Pharmacokinetics and Tolerability of a Single Dose of DN-101, a New Formulation of Calcitriol, in Patients with Cancer

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Abstract

Background: Intermittent administration allows substantial dose escalation of calcitriol but limited bioavailability of the commercially available formulations at high doses is limiting. In this dose escalation study, we sought to evaluate the tolerability and pharmacokinetics of a single oral dose of DN-101, a high-dose calcitriol formulation.

Methods: DN-101 doses were escalated in sequential groups of three to six patients with advanced solid tumors. Dose-limiting toxicity was defined as grade ≥2 hypercalcemia or grade ≥3 persistent treatment-related toxicities. Single-dose administration of 15, 30, 60, 75, 90, 105, 135, and 165 μg was tested.

Results: Thirty-eight patients were enrolled in 2002 and 2003. The median age was 70 years (range, 44-91 years). Dose escalation was stopped at the 165 μg level when the number of capsules required at one time reached 11. No dose-limiting toxicities occurred. Transient and self-limited grade 3 toxicities were hyponatremia (2) and proteinuria (1). A dose-proportional increase in peak concentration (C_{max}) and area under the concentration curve (AUC) was seen across the full range of DN-101 doses tested. At the 165 μg dose, C_{max} was 6.21 ± 1.99 ng/mL, AUC(0-24) was 41.3 ± 9.77 ng h/mL, AUC(0-∞) was 55.4 ± 8.44, and half-life ($T_{1/2}$) was 16.2 hours.

Conclusions: At doses between 15 and 165 μg, DN-101 exhibits linear pharmacokinetics. At 165 μg, DN-101 achieves systemic exposure that is 5- to 8-fold higher than that achieved with commercial formulations of calcitriol, which makes DN-101 comparable to that required for anti-tumor activity *in vivo* in a murine squamous cell carcinoma model.

Numerous studies have shown that calcitriol, the natural ligand of the vitamin D receptor, potently inhibits cell proliferation *in vitro* and *in vivo* in many cell types, including carcinomas of the breast, prostate, colon, skin, and brain, myeloid leukemia cells, and others (1, 2). Recently, calcitriol has also been shown to induce apoptosis and to inhibit angiogenesis, tumor invasion, and metastases (reviewed in ref. 3). Calcitriol or its analogues have been shown to produce additive or synergistic antineoplastic activity with a broad range of agents including dexamethasone (4, 5), retinoids (6, 7), tamoxifen (8–10), and radiation (11, 12), and several cytotoxic chemotherapy drugs, including docetaxel (13), paclitaxel (14), platinum compounds (15, 16), mitoxantrone (17), doxorubicin (18), and etoposide (19), are enhanced by calcitriol or its analogues. These preclinical data have stimulated interest in developing calcitriol for cancer treatment. Antineoplastic effects of calcitriol occur at supraphysiologic concentrations. In *in vitro* models of human prostate (LNCaP and DU145), pancreas (CaPAN-1), lung (MV522), breast (MCF-7), myeloma (H929), leukemia (HL-60), rat prostate (Dunning), and murine squamous cell carcinoma (SCC), IC_{50} values of 1 to 50 nmol/L have been reported (14, 20–22). Johnson and Trump also confirm these findings.[5] Kumagai et al. found that the 1,25 dihydroxycholecalciferol analogue paricalcitol inhibited the proliferation of myeloid leukemia cell lines HL-60, NB-4, and THP-1 by 50% (ED_{50}) at concentrations of 2.4×10^{-9} to 5.8×10^{-9} mol/L. This molecule inhibited the proliferation of colon cancer cell lines by 50% (ED_{50}) at a concentration of 1.7×10^{-8} mol/L for the HT-29 line and 3.2×10^{-8} mol/L for the SW837 line (23).

Initial trials in cancer patients tested calcitriol at a daily dose and substantial dose escalation was precluded by hypercalcemia and hypercalcuria. No significant clinical activity was seen in several tumor types. However, these trials did not test the drug at concentrations that are active in preclinical systems (24–27). In

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another study, s.c. administration of calcitriol every other day allowed some dose escalation and resulted in peak blood calcitriol concentrations (C_{max}) of ~0.7 nmol/L (28).

Weekly administration of oral calcitriol allowed substantial dose escalation. In a phase I trial, doses from 0.06 to 2.8 μg/kg were safely administered (29). Additional safety data were provided by a phase II trial of weekly calcitriol dosed at 0.5 μg/kg in hormone-naïve prostate cancer patients with a rising serum prostate-specific antigen (30). In this trial, calcitriol C_{max} was ~2 nmol/L. Another weekly schedule, with calcitriol given on 3 consecutive days every 7 days, has been tested in a phase I trial of calcitriol combined with paclitaxel (31) and dexamethasone (Trump 2005, in press). Doses up to 38 μg daily × 3 were given without dose-limiting toxicity. Calcitriol C_{max} ranged from 1.4 to 3.5 nmol/L.

Although pulse dosing, both weekly and 3 days per week, allowed substantial dose escalation of calcitriol, both trials found that calcitriol pharmacokinetic variables did not increase in a dose-proportional fashion at higher doses. In the trial of weekly calcitriol, the apparent limitation in bioavailability at higher doses precluded the determination of the maximum tolerated dose. Both trials noted significant interpatient variability in calcitriol pharmacokinetics. Finally, due to the limitations of the available formulations (0.5 μg capsules), both trials required patients to swallow very large numbers of capsules.

To overcome these limitations, DN-101, a new high-dose (15 μg calcitriol per capsule) formulation, was designed for oncology applications (Novacea, Inc., South San Francisco, CA). In this dose escalation study, we sought to evaluate the tolerability and pharmacokinetics of a single oral dose of DN-101.

Methods

Patients

Adult patients with advanced solid tumor malignancies who failed at least one potentially effective therapy were eligible. Patients with prostate cancer who had a rising prostate-specific antigen after therapy with curative intent or while on hormonal therapy were eligible. Patients were required to have an Eastern Cooperative Oncology Group performance status ≤2, life expectancy ≥3 months, WBC count ≥3,000/mm^3, neutrophil count ≥1,500/mm^3, platelet count ≥100,000/mm^3, serum creatinine <1.5 mg/dL, albumin >3.0 gm/dL, and serum calcium less than the upper limit of normal. Women of childbearing potential had to have a negative urine pregnancy test and both men and women had to agree to use effective contraception.

Patients were excluded for any significant medical condition, specifically for uncontrolled heart failure, history of cancer-related hypercalcemia or vitamin D toxicity, calcium salt kidney stones within 5 years, prior investigational therapy within the past 30 days, prior use of calcitriol within 3 months, known hypersensitivity to calcitriol, and concurrent active treatment for cancer (except for androgen deprivation for prostate cancer). Excluded concomitant medications were calcium- or magnesium-containing antacids, bile-resin binders, bisphosphonates, calcium supplements, and ketoconazole or related compounds.

Dose escalation

This was a single-dose phase I study. DN-101 doses were escalated in sequential groups of 3 to 10 patients. Dose levels tested were 15, 30, 60, 75, 90, 105, 135, and 165 μg. The maximum tolerated dose was defined as that dose at which no more than one patient experienced dose-limiting toxicity. Dose-limiting toxicity was defined as grade ≥2 hypercalcemia or grade ≥3 toxicities that were deemed treatment-related by the investigator and persisted. Dose escalation was permitted if none of the at least three patients experienced dose-limiting toxicity within 7 days. If one patient experienced a dose-limiting toxicity, an additional three patients were to be treated at the same dose level. Patients were subsequently offered enrollment in an extension study of repeat dosing. This study is ongoing and will be reported on completion.

Treatment

Patients were admitted overnight, received DN-101 in the morning, were released 24 hours after dosing, and returned to the outpatient clinic 48 hours, 72 hours, and 7 days after dosing. Patients were asked to fast starting at midnight before dosing and continued fasting for 2 hours after the morning dose of DN-101. Patients were asked to drink three to four cups (24-32 oz.) of electrolyte-containing fluids (initially, instructions called for water but were modified to electrolyte-containing solution when hyponatremia was noted) above their usual intake starting 12 hours before dose; oral hydration was continued for 3 days after dosing. Patients were encouraged to limit their intake of high-calcium foods such as milk, cheese, and red meats.

Monitoring

In addition to a medical history and physical examination, the pretreatment evaluation included an electrocardiogram, a complete blood count and chemistry profile, urinalysis, and 24-hour urine collection for creatinine clearance, quantitative protein, β_2-microglobulin, calcium, sodium, and potassium.

The electrocardiogram was repeated 12 and 24 hours after dosing. A blood chemistry profile was repeated at 4, 8, 12, 24, 48, and 72 hours and after 7 days. A complete blood count and a urinalysis were obtained 7 days after dosing. A 24-hour urine collection for creatinine clearance, quantitative protein, β_2-microglobulin, calcium, sodium, and potassium was repeated on days 2 and 6. Adverse events were recorded on the same schedule as the blood draws for pharmacokinetics and on day seven. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (version 2).

Pharmacokinetics

Samples. Specimens for pharmacokinetics were drawn immediately before dosing and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours after the dose. An additional blood sample at 168 hours (7 days) was drawn for the 165 μg dose group. At each time point, one 10.0-mL EDTA lavender top tube was filled, centrifuged for 15 minutes, and 4.0 mL of plasma were placed in a cryovial and frozen at −70°C until testing.

Analytic methods. Calcitriol plasma concentrations were determined by a commercial RIA that uses a double antibody (DiaSorin, S.P.A. Saluggia, Italy). The limit of detection was 4.0 pg/mL for 1,25-$(OH)_2D_3$ and 1,25-$(OH)_2D_2$ with negligible cross-reactivities to 25-(OH)D3, 24,25-(OH)2D3, and 25,26-(OH)2D3; the coefficients of variation were 12% intraassay and 15% interassay; linearity of dilutions ranges from 7 to 87 pg/mL; and recovery was 4 to 31 pg/mL within 80% to 120%. For samples that contained calcitriol concentrations greater than the upper limit of detection of the method, the samples were diluted from 10:1 to 100:1 (with the zero standard buffer provided by the assay manufacturer per instructions of the manufacturer, depending on the expected serum concentration) to a target concentration within the calibration curve and reassayed.

Pharmacokinetic analyses. Descriptive pharmacokinetic variables were determined by standard model independent methods based on the plasma concentration-time data of each patient. Plasma samples with adjusted concentrations (baseline subtraction) below zero were assigned values of zero. Maximum concentration (C_{max}) and time of C_{max} (T_{max}) were determined by visual inspection of the data. Half-life was determined by ln(2) divided by the terminal rate constant, which was calculated from the log-linear portion of the terminal phase. The area under the concentration curve, AUC(0-T), was calculated using

log-linear trapezoidal method where T was the last measurable time point. The area under the curve AUC(0-∞) was the sum of AUC(0-T) and AUC(T-∞). The latter was calculated by dividing the computer estimated value at T by the terminal rate constant. Pharmacokinetic analyses were done using WinNonlin Professional 3.3 (Pharsight Corp., Mountain View, CA). Mean and SD plasma concentrations were generated using Microsoft Excel (Redmond, WA). All group results are expressed as arithmetic mean and SD with the exception of T_{max} and $T_{1/2}$. T_{max} is expressed as the median and range and $T_{1/2}$ is reported as the harmonic mean and pseudo SD based on the jackknife variance technique (32).

Results

Patients. Thirty-eight patients were enrolled between March 2002 and September 2003. All patients completed the safety and pharmacokinetic evaluations. Patients' characteristics are summarized in Table 1. The majority of patients had prostate cancer. The median age was 70 years (range, 44-91 years) and the median Eastern Cooperative Oncology Group performance status was 0.

Treatment. Patients were entered into the study at dose levels of 15, 30, 45, 60, 75, 90, 105, 135, and 165 μg. The expansion of the 45 and 60 μg cohorts was a result of events in a companion repeat dosing trial and not a result of toxicity encountered in the single-dose study reported here. No patient withdrew before completing 7 days of observation. Dose escalation was stopped at the 165 μg level when the number of capsules required to be consumed at one time reached 11. The accrual of an additional patient at the 165 μg level reflects the added caution exercised at a dose level that produced unprecedented calcitriol exposure.

Table 1. Patient characteristics

Number	38
Age	
Median	70
Range	44-91
Eastern Cooperative Oncology Group performance status	
0	21 (55%)
1	15 (39%)
2	2 (5%)
Gender	
Male	34 (89%)
Female	4 (11%)
Body weight, male (kg)	
Median	83.5
Range	61-131.4
Body weight, female (kg)	
Median	72.75
Range	59.5-112
Tumor type	
Adenocarcinoma of the prostate	27 (71%)
Adenocarcinoma of the colon	8 (21%)
Adenocarcinoma of the rectum	1 (3%)
Gastric adenocarcinoma	1 (3%)
Squamous cell carcinoma of the head and neck	1 (3%)
Prior therapy	
Surgery	27 (71%)
Radiation	18 (47%)
Chemotherapy	14 (37%)
Hormonal therapy	18 (47%)
Immunotherapy	2 (5%)
Other*	5 (13%)

*Includes investigational agent, herbal medicine, calcitriol, and corticosteroid.

Toxicity. No dose-limiting toxicities occurred. No serious adverse events related to DN-101 occurred. One patient was hospitalized for grade 1 pyrexia that was determined to be unrelated to DN-101. DN-101 was well tolerated with generally only grade 1 to 2 adverse events up to doses of 165 μg. No grade 4 toxicities occurred. Two episodes of grade 3 hyponatremia occurred, possibly related to study specified hydration, one at the 45 μg dose level and one at the 60 μg dose level. The grade 3 hyponatremia at the 60 μg dose level occurred after the protocol was amended to provide for hydration using electrolyte-containing fluids rather than water. One reversible grade 3 proteinuria (without renal dysfunction) that did not require discontinuation of DN-101 was reported at the 60 μg dose level. Most frequent adverse events are reported in Table 2.

Pharmacokinetics. Plasma samples for pharmacokinetics were collected from all 38 patients enrolled in the study. The mean plasma concentration-time profiles for each dose are presented in Fig. 1. Pharmacokinetic variables are summarized in Table 3.

Relationship between dose and pharmacokinetic variables. Previous efforts to dose escalate oral calcitriol using commercially available calcitriol capsules (29, 31) and calcitriol liquid (33) showed a nonlinear relationship between dose and C_{max} and AUC at higher doses. In contrast, Figs. 2 and 3 show a dose-proportional increase in both C_{max} (linear regression $r = 0.86$, $P < 0.0001$) and AUC (linear regression $r = 0.89$, $P < 0.0001$) across the full range of DN-101 doses tested. Absorption was rapid, with a T_{max} of ~1 to 2 hours. Terminal $T_{1/2}$ values were dose independent and ranged from 5.5 to 16.2 hours.

In an exploratory analysis, we also examined the relationship between dose-normalized AUC and body weight, age, and creatinine clearance. The slope of the linear regression line was not significant for body weight ($P = 0.12$) or age ($P = 0.92$). A modest correlation was seen, demonstrating increased calcitriol exposure, AUC(0-∞) with reduced creatinine clearance ($P = 0.0595$; Fig. 4).

Effect on serum calcium and urinary calcium excretion. DN-101 administration resulted in modest increases in the serum levels of calcium. Mean serum calcium (reference range, 2.20-2.56 mmol/L) increased from 2.26 mmol/L (range, 1.9-2.43 mmol/L) before dosing to 2.44 mmol/L (range, 2.18-2.75 mmol/L) at 48 hours and 2.41 mmol/L (range, 2.03-2.65 mmol/L) at 72 hours ($P < 0.0001$ by repeated measures ANOVA). Serum calcium was in the reference range after 7 days in all patients. There was no apparent effect of DN-101 dose on mean serum calcium at any of the time points.

Urinary excretion of calcium, determined from 24-hour urine collections, was measured before dosing at 48 hours and 7 days for a subset of patients who received doses of 45 μg or higher. Mean 24-hour urinary calcium excretion (reference range, 0-6.2 mmol) increased from 2.3 mmol (SD 1.60, $n = 22$) with a range of 0.27 to 5.84 mmol before treatment to 8.1 mmol (SD 5.90, $n = 22$) with a range of 0.15 to 20.23 mmol at 48 hours and 6.3 mmol (SD 4.59, $n = 16$) with a range of 0.5 to 17.51 mmol

Table 2. Adverse events related to DN-101 reported in more than one patient

Adverse event	Severity grade				Total (N = 38)	Lowest dose at which event occurred (μg)
	1	2	3	4		
Hyponatremia	10 (26.3%)		2 (5.6%)		12 (31.6%)	15
Hypoalbuminemia	3 (7.9%)	5 (13.2%)			8 (21.1%)	45
Hyperphosphatemia	6 (15.8%)	1 (2.6%)			7 (18.4%)	30
Hypercalcemia	5 (13.2%)				5 (13.2%)	60
Proteinuria	3 (7.9%)	1 (2.6%)	1 (2.6%)		5 (13.2%)	45
Creatinine clearance decreased	3 (7.9%)	1 (2.6%)			4 (10.5%)	60
Hypercalciuria*	2 (5.3%)	2 (5.3%)			4 (10.5%)	60
Hyperglycemia nitric oxide synthase	2 (5.3%)	2 (5.3%)			4 (10.5%)	30
Edema peripheral	4 (10.5%)				4 (10.5%)	75
Fatigue	3 (7.9%)				3 (7.9%)	45
Hypercholesterolemia	2 (5.3%)	1 (2.6%)			3 (7.9%)	135
Nausea	3 (7.9%)				3 (7.9%)	15
Blood creatinine increased	2 (5.3%)				2 (5.3%)	75
Diarrhea	2 (5.3%)				2 (5.3%)	30
Dizziness	2 (5.3%)				2 (5.3%)	135
Nasal congestion	2 (5.3%)				2 (5.3%)	45
Thrombocytopenia	2 (5.3%)				2 (5.3%)	60

*Graded by the investigator. No Common Toxicity Criteria grade available.

on day 7 (P = 0.0014 by repeated measures ANOVA). Over the DN-101 dose range studied (45-165 μg), the dose of DN-101 administered did not seem to have any effect on urinary calcium excretion.

Discussion

Encouraging preclinical data have led to growing interest in the development of calcitriol, the active form of vitamin D, for the treatment of cancer. The same preclinical data suggest that antineoplastic activity requires substantially supraphysiologic concentrations of calcitriol. Previous efforts to escalate the doses of oral calcitriol have focused on intermittent dosing and used commercially available forms of this drug.

Significant escalation of the calcitriol dose was accomplished with weekly administration of commercially available capsules. However, studies that examined dosing once a week and for 3 consecutive days each week showed that at higher doses, neither C_{max} nor AUC increased in a dose-proportional fashion. This limited the full evaluation of the antineoplastic activity of calcitriol in humans. Commercially available oral liquid formulation was then tested to exclude the possibility that the nonlinear pharmacokinetics was a feature that was specific to the capsule formulation. This approach did not improve the outcome of the studies conducted with commercially available calcitriol capsules.

DN-101 is a new investigational formulation of calcitriol that is highly concentrated and specifically designed for cancer

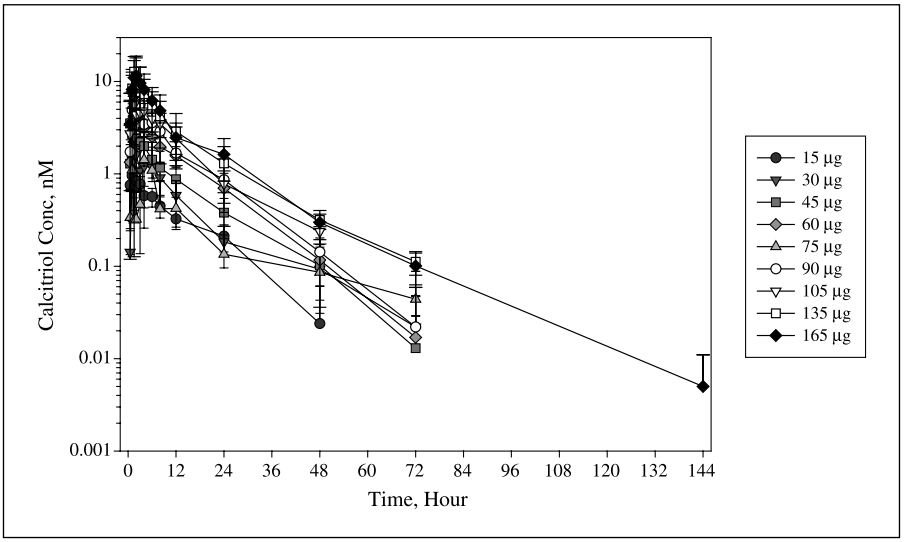

Fig. 1. Mean calcitriol concentration-time profiles following the administration of a single dose of DN-101.

Table 3. DN-101 pharmacokinetic parameters (mean ± SD)

Dose (μg)	C_{max} (nmol/L)	AUC(0-24) (ng·h/mL)	AUC(0-∞) (ng·h/mL)	$T_{1/2}$* (h)
15 (n = 3)	1.1 ± 0.2	9.7 ± 1.3	12.0 ± 2.6	5.4 ± 3.9
30 (n = 3)	2.2 ± 0.8	15.6 ± 6.9	20.5 ± 8.4	11.0 ± 8.1
45 (n = 6)	3.4 ± 0.8	25.3 ± 7.9	32.2 ± 10.5	11.3 ± 2.6
60 (n = 10)	5.2 ± 1.6	42.3 ± 8.0	52.7 ± 12.0	7.9 ± 5.8
75 (n = 3)	3.8 ± 0.8	38.4 ± 5.8	51.3 ± 10.1	10.8 ± 3.1
90 (n = 3)	6.9 ± 1.2	54.3 ± 13.8	65.8 ± 21.3	7.4 ± 1.9
105 (n = 3)	8.1 ± 4.9	64.1 ± 25.9	80 ± 24.8	11.6 ± 3.7
135 (n = 3)	13.7 ± 5.3	93.4 ± 46.7	118.3 ± 52.1	14.3 ± 5.1
165 (n = 4)	14.9 ± 4.8	95.9 ± 21.8	124.6 ± 21.7	16.2 ± 5.5

*Mean ± pseudo SD.

therapy. In this study, we showed that a broad range of doses of DN-101 was safely administered in a single-dose study. Unlike previous reports, we showed dose-proportional increases in C_{max} and AUC of calcitriol. The absence of the apparent absorption ceiling seen with commercially available oral formulations allowed us to reach significantly higher concentrations of calcitriol than had been previously possible. Because most preclinical systems show dose-dependent antineoplastic activity, this may provide a significant advantage as calcitriol is tested for cancer therapy.

In this study, although we achieved markedly higher serum concentrations of calcitriol than had been previously reported, we did not establish a maximum tolerated dose of a single dose of DN-101. Dose escalation was stopped when the number of capsules was thought to hamper feasibility of further dose escalation. More concentrated formulations may allow further dose escalation in future studies. Adverse events with the single dose were modest and were generally limited to mild, asymptomatic changes in serum and urine calcium. Notably, hypercalcemia was limited to grade 1 and seen in a minority

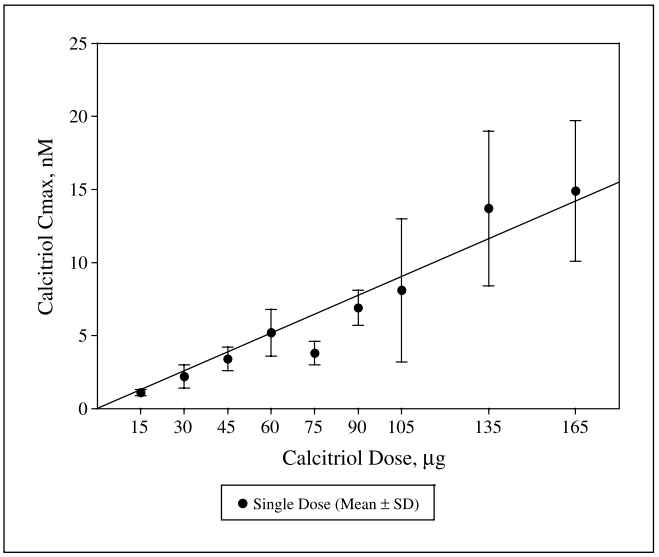

Fig. 2. Mean calcitriol C_{max} over the range of doses tested.

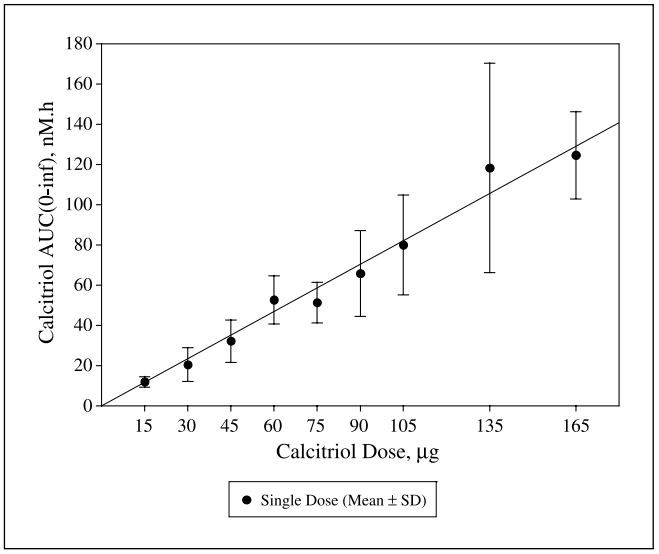

Fig. 3. Mean calcitriol AUC(0-∞) over the range of doses tested.

of patients. Consistent with previously reported data, serum calcium was highest on day 3. Increases in urinary calcium excretion were observed as expected for calcitriol although their clinical significance is unclear. No symptomatic kidney stones were reported although hypercalcuria could have contributed to the reduction in creatinine clearance reported in four patients. The frequency of hyponatremia was greater than expected. The protocol specified vigorous oral hydration, which may have contributed to hyponatremia. However, a shift to electrolyte-containing fluids did not result in the elimination of this adverse event. One might speculate that syndrome of inappropriate antidiuretic hormone secretion, which has been reported in prostate cancer (34), may be present in a subclinical form more frequently than previously suspected.

Muindi and colleagues have done pharmacokinetic studies in tumor-bearing mice treated with a dose of calcitriol that has distinct single agent activity, 0.125 μg/mouse qd × 3. At this dose, the C_{max} and AUC are 9.2 ng/mL (22.1 nmol/L) and 37 ng h/mL (35). By comparison, in humans, C_{max} and AUC

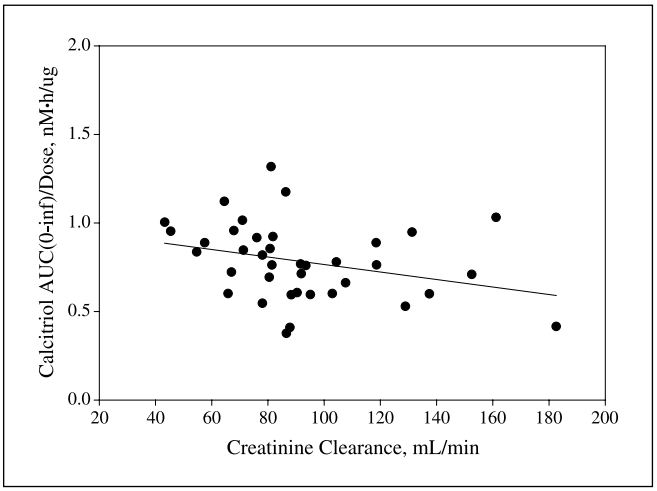

Fig. 4. Calcitriol dose-normalized AUC versus creatinine clearance in individual patients following the administration of a single dose of DN-101.

following 38 μg of the commercially available preparation of calcitriol are 1.4 ng/mL (3.4 nmol/L) and 7.5 ng h/mL, respectively (36). In the study presented here, for DN-101, the 165 μg dose yields drug exposure estimates comparable to those seen in mice at the active dose: AUC of 41.3 ng h/mL and C_{max} of 6.21 ng/mL. These data indicate that the highest dose of DN-101 administered achieves systemic exposure comparable to that required for antitumor activity *in vivo* in one murine tumor model (SCC). This exposure is 5- to 8-fold higher than that achieved and likely to be achievable with the available commercial oral formulations of calcitriol (36).

A phase I evaluation of DN-101 on a weekly schedule is under way. DN-101 is also being examined as a single agent and in combination with several standard anticancer agents in several cancer clinical trials in patients with advanced prostate cancer and non–small-cell lung cancer.

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