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Research Article

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Gallium Nitrate Inhibits Calcium Resorption from Bone and Is Effective Treatment for Cancer-related Hypercalcemia

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Abstract. Approximately two-thirds of patients who receive the anticancer drug gallium nitrate develop mild hypocalcemia. To evaluate the mechanism of drug-induced hypocalcemia, we tested the effects of gallium nitrate upon in vitro release of $^{45}Ca^{++}$ from explanted fetal rat bones. The drug significantly inhibited $^{45}Ca^{++}$ release in response to stimulation with both parathyroid hormone and a lymphokine preparation with osteoclast activating factor activity. The inhibitory effects on bone resorption were both time- and dose-dependent. Later, in a pilot study, we treated 10 patients who had cancer-related hypercalcemia with gallium nitrate administered by continuous infusion. All patients responded by a reduction of total serum calcium to normal or subnormal concentrations (13.8 ± 1.05 mg/dl, mean$\pm$SD pretreatment, to 8.03 ± 1.03 mg/dl, mean posttreatment nadir). Our results indicate that gallium nitrate effectively treats cancer-related hypercalcemia and that it probably acts by inhibiting calcium release from bone.

Introduction

Gallium nitrate is a new experimental antitumor compound. During the initial clinical studies, hypocalcemia was occasionally

observed and the condition was attributed to increased urinary calcium excretion (1). During a recent study, we observed that approximately two-thirds of patients developed transient hypocalcemia (2). Four of those patients who underwent metabolic study were actually in positive calcium balance while receiving the drug. Each showed reduced urinary calcium excretion relative to his base line (3).

Because hypocalcemia in our patients could not be explained on the basis of hypercalciuria, as had been suggested (1), several studies were initiated to evaluate the mechanisms of drug-induced hypocalcemia. We have found that gallium nitrate causes hypocalcemia primarily by decreasing calcium resorption from bone and that the drug is effective treatment for cancer-related hypercalcemia.

Methods

Reagents. Pharmaceutical-grade gallium nitrate was obtained from the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Each 1 ml of drug solution contained 10 mg anhydrous gallium nitrate and 11.5 mg trisodium citrate dihydrate that had been brought to volume and neutral pH by the addition of 0.9% solutions of sodium chloride and sodium hydroxide. Lymphokine, with a bone-resorbing activity similar to that of osteoclast activating factor, was prepared as previously described (4). Partially purified parathyroid hormone (PTH)[1] was prepared by Sephadex column chromatography from urea-HCl-cysteine, TCA-precipitated bovine parathyroid gland extracts (5).

In vitro study of bone calcium release. Pregnant rats were injected with 0.2–0.4 mCi of $^{45}CaCl_2$ on the 18th day of gestation. After 2 d of bone mineralization in utero, the radii and ulnae of fetal rats were explanted and placed on stainless steel rafts in BGJ media. Calcium release from bone was stimulated by the addition of bovine PTH (2 μM, final concentration) or of a lymphokine preparation (10% of final volume) (5). Gallium nitrate was added to the culture media at final concentrations of 1, 5, and 10 μg/ml either simultaneously with, or 48 h before, the addition of the bone-resorbing factors. After 48 h of exposure to lymphokine or PTH, calcium release was determined by counting

1. *Abbreviation used in this paper:* PTH, parathyroid hormone.

the supernatant media in a liquid scintillation counter. Data were expressed as the ratio of calcium released in counts per minute by the experimental bone (untreated or treated with gallium nitrate and a resorbing factor) to counts per minute released by a paired control bone (untreated or treated with gallium nitrate). 49 bones were used to establish control values; 4–22 bones were used to obtain the experimental points. Statistical testing for differences between means was done by a two-sided t test. Samples of cultured bones were fixed and histologic sections were examined by light microscopy.

Biochemical analyses. Total serum calcium was measured by atomic absorption spectrophotometry and serum creatinine was measured with fuller's earth (6).

Design of clinical study in patients with hypercalcemia. 10 patients with advanced cancer and hypercalcemia were hospitalized and aggressively treated for at least 48 h. Treatment included vigorous saline hydration and the administration of diuretics and oral phosphates. Three patients were receiving stable doses of corticosteroids. Patients had been given neither mithramycin nor other cytotoxic chemotherapy within the preceding 7 d. The persistence of a total serum calcium concentration ≥ 12 mg/dl despite treatment, a serum creatinine concentration ≤ 2 mg/dl, and the maintenance of a urinary volume ≥ 2,000 ml/d were also required for a patient's inclusion in this study. Patients received a continuous intravenous infusion of gallium nitrate (200 mg/m² per d) for 5–7 d. Daily measurements of total serum calcium and creatinine were made during the infusion and for several days thereafter. Patients who participated in this study (or their legal next-of-kin) gave written, informed consent, and the study was approved in advance by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center.

Results

In vitro effects upon bone resorption. Using explants of fetal rat bones, we found that the inhibitory effect of gallium nitrate upon bone resorption was time dependent. The simultaneous addition of gallium nitrate (10 μg/ml) and lymphokine decreased $^{45}Ca^{++}$ release by 15%, relative to control bones incubated with lymphokine only; however, the reduction was not statistically significant. Preincubation of bones with gallium nitrate (10 μg/ml) for 48 h before the addition of lymphokine caused a 40% reduction in $^{45}Ca^{++}$ release, which was highly significant ($P < 0.0005$). In the absence of resorbing factors, the addition of gallium nitrate to media did not affect $^{45}Ca^{++}$ release from treated bones as compared with untreated controls.

The inhibitory effects of gallium nitrate on in vitro bone resorption were also dose dependent. After 48 h of incubation, 1 μg/ml of gallium nitrate caused no significant change in augmented $^{45}Ca^{++}$ release after bones were stimulated with PTH or lymphokine. Significant dose-related reductions in $^{45}Ca^{++}$ release were observed, however, when concentrations of 5 and 10 μg/ml were used ($P < 0.025$) (Fig. 1). Cytological studies of bones exposed to gallium nitrate (10 μg/ml) for up to 72 h showed no significant change in cell morphology or number. There was no significant difference in the number of activated osteoclasts observed in gallium-treated bones as compared with untreated controls after exposure to lymphokine or PTH.

Effects in hypercalcemic patients. 10 patients with various histologic types of cancer received gallium nitrate to treat hypercalcemia. Despite the aggressive conventional therapy detailed
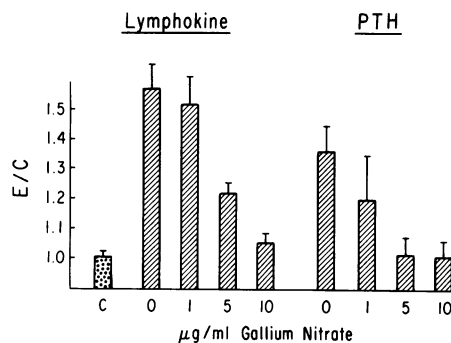

Figure 1. Dose-dependent inhibition of bone resorption in vitro by gallium nitrate. Explanted fetal rat bones were incubated with 0, 1, 5, and 10 μg/ml of gallium nitrate for 48 h before the addition of lymphokine or PTH. After 48 h of exposure to the bone-resorbing factors, significant inhibition of $^{45}Ca^{++}$ release was observed at drug concentrations of 5 and 10 μg/ml, $P < 0.025$. E/C, counts per minute exposed bone/counts per minute control bone.

previously, each patient persistently had levels of serum calcium ≥ 12.3 mg/dl. The sites of primary cancer in these patients and the change in serum calcium in response to gallium nitrate are shown in Table I. Total serum calcium was reduced to normal, and frequently to subnormal, levels in all patients. Two patients required parenteral calcium supplements after they abruptly developed severe hypocalcemia (≤7.0 mg/dl). With one exception, the initial fall in serum calcium concentration occurred within 48 h of initiating therapy.

The persistence of normocalcemia was more difficult to assess, since several patients whose hypercalcemia had responded to gallium nitrate later received cytotoxic chemotherapy and three patients died from progressive cancer within 2 wk after completing the trial. With these reservations, the median duration of the normocalcemic control was 10 d (range, 6–19+ d) (Table I). The only patient (with lymphoma) who showed any direct antitumor response later developed rapidly progressive disease without a recurrence of hypercalcemia. In the rest of the patients, hypercalcemia was controlled without evidence of any other anticancer effect.

Typical effects of gallium nitrate in two patients with cancer-related hypercalcemia are shown in Fig. 2. The relative effects of gallium nitrate and mithramycin were evident in one patient with epidermoid lung cancer and resistant hypercalcemia who received both drugs sequentially (Fig. 2, *bottom*). Serum calcium was normalized for 7 d by the infusion of gallium nitrate but for only 1 d despite four infusions of mithramycin.

Treatment with gallium nitrate at these dosages was very well tolerated. No patient experienced nausea or myelosuppression. The serum creatinine concentration in one patient with preexisting renal insufficiency increased transiently from 1.9 to 2.3 mg/dl, with an improvement to 1.6 mg/dl after hypercalcemia was controlled. Three other patients showed a minor increase in serum creatinine over the base line during the treatment period. In each case the increase was ≤0.2 mg/dl. Renal function improved in six other patients after serum calcium was normalized. The median

Table I. Control of Cancer-related Hypercalcemia in 10 Patients Treated with Gallium Nitrate

Primary site	Dosage	Total serum calcium*		Day normalized	Day increased
		Initial	Nadir		
	(mg/m²)(d)	*mg/dl*	*mg/dl*		
Breast	200 × 5	13.8 (14.2)	8.9 (10.5)	7	13
Breast	200 × 5	15.2 (16.1)	8.5 (9.5)	6	15
Lymphoma	200 × 7	15.6 (16.6)	6.6 (5.9)	5	26+
Head and neck	200 × 5	12.3 (13.6)	8.5 (9.8)	6	15
Breast	200 × 6	13.5 (14.8)	7.7 (9.0)	6	12+
Lymphoma	200 × 7	14.0 (15.2)	6.7 (7.5)	4	18+
Lung	200 × 5	14.0 (15.0)	7.0 (8.7)	4	18
Penis	200 × 5	12.3 (14.0)	9.3 (10.7)	6	14
Head and neck	200 × 5	13.5 (14.2)	7.8 (9.3)	5	11+
Lung	200 × 5	13.7 (13.6)	9.3 (9.7)	6	11
Mean		13.8 (14.7)	8.0 (9.1)	5	15

Normal range, 9.2–10.8 mg/dl. Numbers in parentheses represent values corrected for serum albumin concentration using the formula: corrected $[Ca^{++}]$ = observed $[Ca^{++}]$ − [albumin (g/dl)] + 4.0 (reference 12).

change in serum creatinine concentration for all 10 patients was 0 (range, +0.4 to −0.8 mg/dl).

Discussion

At the dosage used in this study, gallium nitrate proved highly effective for the control of cancer-related hypercalcemia. Moreover, the treatment was associated with notably few side effects. At the high dosages used for cancer chemotherapy, gallium nitrate can cause nephrotoxicity; this risk can be minimized, however, if adequate urinary output is assured (1, 2). Unlike cisplatin, a widely used metal-based anticancer drug, gallium

Figure 2. Typical effects of gallium nitrate in two patients with hypercalcemia. The patient represented by the top graph had malignant lymphoma. The bottom graph depicts the comparative effects of gallium nitrate and four infusions of mithramycin in a patient with epidermoid lung cancer and resistant hypercalcemia. (Shaded areas indicate normal range; bars indicate gallium nitrate infusion; arrows indicate mithramycin infusions, 25 μg/kg.)

nitrate produces substantially less hypomagnesemia and does not produce cumulative nephrotoxicity when administered by prolonged infusion (2, 7). Although the patients in our study had relatively resistant hypercalcemia, they were already well hydrated and had initial serum creatinine concentrations ≤ 2 mg/dl. Moreover, the dose received by most patients (200 mg/m² per d × 5 d) is <50% of the anticancer dosage (2). The results of this pilot study suggest that lower dosages, administered by more convenient routes and schedules, may also be effective. The absence of nausea and myelosuppression produced by this drug are important for patients who have usually received extensive anticancer therapy.

The mechanism by which gallium nitrate inhibits bone resorption is unclear. Like mithramycin, gallium nitrate is an effective antitumor drug for certain neoplastic diseases (2). It is unlikely, however, that the inhibition of bone resorption occurs by a cytotoxic effect. With a single exception, hypercalcemia was controlled in our patients without evidence of other antitumor effects. Mithramycin is directly toxic to osteoclasts. Bones exposed to this drug in vitro show multiple histologic abnormalities (8). By contrast, we observed no histologic abnormalities in bones exposed to pharmacologic concentrations of gallium nitrate. Furthermore, we recently showed that the drug only minimally affects lymphokine-mediated prostaglandin production by bone cells (9). Certain data suggest that radioactive gallium-67 can be incorporated into hydroxyapatite (10). Such an interaction might render bone calcium less susceptible to dissolution. Alternatively, gallium nitrate might impair binding of osteoclasts to the bone matrix. Either mechanism (or others) could account for the relatively unaltered histologic appearance of bones treated with this agent.

Hypercalcemia in cancer patients may arise from several different pathways (11). Because our initial clinical study was
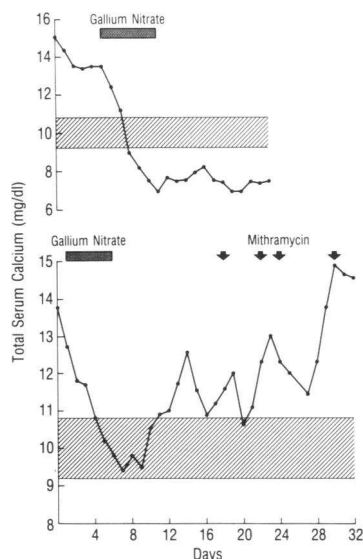
a pilot study, we did not evaluate specific mechanisms of hypercalcemia. Nonetheless, our results indicate that infusions of gallium nitrate effectively treat cancer-related hypercalcemia in patients with various types of cancer that presumably have different etiologies. Further study should establish the mechanism by which bone resorption is inhibited in vitro and the clinical correlations of dose and response. Studies of bone turnover in cancer patients with lytic bone disease, evaluation of alternative dose-schedules and routes of drug delivery, and comparative trials of gallium nitrate with standard hypocalcemic agents are important and will be initiated shortly.

Acknowledgments

References

1. Krakoff, I. H., R. A. Newman, and R. S. Goldberg. 1979. Clinical toxicologic and pharmacologic studies of gallium nitrate. Cancer (Phila.). 44:1722–1727.

2. Warrell, R. P., Jr., C. J. Coonley, D. J. Straus, and C. W. Young. 1983. Treatment of patients with advanced malignant lymphoma using gallium nitrate administered as a seven-day continuous infusion. Cancer (Phila.). 51:1982–1987.

3. Warrell, R. P., Jr., R. S. Bockman, C. J. Coonley, M. Isaacs, and H. Staszewski. 1983. Gallium nitrate inhibits calcium resorption from bone and is effective treatment for cancer-related hypercalcemia. Clin. Res. 31:511A. (Abstr.)

4. Bockman, R. S., and M. A. Repo. 1981. Lymphokine-mediated bone resorption requires endogenous prostaglandin synthesis. J. Exp. Med. 154:529–534.

5. Bockman, R. S., and W. P. L. Myers. 1977. Osteotropism in human breast cancer. In Cancer Invasion and Metastasis: Biologic Mechanisms and Therapy. S. B. Day, W. P. L. Myers, P. Stansly, S. Garattini, and M. G. Lewis, editors. Raven Press, NY. 431–450.

6. Owen, J. N., B. Iggo, T. J. Scandrett, and C. P. Stewart. 1954. Plasma or serum creatinine using Fuller's earth. Biochem. J. 58:426–437.

7. Leyland-Jones, B., R. B. Bhalla, F. Farag, L. Williams, C. J. Coonley, and R. P. Warrell, Jr. 1983. Administration of gallium nitrate by continuous infusion: lack of chronic nephrotoxicity confirmed by studies of enzymuria and β_2-microglobulinuria. Cancer Treat. Rep. 67:941–942.

8. Minkin, C. 1973. Inhibition of parathyroid hormone stimulated bone resorption in vitro by the antibiotic mithramycin. Calcif. Tissue Int. 13:249–257.

9. Bockman, R. S., M. A. Repo, and R. P. Warrell, Jr. 1983. Inhibition of in vitro bone resorption by gallium nitrate. Calcif. Tissue Int. 35:637.

10. Anghileri, L. 1971. Studies on the accumulation mechanisms of radioisotopes used in tumor diagnostic. Strahlentherapie. 142:456–462.

11. Stewart, A. F., R. Horst, L. F. Deftos, E. C. Cadman, R. Lang, and A. E. Broadus. 1980. Biochemical evaluation of patients with malignancy-associated hypercalcemia; evidence for humoral and non-humoral groups. N. Engl. J. Med. 303:1377–1383.

12. Payne, R. B., M. E. Carver, and D. B. Morgan. 1979. Interpretation of serum total calcium: effects of adjustment for albumin concentration on frequency of abnormal values and on detection of change in the individual. J. Clin. Pathol. (Lond.). 32:56–60.