 2).

This constellation of findings is an idealized one, and these changes can be modified in an individual patient by the degree of preexisting renal impairment, dietary intake of calcium and fluids, and, most importantly, the interacting influences of other active mediators. There is considerable evidence that other less well-understood cytokines have a role in the causation or modulation of the hypercalcemia mediated by calcitriol. Before these interactions can be fully understood, the underlying physiological regulation of vitamin D production, and its major metabolic actions, must be thoroughly understood.

NORMAL PHYSIOLOGY AND METABOLISM OF CALCITRIOL

A thorough review of the normal physiology of calcitriol has recently been published, and only the details directly relevant to the understanding of hypercalcemia in hematologic malignancies will be outlined here.

Synthesis and measurement. Exposure of the skin to ultraviolet light (UV) facilitates the conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D3), which then undergoes hepatic hydroxylation to 25-OH-D3. This compound is not known to have biologic activity, and the hepatic 25-hydroxylase is not tightly regulated. Blood levels of 25-OH-D3 vary greatly depending on dietary intake and UV exposure. The metabolically active derivatives are produced by a second hydroxylation in the kidney. The major metabolic product is 1,25-(OH)2-D3 (calcitriol). The enzyme involved, 1a-hydroxylase, is tightly regulated, and its activity varies inversely with that of the associated 24-hydroxylase, which yields the second significant metabolite: 24,25-(OH)2-D3, whose biologic actions are not well characterized. The principal stimuli to enhanced 1a-hydroxylase activity are hypocalcemia (mediated via parathyroid hormone) and hypophosphatemia (Table 3). Under normal physiologic circumstances the kidney is the only significant source of circulating calcitriol. Calcitriol itself also directly inhibits the renal 1a-hydroxylase. Given that PTH stimulates the activity of the renal 1a-hydroxylase, it is not surprising to find that 1,25-(OH)2-D3 levels are often significantly elevated in patients with primary hyperparathyroidism. PTHrP shares the stimulatory action of PTH on renal 1a-hydroxylase and one of the potential sources of calcitriol in malignancy-associated hypercalcemia is from dysregulated renal production secondary to PTHrP stimulation. However, as discussed below, for poorly understood reasons this is rarely observed.

Calcitriol in plasma is 85% bound to the vitamin D-binding protein, 15% bound to albumin, and less than 1% is in

| Table 1. General Features of Categories of Hypercalcemia of Malignancy |
|----------------|----------------|----------------|
| **Calcitriol-Mediated** | **Humoral Hypercalcemia** | **Local Osteolytic** |
| Common tumor types | Hodgkin’s disease, NHL | Epidermoid, renal cell, breast | Lung, breast, myeloma |
| Sites of tumor | Variable, often advanced stage | Localized, some metastatic | Always metastatic to bone |
| Prominent mediators | Calcitriol | PTHrP | Various: IL-1, TNF-α/β, IL-6, prostaglandins, UFP, PTHrP, TGF-α |
| Mode of action | Systemic humoral | Systemic humoral | Local paracrine |

Abbreviation: UFP, leukemia-inhibitory factor.

| Table 2. Biochemical Features of Patients With Various Categories of Hypercalcemia of Malignancy |
|----------------|----------------|----------------|
| **Calcitriol-Mediated** | **Humoral Hypercalcemia** | **Local Osteolytic** |
| Serum | Parathyroid hormone | decreased | decreased | decreased |
| PTHrP | undetectable | increased | undetectable |
| Calcitriol | increased | decreased | decreased |
| Phosphate | normal/ increased | decreased | normal |
| Urine | cAMP | normal | increased | normal |
| Renal phosphate resorption | normal | decreased | normal |
| Gut | Calcium absorption | increased | decreased | decreased |
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...the free state. It is this free fraction that is most biologically active.35,36 Although sensitive and specific assays for total 1,25-(OH)2-D3 are readily available,37,38 a clinically useful assay for vitamin D-binding protein has only recently been described.39 Although 25-OH-D3 has a half-life of 21 days, calcitriol, the active form, is rapidly degraded with a circulating half-life of 3 to 6 hours. This has not been shown to be significantly prolonged in disease states, and thus any elevations of plasma levels of calcitriol are the result of enhanced production rather than of impaired clearance.

Metabolic effects of calcitriol. Calcitriol binds to a specific intracellular receptor present in many tissues, particularly intestine and bone. Ligand-receptor interaction results in increased intestinal absorption of calcium and phosphate, and enhanced osteoclast adherence to bone stroma, osteoclast activity, and maturation, stimulating osteolysis. These effects add calcium and phosphate to the serum and counterbalance any hypocalcemic stimulus to PTH secretion. Vitamin D receptors have also been shown in a variety of other tissues, including, but not limited to, pancreas, breast, pituitary gland, and most hematopoietic cells.41 The exact role of vitamin D in the function of these tissues remains unclear. However, in monocytes ligand-binding stimulates differentiation toward macrophage lineage and induces and potentiates IL-1 secretion.42

Table 3. Major Physiological Modulators of Renal Calcitriol Production

| Stimulators | hypophosphatemia | hypocalcemia (mediated via PTH) | PTH | PTHrP |
| Inhibitors  | calcitriol | hyperphosphatemia | hypercalcemia | metabolic acidosis |

THE ROLE OF MONOCYTES AND MACROPHAGES IN THE EXTRA-RENAL PRODUCTION OF CALCITRIOL

Macrophages are one of the few extra-renal cell types with significant 1α-hydroxylase activity, and evidence strongly implicates them as the source of calcitriol in the hypercalcemia-complicating both sarcoidosis and tuberculosis. Monocytes and macrophages probably have a similar pivotal role in the production of calcitriol in the hypercalcemia-complicating lymphomas. Although detailed studies of calcitriol production in hematologic malignancies have not been reported, the cellular mechanisms involved in the hypercalcemia of sarcoidosis provide a potential model. In sarcoidosis and tuberculosis, neither PTH43 nor functional renal tissue44,45 is necessary for the development of an elevated calcitriol level and hypercalcemia. Indeed, macrophages are poorly responsive to PTH in vitro.46 Both sarcoid lymph node homogenate47 and cultured isolated alveolar macrophages from patients with sarcoidosis48,49 are able to synthesize calcitriol in vitro. Calcitriol synthesis by macrophages is enhanced by interferon-γ (IFN-γ)50,51; however, complex regulatory mechanisms are involved. The 1α-hydroxylase activity macrophages is inhibited by calcitriol, but to a lesser degree than the renal 1α-hydroxylase.46 and, normally IFN-γ secretion by T lymphocytes is also inhibited by calcitriol.52,53 These regulatory mechanisms may serve to prevent excessive IFN-γ production and continuing extrarenal calcitriol synthesis. However, these inhibitory stimuli can be overcome, as pulmonary T lymphocytes from patients with sarcoidosis spontaneously release IFN-γ54 despite elevated calcitriol levels.

IFN-γ alone, although a powerful inhibitor of bone formation,55 is insufficient to cause hypercalcemia, as evidenced by the numerous infectious diseases that are associated with prolonged systemic elevations of IFN-γ without hypercalcemia56 and the absence of hypercalcemia complicating phase-I studies of IFN-γ.57,58 Indeed, IFN-γ actually inhibits IL-1-mediated bone resorption.59 Thus, it is clear that additional factors are involved. IL-10 shares some of the monocyte stimulatory properties of IFN-γ50 and it has recently been shown that certain phorbol esters can overcome the inhibitory effect of calcitriol on IFN-γ production from lymphocytes.60 Both IL-1 and TNF have been reported to increase the expression of IFN-γ receptors on monocytes and macrophages,61 potentially enhancing cellular responsiveness to IFN-γ. However, despite increasing the number of IL-1 receptors,62 calcitriol has also been reported to inhibit the production of IL-1 by T lymphocytes.63 Thus, there are many complex cytokine interactions that are yet to be fully integrated into a complete understanding of the regulation of calcitriol production by macrophages.

NEOPLASTIC DISORDERS IN WHICH CALCITRIOL IS IMPLICATED AS A CAUSE OF HYPERCALCEMIA

Hodgkin's disease, NHL and HTLV-1-related ATL are the major diseases in which calcitriol is involved in the pathogenesis of hypercalcemia. In each disease the incidence, clinical associations, and prognostic implications of hypercalcemia will be described and the evidence implicating the involvement of other mediators discussed. There have also been isolated reports describing elevated calcitriol levels in acute lymphoblastic leukemia (ALL)64 and myelofibrosis.65 In ALL, although no large series have been reported, hypophosphatemia and lytic bone lesions often accompany hypercalcemia.66 These features, and the recent report of a patient with ALL and an elevated PTHrP level,67 suggest that factors other than calcitriol are likely to be involved. Similarly, there is no evidence implicating calcitriol in the hypercalcemia associated with acute myeloid leukemia (AML),68 multiple myeloma,14,69 chronic myeloid leukemia (CML),70 chronic lymphocytic leukemia,71 or Waldenström's macroglobulinemia.72

HODGKIN'S DISEASE

Hodgkin's disease is the disorder in which calcitriol is most consistently implicated as the mediator of hypercalcemia and in which the most extensive clinical studies have been conducted. Hypercalcemia was first reported to com-
complicate Hodgkin’s disease by Plimpton and Gellhorn in 1956, and the association with increased vitamin D sensitivity was described one year later by Kabakow et al. Since that time more than 60 cases have been described.

Only three studies report the incidence of this complication, and these used a variety of methods and definitions of hypercalcemia. Using hospital admission data and the thresholds of 10.8 mg/dL and 10.3 mg/dL for hypercalcemia, respectively, incidences of 1.6% (1/61) and 5.4% (15/276) have been reported. Limiting his analysis only to patients with stage IV Hodgkin’s disease and using an unspecified threshold, Canellos reported a 0.9% (1/109) incidence of hypercalcemia. The incidence of significant hypercalcemia is probably higher. In an autopsy study Moses and Spencer found nephrocalcinosis, an indicator of longstanding hypercalcemia, in 8 of 19 patients with lymphoma (including 11 with Hodgkin’s disease) who had not been known to be hypercalcemic during life.

In 38 patients the actual peak serum calcium levels were reported and the distribution of these values is shown in Fig 1. The peak serum calcium values ranged from 10.9 to 23.1 mg/dL (median 14.4 mg/dL), 84% of patients had a peak serum calcium above 12 mg/dL, showing that the hypercalcemia is usually severe and clinically significant. In contrast to the hypercalcemia of sarcoidosis, the angiotensin-converting enzyme level has been normal in the four patients measured.

From those reports in which details are provided, the ages of the patients were 15 to 83 years (median 50), with 15 of 42 (36%) 60 years old or more (in 9 cases no individual data was provided). Forty-one of 51 (80%) were male, and for 29 patients the histologic subtype was described (Table 4). In 38 patients the Ann Arbor stage at the time of diagnosis of hypercalcemia was described (Fig 2). The clinical features and histologic subtypes in these hypercalcemic patients contrast with those reported from large series of patients with Hodgkin’s disease, in which the age distribution is bimodal and the majority of cases occur in the 15- to 35-year-old age group. Of the reported patients with hypercalcemia, only 11 of 42 (26%) were within this age range. The incidence of Hodgkin’s disease is greater in males, constituting up to 60% of cases. This is notably less than the 80% of males among those patients with hypercalcemia. Table 4 shows the distribution of the histologic subtypes of patients with Hodgkin’s disease seen at the National Cancer Institute and clearly shows that the nodular-sclerosing subtype is significantly under-represented among those patients with hypercalcemia. As discussed below, many of the patients with hypercalcemia presented with predominantly infradiaphragmatic disease. Some reports have described an older age at diagnosis, a greater proportion of males, and a lower incidence of nodular-sclerosing histology among patients presenting with infradiaphragmatic Hodgkin’s disease. A second possible explanation for these significant differences is pathologic mis-classification in some instances, or the inclusion of cases of NHL. Indeed, in 1986 a pathologic review of the cases of lymphocyte-depleted Hodgkin’s disease seen at the National Cancer Institute between 1964 and 1976, two patients who presented with hypercalcemia were retrospectively reclassified as having NHL and the presence of bulky

Table 4. Distribution of Histologic Subtype in Reported Cases of Hypercalcemia Complicating Hodgkin’s Disease

<table>
<thead>
<tr>
<th>Histologic Subtype</th>
<th>N.C.I. Series (%)</th>
<th>Hypercalcemic (n = 29)</th>
</tr>
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<tbody>
<tr>
<td>Nodular sclerosing</td>
<td>70</td>
<td>9</td>
</tr>
<tr>
<td>Lymphocyte predominant</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Mixed cellularity</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Lymphocyte depleted</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

* Distribution of histologic subtypes from National Cancer Institute series.

† Twenty-nine cases of hypercalcemia complicated by hypercalcemia with histologic subtyping given: see text for references.

Fig 1. (A) Peak serum calcium for 38 patients with hypercalcemia complicating Hodgkin’s disease from the literature. Normal range for calcium shaded. (B) Calcitriol level in 11 patients with Hodgkin’s disease and hypercalcemia from the literature. Six other patients with levels measured but no numerical values reported are not included. See text for references.

Fig 2. Distribution of Ann Arbor stage of hypercalcemic patients with Hodgkin’s disease from the literature. See text for references.
abdominal adenopathy was also present in two other patients who were reclassified as having NHL. It is not possible to clarify this issue in a retrospective literature review. Contrary to the general belief that hypercalcemia is usually a complication of extensive osseous disease, only 3 of the 23 patients for whom sufficient data are available had radiologic evidence of lytic bone lesions and one had diffuse osteoporosis. Some patients displayed diffuse enhancement of radiotracer uptake on nuclear bone scanning in the absence of distinct radiologic bone lesions.

As suggested above, the metabolic profile of these patients is not consistent with the humoral hypercalcemia of malignancy mediated by PTHrP. Only 9 of 35 reported patients had a phosphate level below the normal range during the hypercalcemic episode. Cyclic AMP levels have consistently been low or normal. Normal or suppressed PTH levels have been reported, with normal parathyroid glands documented at autopsy or neck exploration.

The lack of any large series of patients administered uniform treatment precludes any meaningful analysis of the prognostic significance of hypercalcemia in Hodgkin’s disease. In many of the reported patients independently poor prognostic features were present. Disease was often described as bulky. Twenty-eight of 38 patients (74%) had Ann Arbor stage III or IV disease (Fig 2). Further, 17 of 25 patients (68%) had “B” symptoms. Many of the reported patients did not receive what would currently be considered optimum therapy. However, the presence of hypercalcemia at presentation does not preclude a complete remission to therapy and a long-term disease-free survival with standard therapy.

The calcitriol levels of 17 patients, measured while hypercalcemic, have been reported (Fig 1). In only one was the level not elevated. This patient was positive for antibodies to the HIV-1. Although no details are provided, Rosenthal et al also refer to an unpublished instance of a hypercalcemic patient with Hodgkin’s disease and a normal calcitriol level. The available data show that nearly all patients with hypercalcemia complicating Hodgkin’s disease have elevated calcitriol levels, indicating that this mediator is intimately involved in the pathogenesis of this syndrome.

Evidence implicating additional mediators in the hypercalcemia of Hodgkin’s disease. Two patients are described for whom it appears calcitriol was the sole factor responsible for the hypercalcemia. These patients were normocalcemic, with normal calcitriol levels, until they were exposed to UV or received vitamin D supplementation. These maneuvers resulted in hypercalcemia and concurrent elevations of calcitriol levels that normalized after the causative agent was withdrawn.

However, in most instances, the data strongly implicate the action of additional factors. Jacobson et al studied the bone-resorbing activity of lymph node homogenate from a patient with hypercalcemia complicating Hodgkin’s disease. Significant bone-resorbing activity remained after removal of calcitriol, and bone-resorbing ability did not parallel 1,25-(OH)₂-D₃ concentration in dilution studies. In reported cases the calcitriol levels range from 1.12 to 5.4 (mean 2.0) times the upper limit of normal (Fig 1). In studies of oral calcitriol administration to normal volunteers, plasma levels up to twice the upper limit of normal for brief periods did not result in significant hypercalcemia. Further, in patients with sarcoidosis and hypercalcemia, the levels of calcitriol associated with comparable degrees of hypercalcemia are significantly higher.

The identities of these putative additional mediators are uncertain. There is no evidence available to date to implicate PTHrP in Hodgkin’s disease. However, IL-1, a cytokine with independent osteolytic activity, has been implicated. IL-1 mRNA is present within Reed-Sternberg cells and the IL-1 protein has been shown by immunohistochemistry to be present within both Reed-Sternberg cells and the infiltrating host macrophages. Circulating levels of IL-1 are also elevated in a minority of patients with untreated Hodgkin’s disease. As discussed earlier, there is also a possible link between IL-1 and the stimulation of IFN-γ–induced macrophage calcitriol production. Any possible correlation between hypercalcemia and IL-1 levels in Hodgkin’s disease has not been investigated.

NHL

Hypercalcemia occurs most commonly in aggressive histologic types of NHL. In a recent study from the M.D. Anderson Cancer Center, 9 of 219 (4.1%) newly registered patients with NHL of all grades were hypercalcemic (total calcium ≥10.8 mg/dL); in 3 patients (1.4%) the calcium level was >12.0 mg/dL. The incidence of hypercalcemia in high- and intermediate-grade NHL (according to the International Working Formulation) may be as high as 30%. This rising incidence with increasing histologic grade correlates with the degree of host macrophage infiltration of lymphomatous tissue, recognized histologically by the “starry sky” appearance, which is limited to the more aggressive histological subtypes. In contrast, hypercalcemia is very uncommon in low-grade NHL, only complicating 1% to 2% of cases. Except in ATLL, in which hypercalcemia is a powerful adverse prognostic factor, no data is available concerning the prognostic significance of hypercalcemia in NHL.

An unknown percentage of patients who have NHL without overt hypercalcemia have hypercalcioria, suggesting the presence of a calcitropic mediator, perhaps inappropriately elevated levels of 1,25-(OH)₂-D₃, even in the absence of hypercalcemia. In these patients with hypercalcioria, the risk of subsequent hypercalcemia may be increased, although this issue has not been systematically investigated.

The literature contains reports of at least 19 instances of elevated serum calcitriol levels associated with hypercalcemia in patients with NHL. Fourteen (74%) of these patients were male, their median age was 58 years (range 4 to 81 years), and only one patient had low-grade histology, and one an uncommon angiocentric lymphoma. The small number of patients and the anecdotal nature of these reports, combined with the broad range of clinical features in NHL, precludes a meaningful comparison of these patients with nonhypercalcemic NHL patients.
However, a few points are noteworthy. Firstly, although many patients had bulky or advanced-stage disease, none had bone lesions identified clinically or radiographically. This excludes direct tumoral osteolysis as a major contributor to the hypercalcaemia. Both B- and T-cell phenotypes have been reported to be associated with elevated calcitriol levels. It is not known if immunophenotype influences the incidence of hypercalcemia. All patients had normal or elevated phosphate levels, in contrast to PTHrP-mediated humoral hypercalcemia of malignancy. Finally, where measured, levels of urinary cyclic AMP were normal or suppressed. The lack of elevations of PTH, the absence of high cyclic AMP levels, and the normal to elevated phosphate levels all support an extrarenal origin of the calcitriol in these patients. The probability of an extrarenal source of calcitriol is further strengthened by the presence of severe renal failure in a number of instances, the demonstration of in vitro conversion of 25-OH-D$_3$ to 1,25-(OH)$_2$-D$_3$ by excised lymph node homogenate, the prompt decline of calcitriol levels to normal within 24 hours of excision of an isolated splenic lymphoma, and normal PTHrP levels in all cases where measured. A few cases of hypercalcemia in NHL associated with elevated levels of PTHrP have been reported. In only three of these patients were calcitriol levels reported, and all were appropriately suppressed. It appears that simultaneous elevations of the levels of both PTHrP and calcitriol do not commonly occur in NHL. Although both PTHrP- and calcitriol-mediated syndromes may occur in patients with NHL, calcitriol-mediated hypercalcemia is the more common mechanism.

**HTLV-1–RELATED ATLL**

The understanding of the complex mechanisms of hypercalcemia in HTLV-1–related ATLL is incomplete. However, PTHrP is most strongly implicated as the major mediator.

An early report suggested HTLV-1-transformed lymphocytes possessed significant 1α-hydroxylase activity, although this capability was substantiated in only one of three HTLV-1–transformed cell lines examined. Most studies have shown suppressed calcitriol levels in the hypercalcemia associated with ATLL. However, there are two well-documented instances of elevated calcitriol levels. In the first case PTHrP levels were not available. The second case involved concomitant elevations of calcitriol and PTHrP, suggesting the possibility of increased renal 1α-hydroxylase activity secondary to stimulation by PTHrP.

**INTERACTION OF CALCITRIOL WITH OTHER MEDIATORS**

There are data clearly showing dysregulated renal production of calcitriol in response to tumor-derived PTHrP, which shares the 1α-hydroxylase–stimulating capability of PTH. Infusion of synthetic PTHrP(1-34) or PTHrP(1-36) produces significantly elevated levels of circulating calcitriol, and dogs with malignant lymphoma and hypercalcemia often exhibit elevations of both PTHrP and calcitriol. Patients with an elevated PTHrP level would be expected to have increased renal 1α-hydroxylase activity and an elevated calcitriol level in the presence of sufficient precursor 25-OH-D$_3$. However, in most patients with solid tumors complicated by humoral hypercalcemia of malignancy calcitriol levels are, with some exceptions, depressed. One of the most intriguing questions about the involvement of calcitriol in the hypercalcemia of malignancy is why levels are not consistently elevated in association with high levels of PTHrP.

There is some recent evidence that the phosphaturic and 1α-hydroxylase–stimulating activities of PTH can be separated using synthetic analogues. However, both the cyclic AMP-generating and the 1α-hydroxylase-stimulating activities of PTHrP are localized with the PTHrP(1-34) fragment, and it is very unlikely that cleavage within this region can separate these activities.

It is probable that the failure to exhibit elevations of calcitriol in response to PTHrP is caused by inhibition of the renal 1α-hydroxylase. Evidence suggests that when acting systemically, IL-1 may have such properties. Although able to mediate osteolytic bone resorption in isolation and to act synergistically with PTHrP, IL-1 is also able to downregulate PTH receptors and to strongly inhibit PTHrP(1-34)-induced renal phosphate wasting and calcium resorption without diminishing urinary cyclic AMP excretion. However, it is yet to be shown that IL-1 is able specifically to inhibit renal 1α-hydroxylase.

In humoral hypercalcemia of malignancy mediated by PTHrP, the elevated serum calcium is one factor contributing to the suppression of calcitriol. After normalization of the serum calcium by bisphosphonate treatment, which does not have any known antitumor activity or effect on PTHrP levels, calcitriol levels have been shown to increase towards normal. The demonstration of IL-1 cosecretion by PTHrP-producing squamous carcinoma cell lines further strengthens the hypothesis that IL-1 is intimately involved in the causation and modulation of the manifestations of PTHrP-mediated hypercalcemia in some solid tumors.

Other substances have similar activities. TNF-α can downregulate PTH receptors, inhibiting renal PTHrP-induced cyclic AMP formation. Levels of TNF-α adequate to produce such effects have been detected in the serum of hypercalcemic patients with ATLL. Similarly, transforming growth factor-α (TGF-α) can inhibit the PTH-responsiveness of an osteoblast cell line, but it is not known if TGF-α similarly inhibits renal PTH-responsiveness or 1α-hydroxylase activity.

As discussed above, both IL-1 and TNF have potentially stimulatory actions on macrophage calcitriol production, and it is possible that the local paracrine, and systemic humoral actions of these factors may be distinct, or even counteracting. Elevated systemic levels of IL-1, TNF-α, or TGF-α may also explain the failure to frequently observe elevated calcitriol levels in HTLV-1–related ATLL where PTHrP is active. ATLL cells both express the IL-1 gene product and secrete the active peptide, and both PTHrP and IL-1 are found in the culture supernatant of ATLL cells.
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THERAPY OF CALCITRIOL-MEDIATED HYPERCALCEMIA

Corticosteroids are the most effective agents in the therapy of calcitriol-mediated hypercalcemia. There is strong evidence that corticosteroids have specific activity in alleviating calcitriol-mediated hypercalcemia independent of any antitumor cytotoxic activity. The literature contains accounts of at least 15 patients with Hodgkin's disease and hypercalcemia, or NHL and calcitriol-mediated hypercalcemia, who were treated with rehydration and single agent corticosteroids.74-76,79,82,87,89,94,100,117,119,121,122 The time course of response in serum calcium in these patients is shown in Fig 3. In one instance, the response to corticosteroids was very brief.74 In seven of these patients serial calcitriol measurements were available and the resolution of hypercalcemia with corticosteroid therapy was documented to parallel a falling calcitriol level.87,89,94,117,119 In a number of patients this calcium-lowering effect was seen in the absence of objective tumor response or alleviation of "B" symptoms.75,122 Even a patient with disease progressing on treatment has shown a lowering of the calcium level with the administration of corticosteroids. One case is particularly illustrative.121 After liver transplantation a child developed a B-cell NHL while receiving immunosuppressive therapy with cyclosporine A and prednisone. Withdrawal of the cyclosporine A did not influence the progression of the lymphoma or the calcium level. In spite of active lymphoma the patient remained normocalcemic until prednisone was withdrawn, at which time hypercalcemia first developed. After surgical debulking, subsequent intermittent administration of therapy including pulse steroids maintained normocalcemia until death despite tumor progression.

The minimum effective dose of corticosteroid is uncertain. However, hypercalcemia has recurred while patients were taking prednisone daily, 10 mg and 40 mg.119 However, another patient, maintained normocalcemia despite tumor progression with 30 mg of prednisone daily.89 Even for patients with terminal or refractory disease complicated by calcitriol-mediated hypercalcemia, prednisone in doses of greater than 40 mg daily, or equivalent, should be effective palliation of calcitriol-mediated hypercalcemia.

These reports clearly show a calcium-lowering effect of corticosteroids distinct from any cytotoxic activity. Although corticosteroids may have some effect on osteoblasts148 and intestinal calcium absorption,149 the illustrated reductions in calcitriol levels that accompany therapy suggest inhibition of calcitriol production. Corticosteroids have pleiotropic effects on lymphocytes, including inhibition of the secretion of the potentially important mediators IFN-γ, IL-1, and IL-10.150,152 The 1α-hydroxylase activity of pulmonary alveolar macrophages is also directly inhibited by corticosteroids,153 and the addition of leukotriene C4 reverses this inhibition.154 It is feasible that both the inhibition of arachidonate metabolism in macrophages, and the inhibition of stimulatory cytokine production by lymphocytes is responsible for reducing calcitriol levels and improving hypercalcemia in patients with lymphoma.

For patients with sarcoidosis who are intolerant of corticosteroids, chloroquine has been shown to reduce calcitriol levels and alleviate hypercalcemia.155,156 This effect may be mediated by inhibition of macrophage IL-1 production or by a reduced responsiveness to stimulatory cytokines.157 Ketoconazole has also been shown to reduce calcitriol levels in normal men158 and in patients with primary hyperparathyroidism.159 Whether these agents have similar efficacy in calcitriol-mediated hypercalcemia complicating hematologic malignancies is unknown.

OTHER THERAPEUTIC MODALITIES EFFECTIVE IN CALCITRIOL-MEDIATED HYPERCALCEMIA

Unfortunately, serum calcitriol levels are not likely to be readily available when decisions on the management of hypercalcemia need to be made. Although calcitriol is implicated in all well-documented cases of hypercalcemia in immunocompetent patients with Hodgkin's disease, perhaps only 30% of hypercalcemic patients with NHL will have calcitriol as the major mediator.14 Thus, it may not be prudent to limit therapy specifically to calcitriol mediated effects if calcitriol levels are not known. Therapy should also address other possible sources of elevated serum calcium. Thus, there are four main targets for therapeutic intervention available (Table 5); the inhibition of calcium mobilization from bone, the reduction of calcium absorption from the intestine, the enhancement of renal calcium excretion, and the prevention of calcitriol production.

Table 5. Sites of Intervention in the Therapy of Calcitriol-Mediated Hypercalcemia

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<th>Intervention</th>
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<tr>
<td>Inhibition of calcium mobilization from bone</td>
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<tr>
<td>Reduction of calcium absorption from the gut</td>
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<tr>
<td>Enhancement of renal calcium excretion</td>
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<tr>
<td>Inhibition of calcitriol production</td>
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and interference with calcitriol production or action. As discussed above, the most effective inhibitors of calcitriol production are the corticosteroids.

Many agents are available that either stabilize bone to the action of osteoclastic resorption, or inhibit osteoclast function. These include calcitonin, mithramycin, gallium nitrate and the bisphosphonate class of drugs. These agents are the mainstay of the therapy of humoral hypercalcemia of malignancy and the bisphosphonates have also shown activity in calcitriol-mediated hypercalcemia. Pamidronate is the most effective of the currently available agents and should be a component of the therapy of calcitriol-mediated hypercalcemia, optimally in combination with corticosteroids and specific cytotoxic agents.

In contrast to the humoral hypercalcemia of solid tumors, intestinal calcium absorption is enhanced in calcitriol-mediated hypercalcemia. Intestinal calcium absorption can be reduced by the application of a low-calcium diet, or the use of calcium binding resins. As discussed above, a part of the activity of glucocorticoids may also be to reduce calcium absorption. However, severe hypercalcemia associated with elevated calcitriol levels can develop while patients are taking nothing by mouth, so additional measures should always be implemented.

Calcitriol does not promote renal tubular calcium resorption, unlike PTH-like factors. However, hypovolemia and renal impairment are common in calcitriol-mediated hypercalcemia, as in any form of hypercalcemia. Thus, volume replacement, preferentially with saline, to ensure restoration of the intravascular volume and a good urine output, is an important fundamental component of therapy. A large volume diuresis induced by loop diuretics is difficult to safely implement and is not of major additional therapeutic benefit.

In most cases calcitriol production is not limited by 25-OH-D$_3$ supply. However, some patients have manifested hypercalcemia only following significant sun exposure, presumably secondary to enhanced production of 25-OH-D$_3$. It would be prudent to advise these patients to avoid excessive UV exposure and dietary vitamin D supplements until the hypercalcemia is controlled. In some geographic areas this precaution may be adequate to prevent hypercalcemia.

UNANSWERED QUESTIONS

The data reviewed clearly show that calcitriol is implicated in nearly all instances of hypercalcemia complicating Hodgkin’s disease and perhaps 30% to 40% of cases of hypercalcemia in NHL. It is probable, but not definitively proven, that activated infiltrating nonmalignant host macrophages are the cells responsible. Currently, the most effective treatment to inhibit calcitriol production is the use of corticosteroids. These agents have pleitropic effects and their critical site of action remains uncertain. Before it will be possible to more precisely and effectively treat these disorders it will be necessary to more accurately define the host-tumor interactions that initiate and sustain this dysregulated calcitriol production. It remains entirely unknown how the elevated calcitriol levels, and the interactions that produce them, influence the growth, differentiation, and progression of these tumors.

Finally, the question of why renal calcitriol production and serum levels are not routinely elevated in response to PTHrP remains unanswered. Clarification of this issue will increase our understanding of the pathophysiology of both the hypercalcemia of ATLL and the broad field of humoral hypercalcemia of malignancy associated with solid tumors.

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REFERENCES

CALCITRIOL, HYPERCALCEMIA, AND LYMPHOMA

29. Mundy GR, Martin TJ: The hypercalcemia of malignancy: Pathogenesis and management. Metabolism 31:1247, 1982
52. Koefler HP, Reichel H, Bishop JE, Norman AW: γ-inter-


62. Krakauer T, Oppenhaim JJ: IL-I and tumor necrosis factor-α each up-regulate both the expression of IFN-γ receptors and enhance IFN-γ-induced HLA-DR expression on human monocytes and a human monocytic cell line (THP-1). J Immunol 150:1205, 1993


CALCITRIOL, HYPERCALCEMIA, AND LYMPHOMA


105. Leibenhaut MH, Hoppe RT, Varghese A, Rosenberg SA: Cytokine production (IL-1a, IL-1b, and TNF-a) in the systemic staging and treatment results in 49 patients. J Clin Oncol 5:1050, 1987


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