Cancer Chemother Pharmacol (2009) 65:33–40
DOI 10.1007/s00280-009-1000-2

ORIGINAL ARTICLE

A phase I and pharmacokinetics study of intravenous calcitriol in combination with oral dexamethasone and gefitinib in patients with advanced solid tumors

Josephia R. Muindi · Candace S. Johnson · Donald L. Trump · Renee Christy · Kristie L. Engler · Marwan G. Fakih

Received: 10 December 2008 / Accepted: 5 April 2009 / Published online: 26 April 2009

Abstract
Purpose The primary objective of this study was to determine the maximum tolerated dose (MTD) of intravenously (i.v.) calcitriol administered in combination with a fixed oral dose of dexamethasone and gefitinib in patients with refractory solid tumors.
Methods A fixed oral dose of dexamethasone of 4 mg/day was given every 12 h × 3 doses starting 12 h prior to i.v. calcitriol administration. Calcitriol was administered i.v. over 1 h on weeks 1, 3, and weekly thereafter. The starting calcitriol dose level was 57 μg and escalation occurred in cohorts of three patients until the MTD was defined. Gefitinib was given at a fixed oral daily dose of 250 mg starting at week 2 (day 8). Serum calcitriol PK studies were performed on day 1 (calcitriol + dexamethasone) and on day 15 (calcitriol + dexamethasone + gefitinib).
Results A total of 20 patients were treated. Dose-limiting hypercalcemia was observed in two out of the four patients receiving 163 mcg/week of calcitriol. Mean (±SE) peak serum calcitriol concentration (C_{max}) at the MTD (125 μg/week calcitriol) was 11.17 ± 2.62 ng/ml and the systemic exposure ($AUC_{0-72\,h}$) of 53.30 ± 10.49 ng h/ml. The relationship between calcitriol dose and either C_{max} or AUC was linear over the 57–163 μg dose range.
Conclusions The addition of a low dose of dexamethasone allowed the safe escalation of calcitriol to the MTD of 125 μg/week. This dose level resulted in serum calcitriol concentrations that are associated with pre-clinical antitumor activity. However, no antitumor activity was noted clinically in patients with solid tumors.

Keywords Calcitriol · Dexamethasone · Pharmacokinetics · Tolerability

J. R. Muindi · D. L. Trump · R. Christy · K. L. Engler · M. G. Fakih (✉)
Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA
e-mail: marwan.fakih@roswellpark.org

C. S. Johnson
Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

Introduction

Calcitriol, the biologically most active metabolite of vitamin D_3, has potent antiproliferative, differentiating and apoptotic and anti-angiogenic effects in vitro and in vivo tumor models [1–4]. The molecular mechanisms of calcitriol antitumor activity are not fully understood. However, calcitriol is known to induce G0/G1 arrest mediated by over expression of cyclin-dependent kinase p21 and p27, to inhibit MEK- and Akt-mediated survival signaling pathways and to up-regulate pro-apoptotic signaling pathways [5–8]. Recent reports suggest that calcitriol may exert antitumor activity by targeting the epidermal growth factor receptor (EGFR) pathway [9]. Our preclinical studies, in a murine squamous cell carcinoma model system, demonstrated synergistic antitumor activity when calcitriol was used in combination with gefitinib, an EGFR tyrosine kinase inhibitor [10]. Based on these preclinical observations, we conducted a phase I study to determine the maximum tolerated dose (MTD) of weekly intravenous calcitriol with a fixed dose of gefitinib of 250 mg/day [11]. The result of this study identified 74 μg/week as the maximum tolerated calcitriol dose and that the administration of gefitinib had no effect on serum calcitriol pharmacokinetics. These

results also demonstrated that systemic calcitriol exposure associated with antitumor activity in murine squamous cell carcinoma model was not consistently achieved in all patients treated at the MTD. Therefore, new approaches to optimize systemic calcitriol exposure are required.

The administration of calcitriol in combination with dexamethasone has the potential to augment the safety and tolerability of calcitriol in cancer patients for two reasons. First, glucocorticoids, especially dexamethasone, have been extensively used in the treatment of hypercalcemic states including calcitriol-induced hypercalcemia [12, 13]. The mechanisms of dexamethasone antihypercalcemic effects include decreased gastrointestinal calcium absorption and increased urinary calcium excretion [14, 15]. Second, the administration of dexamethasone has been shown to enhance calcitriol antitumor effects in some tumor models [16]. The enhancement of calcitriol antitumor effects when administered in combination with dexamethasone is thought to be mediated by differential up-regulation of the vitamin D receptor (VDR) in tumor tissues compared to the gastrointestinal mucosa [16]. Therefore, this phase I study was designed as a sequel to our prior phase I study of calcitriol and gefitinib to identify the MTD of weekly i.v. calcitriol when administered in combination with gefitinib at 250 mg/day and a fixed dose of dexamethasone at 4 mg/day every 12 h × 3.

Materials and methods

Study schedule and drug administration

Patients received a fixed dose of oral dexamethasone of 4 mg administered orally 12 h prior, at the time of, and 12 h after to each dose calcitriol. Calcitriol (Calcijex®) supplied in 1 μg vials by Abbott Laboratories (Abbott Park, IL) was administered as i.v. infusion over 60 min once weekly except week 2. The first dose level of calcitriol was 57 μg. Subsequent dose escalation levels were 74, 96, 125, and 163 μg/week. No intra-patient calcitriol dose escalation was allowed. Gefitinib (Iressa) was supplied by AstraZeneca (London, United Kingdom) in 250 mg tablets and was given once daily starting 1 week after the first dose of calcitriol. Dexamethasone was given 12 h prior to, at the time of, and 12 h after each calcitriol dose administration. Each 4 weeks of treatment constituted one cycle.

During the first cycle, calcitriol was given on weeks 1, 3, and 4, while gefitinib was started on week 2 of treatment. This study design allowed for the evaluation of calcitriol pharmacokinetics with and without gefitinib. From cycle 2 and beyond, calcitriol was given on a weekly basis (along with dexamethasone), while gefitinib was continued daily. Schematic drug administration schedule is shown in Fig. 1.

Study eligibility

Only patients with histologically confirmed metastatic or unresectable solid tumors for which there was no standard curative or palliative treatment were enrolled in the study. Patients had to be over 18 years of age, with Eastern Cooperative Oncology Group performance status ≤2, estimated life expectancy >12 weeks, and off-cancer chemotherapeutic or radiation treatment for at least 4 weeks (6 weeks for nitrosoureas or mitomycin C). Adequate bone marrow (neutrophils ≥1,500/μl, hemoglobin ≥8.0 g/dL, and platelets ≥100,000/μl), hepatic function (serum bilirubin ≤upper limit of the reference range, serum AST and ALT ≤2.5 × upper limit of the reference range), and renal function (creatinine ≤1.5 upper limit of the reference range or creatinine clearance ≥60 ml/min) were a prerequisite for enrollment in the study. Patients unable to take oral medications; with brain metastases; history of hypercalcemia or genitourinary stones; patients on digoxin, thiazide, calcium supplements, supra-physiologic doses of glucocorticoids

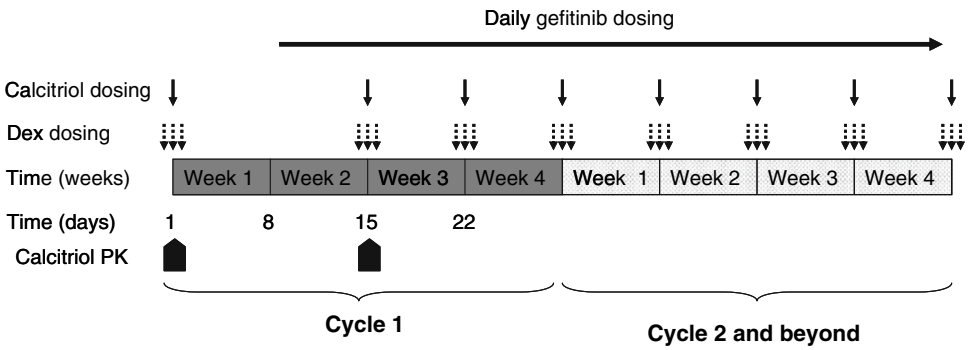

Fig. 1 Treatment schema. On cycle 1, calcitriol (*solid vertical black arrow*) was given on days 1 and 15 and weekly thereafter. Dexamethasone (*broken vertical black arrows*) was given orally 12 h prior, at the time of, and 12 h after to each dose calcitriol. Gefitinib started on day 8 (*solid black horizontal line*). Calcitriol pharmacokinetics (*black pointed rectangle*) was done on days 1 and 15. On cycles 2 and beyond, calcitriol was administered weekly along with daily gefitinib; dexamethasone was given 24 h and at the time of calcitriol dosing

(exceeding the equivalent of 15 mg of hydrocortisone/day), and/or allergic to calcitriol or gefitinib were excluded from study. HIV-positive patients were not eligible because of possible pharmacokinetic interaction with antiretroviral drugs. Patients with reproductive potential had to agree to use adequate contraception before study entry and for the duration of study participation.

The study and consent form was approved by the Institutional Scientific and Review Committee and the Institutional Review Board before its activation. All patients provided signed informed consent before study entry. The study was conducted in accordance with the Good Clinical Practice Guidelines as issued by the International Conference on Harmonization and the Declaration of Helsinki.

Dose escalation and dose limiting toxicity (DLT) definition

Three patients were entered at each dose level. In the absence of DLT, the next dose level was explored. If DLT was seen in one patient, further three patients were added at that dose level and, if no additional DLT was seen, escalation to the next dose level occurred. If at least two patients had DLT at a given dose level, accrual to that dose level was stopped; this was the maximally administered dose. Further patients were then added, as required, to the previous dose level (and if necessary to lower dose levels) to establish the highest dose at which less than two out of six patients had DLT. This was the MTD.

A DLT was any of the following toxicities that were attributable to study treatment on cycle 1 (first 4 weeks of treatment): any grade 3 or 4 toxicity except for grade 3 anemia, any grade 2 or above hypercalcemia (corrected calcium >11.5 mg/dL) if confirmed on repeat blood draw (>72 h), any grade 2 or above hypercalcemia that is associated with serious hypercalcemic symptoms, any treatment interruption that lasts for >2 weeks that is related to treatment toxicity, sustained increase (>72 h) in creatinine to >2x baseline and >2 g/dL, and clinical or radiological evidence of new genitourinary stones.

Dose modifications

No dose modifications were allowed for gefitinib; however, treatment interruptions were allowed for periods not exceeding 2 weeks for grade 3 and above skin toxicity, grade 3 and above diarrhea, grade 3 and above nausea unrelated to hypercalcemia, and grade 4 neutropenia. In case of dose interruption secondary to toxicity, treatment was resumed upon improvement to grade 1 or less with the exception of neutropenia, in which case retreatment was allowed when the count exceeded 500 cells/μl. No dose modifications were allowed for DLTs related to calcitriol, in which case patients had to be discontinued from study. No growth factors were allowed on the study with the exception of recombinant erythropoietin.

Clinical evaluation and follow-up

A complete medical history, physical examination, pregnancy test for women with reproductive potential, complete blood count, and comprehensive chemistry profile (electrolytes, blood urea nitrogen, creatinine, magnesium, LDH, ALT, AST, and bilirubin) were obtained within 1 week before treatment initiation. Baseline computed tomography scans of the chest, abdomen, and pelvis were obtained within 4 weeks before initiation of treatment. Complete blood count and comprehensive chemistry were repeated on a weekly basis. Calcium, phosphorous, and creatinine levels were measured before and 3 days after each calcitriol infusion. Medical history, physical examination, and toxicity assessment as per National Cancer Institute Common Toxicity Criteria 2.0 were done on the first day of the 1st and 3rd weeks of cycle 1 and on day 1 of week 1 of each subsequent cycle. Corrected calcium was used to grade hypercalcemia (corrected calcium = serum calcium + (4 − serum albumin) × 0.8). Computed tomography scans of chest, abdomen, and pelvis were repeated every two cycles (8 weeks) to assess response. Responses were categorized according to the Response Evaluation Criteria in Solid Tumors [17].

Calcitriol pharmacokinetics

About 10 ml of blood was collected in a non-heparinized (red-top) tube for days 1 (calcitriol + dexamethasone treatment) and 15 (calcitriol + dexamethasone + gefitinib treatment) serum calcitriol pharmacokinetic studies. On day 1 PK samples were obtained at baseline; at 30 and 45 min during the infusion; and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, and 72 h after calcitriol infusion; the sample collection on day 15 was up to 24 h only. Serum samples prepared immediately by a 10-min centrifugation at 2,000*g* were batched and stored in 1 to 2 ml aliquots at −20°C until assayed for calcitriol levels. Serum calcitriol concentrations were determined using 1,25-dihydroxyvitamin D_3-[I^{125}] RIA kit from DiaSorin Co. (Stillwater, MN). The analytic characteristics of this assay have previously been described [18]. Non-compartmental analysis of pharmacokinetic data was done using WinNonlin® Version 5.1, Pharmasight (Mountain View, CA, USA). The serum calcitriol pharmacokinetic parameters estimated were: peak levels (C_{max}), area under the concentration–time curve from time 0 to 72 h ($AUC_{0-72\,h}$), terminal half life ($t_{1/2}$); the apparent clearance (CL/F) was calculated using the equation CL/F = Dose/$AUC_{0-72\,h}$. The relationship between pharmacokinetic variables C_{max}, $AUC_{0-24\,h}$, and CL/F and calcitriol dosage were

evaluated by use of linear regression analysis, and the correlation coefficient was defined.

Results

Study patient characteristics

A total of 20 patients were entered on this study between June 2005 and October 2006. Demographic characteristics of these patients are detailed in Table 1.

Treatment administration

As shown in Table 2 five calcitriol dose levels were evaluated with the number of patients treated indicated at each dose level. The total number of cycles received on study was 41. The median number of cycles administered per patient was 2 (range 1–4). All patients without dose limiting toxicity (DLT) were able to receive all the intended dosage on cycle 1.

Table 1 Patient characteristics

N = 20	
Gender (male/female)	8/12
Age (median/range), years	58 (33–74)
ECOG 0	3
ECOG 1	14
ECOG 2	3
Cancer diagnosis	
Colorectal	6
Head and neck	4
Prostate	3
Sarcoma	2
Breast	1
Stomach	1
Non-small cell lung cancer	1
Gastrointestinal stromal tumor	1
Urachal	1

Table 2 Calcitriol dose levels and number of patients enrolled

Dose level	Dose of calcitriol (μg)	Number of patients enrolled
DL1	57	3
DL2	74	4
DL3	96	3
DL4	125	6
DL5	163	4

Dose escalation, DLT, and MTD

No dose-limiting toxicities were noted on dose-levels 1–4. On dose-level 2, one patient experienced chest pain 2 days after the first dose of calcitriol. Cardiac work-up revealed an elevated troponin level consistent with a myocardial infraction. A cardiac catheterization confirmed severe coronary artery disease. The patient's calcium level was normal at the time of occurrence of chest pain and his cardiac adverse event was deemed unrelated to treatment. His treatment was delayed for 4 weeks following which he was restarted on study treatment without any adverse events. He was deemed to have received insufficient treatment on cycle 1 to assess a DLT. Therefore, one additional patient was enrolled on dose-level 2 for a total of four patients. Three additional patients were enrolled on each of dose-levels 3 and 4 without any DLT. At dose-level 5, one patient developed grade 3 dose-limiting hypercalcemia among the first 3 treated patients. A fourth patient was enrolled at this dose level and experienced another DLT grade 3 hypercalcemia. Dose-level 5 was declared as non-tolerable and dose-level 4 was expanded to a total of six patients without any DLT. Dose-level 4 (calcitriol 125 mcg/week) was declared the MTD.

Toxicity

All 20 treated patients were evaluable for toxicity. Only grade 2 and above toxicity is reported except for hypercalcemia—for which all grades were collected and reported. The National Cancer Institute (NCI) Clinical Toxicity Criteria (CTC) version 2.0 was used for toxicity grading and reporting.

Hematological toxicity

The combination of calcitriol + dexamethasone + gefitinib did not result in any significant myelosuppression. None of the patients treated on this study developed ≥grade 2 neutropenia or thrombocytopenia. Five patients developed grade 2 anemia and another two patients developed a grade 3 anemia on cycles 2 and beyond.

Non-hematological toxicity

The most common non-hematological adverse events were hyperglycemia, diarrhea, rash, fatigue, and nausea and vomiting (Table 3). Hyperglycemia was predominantly grade 1–2 and was attributed to dexamethasone. This toxicity may have been over-estimated as patients were not required to be fasting at the time of blood draws. Fatigue, diarrhea, rash, and nausea and vomiting were predominantly grade ≤2 and were attributed to gefitinib. Skin rash typically developed in

Table 3 Grade ≥2 study treatment-related non-hematological toxicities

Toxicity	Dose level 1 3 patients			Dose level 2 3 patients			Dose level 3 4 patients			Dose level 4 6 patients			Dose level 5 4 patients		
	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4
Cycle 1															
Hyperglycemia	2	0	0	3	0	0	1	0	0	4	0	0	0	1	0
Fatigue	1	0	0	0	0	0	1	0	0	0	0	0	3	0	0
Diarrhea	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
Rash	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Nausea/Vomiting	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
Hypophosphatemia	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Vasculitis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Any cycle															
Hyperglycemia	1	2	0	3	0	0	1	0	0	5	0	0	0	1	0
Fatigue	2	0	0	1	0	0	2	1	0	2	0	0	3	0	0
Diarrhea	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0
Rash	1	0	0	1	0	0	0	0	0	2	0	0	1	0	0
Nausea/Vomiting	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0
Hypophosphatemia	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Vasculitis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

G2, G3, and G4 refer to grade of toxicity and dose levels refer to calcitriol doses

the first 3 weeks of treatment of gefitinib (cycle 1) and did not seem to worsen with continuation of treatment. Diarrhea was manageable in all patients with loperamide use and did not require any dose modification.

Hypercalcemia

Hypercalcemia occurred at all dose levels but increased in frequency and severity at higher DLs and with repeated dosing (Table 4). At DLs 1–3, only 2 patients out of 10 experienced grade 2 hypercalcemia. At DL-4, three out of six patients developed grade 2 hypercalcemia and one patient developed grade 3 hypercalcemia (on cycle # 2). At the highest DL-5 of 163 μg of calcitriol/week, two out of four patients developed an asymptomatic grade 3 dose-limiting hypercalcemia. The mean increase in cycle one calcium levels between day 1 and 4 on the first calcitriol week ([Calcium D4 – Calcium D1] was 0.16 mg/dL (range: −0.2–0.6) on DL-1, 0.36 mg/dL (range: −0.3–1) on DL-2, 0.53 mg/dL (range: 0–1.1) on DL-3, 0.5 mg/dL (range: −0.6–1.7) on DL-4, and 1.1 mg/dL (range: 0.1–2.4) on DL-5. Hypercalcemia always resolved completely within 7 days from calcitriol dosing.

Antitumor activity

No significant antitumor activity was seen with this regimen. All patients were taken off study for clinical progression, radiographic progression, or clinical toxicity (two patients had a grade 3 dose-limiting hypercalcemia). Seventeen patients progressed after 2 months and three patients progressed after 4 months of therapy.

Table 4 Grade ≥1 hypercalcemia (cycle 1 and any cycle)

Toxicity	Dose level 1 3 patients				Dose level 2 3 patients				Dose level 3 3 patients				Dose level 4 4 patients				Dose level 5 6 patients			
	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
Hypercalcemia (cycle 1)	2	0	0	0	2	0	0	0	3	0	0	0	3	2	0	0	1	0	2[a]	0
Hypercalcemia (cycle 2)	1	1	0	0	1	1	0	0	3	0	0	0	2	3	1[b]	0	1	0	2	0

[a] Two dose-limiting toxicities (occurred during the first cycle, refer to DLT section)

[b] No dose limiting toxicity (occurred during the second cycle, refer to DLT section)

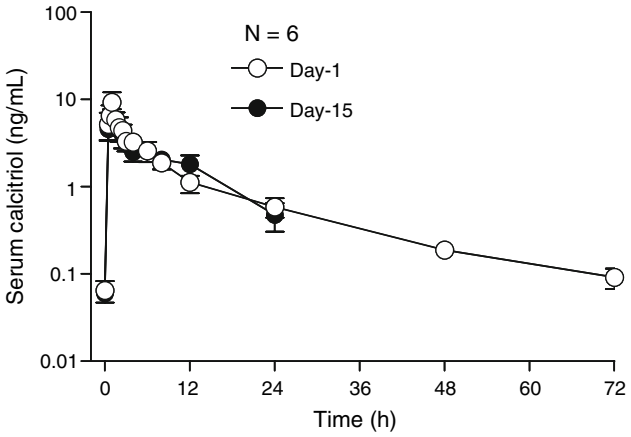

Fig. 2 Day 1 and 15 serum calcitriol concentration over time plots after i.v. administration of 125 μg calcitriol, the maximum tolerated calcitriol dose when administered with dexamethasone

Serum calcitriol pharmacokinetics

Serum calcitriol pharmacokinetics was evaluated in all 20 patients treated with calcitriol + dexamethasone alone on day 1. Fifteen patients had paired serum calcitriol PK evaluated on day 1 (calcitriol + dexamethasone) and day 15 (calcitriol + dexamethasone + gefitinib) of cycle 1. Plots of the day 1 and 15 serum calcitriol concentration over time in the six patients treated at MTD of 125 μg/week calcitriol are shown in Fig. 2. The day 15 plot shows that gefitinib had no effect on serum calcitriol PK. Calcitriol C_{max} was observed during the infusion and started decreasing immediately after the infusion and was within the pretreatment range at 72 h. A summary of the day 1 serum calcitriol PK parameters is shown in Table 5. The relationship between dose and either C_{max} or $AUC_{0–72\ h}$ was linear over the 57–163 μg calcitriol dose range (Fig. 3).

Discussion

Calcitriol has significant antitumor activity in a variety of preclinical tumor models. Preclinical data indicate that antitumor activity is calcitriol concentration dependent—antitumor activity increases as concentrations are escalated from 1 to 100 nmol/l [19, 20]. Multiple trials have shown that high doses of calcitriol can be safely administered on intermittent oral dosing (once weekly or daily for 3 consecutive days repeated every week) when compared to daily calcitriol dosing schedule [21, 22]. However, the intermittent administration of high doses of the commercially available oral calcitriol formulations was associated with limited bioavailability and loss of linear relationship between dose and exposure (AUC, C_{max}) [21, 22]. A novel calcitriol formulation, DN101, has been shown to overcome the bioavailability issues seen with oral commercially available calcitriol [23, 24]. Another approach to overcome reduced bioavailability is through the intravenous administration of calcitriol.

Our group was the first to conduct a phase I clinical trial of i.v. calcitriol. We previously evaluated escalating doses of i.v. weekly calcitriol in combination with a fixed dose of oral gefitinib of 250 mg/day based on previously described synergy data [11]. Dose-limiting hypercalcemia was noted at 96 μg/week of calcitriol and the MTD was confirmed at 74 μg of calcitriol/week. In the present study, we evaluated whether we could attenuate calcitriol-induced hypercalcemia by the addition of intermittent dexamethasone. We show that the co-administration of calcitriol and dexamethasone increased the MTD of i.v. calcitriol from 74 to 125 μg/week in patients with advanced solid tumors. This confirms our hypothesis that dexamethasone protects against calcitriol-induced hypercalcemia. Hypercalcemia was the dose-limiting toxicity in the present study; DLT occurred at 163 mcg/week. We have attributed the higher incidence of hyperglycemia observed in this study to the co-administration of dexamethasone. Since only random blood glucose levels were measured in the current study (fasting blood glucose levels were not determined) the true incidence of hyperglycemia in patients treated with a combination of calcitriol and dexamethasone remains to be determined. The addition of dexamethasone did not appear to impact other known gefitinib-related toxicities.

Table 5 Day 1 serum calcitriol PK parameters of the calcitriol + Dexamethasone combination treatment

Dose of calcitriol (μg)	*N*	Pharmacokinetic parameters			
		$t_{1/2}$ (h)	C_{max} (ng/ml)	$AUC_{0–72\ h}$ (ng h/ml)	CL/F (ml/min)
57	3	16.3 ± 2.0	4.16 ± 1.78	26.90 ± 5.00	38.57 ± 8.75
74	4	18.6 ± 3.9	4.74 ± 1.13	30.94 ± 6.61	56.42 ± 14.28
96	3	8.7 ± 2.3	10.12 ± 2.17	54.41 ± 15.50	20.21 ± 3.27
125	6	14.6 ± 0.6	11.17 ± 2.62	53.30 ± 10.49	51.01 ± 12.92
163	4	11.1 ± 1.7	12.56 ± 1.31	72.22 ± 6.92	38.73 ± 3.90

P-values for $t_{1/2}$ and apparent clearance (CL/F) >0.05 (One-way ANOVA)

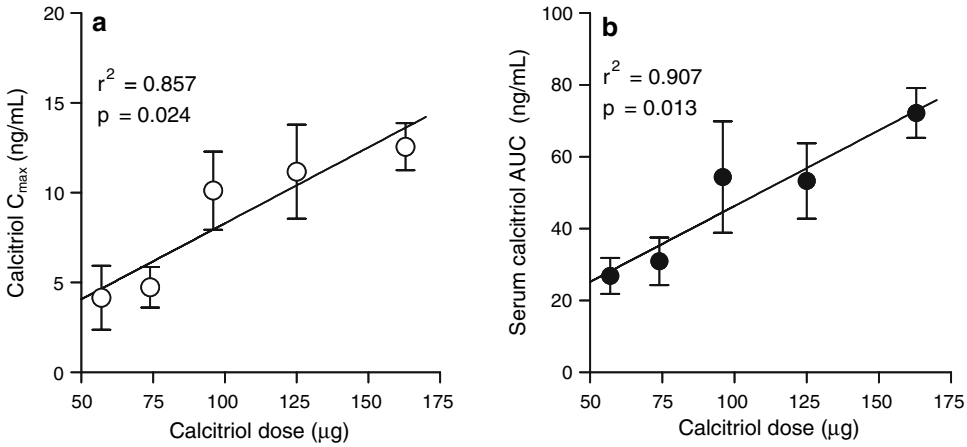

Fig. 3 Plots showing relationship between calcitriol dosage and serum calcitriol C_{max} (**a**) and $AUC_{0–72\,h}$ (**b**)

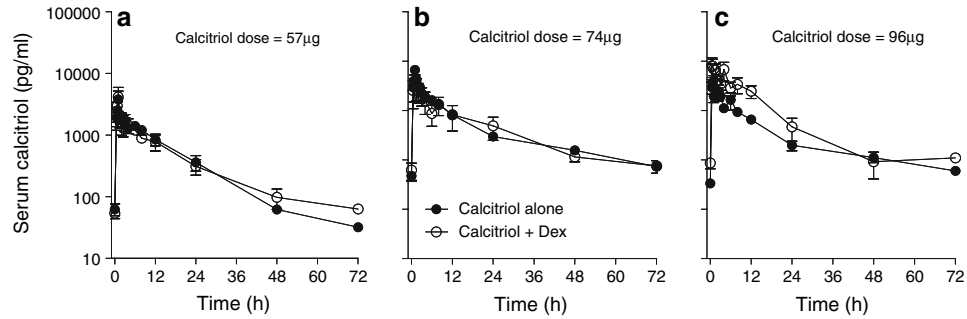

Fig. 4 Plots of serum calcitriol concentration over time in cancer patients treated with 57 (**a**), 74 (**b**), and 96 μg (**c**) of intravenously administered calcitriol with and without dexamethasone

We have previously reported that doses of calcitriol that result in $C_{max} > 10.0$ ng/ml and AUC >40.0 h ng/ml are effective at suppressing tumor growth in a murine squamous cell carcinoma model, [25]. The C_{max} of calcitriol of 11.17 ng/ml and $AUC_{0–72\,h}$ of 54.89 ng h/ml observed at the MTD (125 μg/week i.v. for calcitriol + dexamethasone treatment) indicate that target antitumor serum calcitriol PK parameters were achieved. Therefore, based on these pharmacokinetics results, we suggest that superior calcitriol antitumor activity could potentially be achieved using 125 μg/week i.v. calcitriol + dexamethasone treatment. These target serum calcitriol PK parameters were not consistently and uniformly achieved when the MTD of 74 μg/week i.v. calcitriol alone is utilized (C_{max} = 6.68 ng/ml; $AUC_{0–24\,h}$ = 35.65 ng h/ml) [11]. To evaluate if the co-administration of dexamethasone modulates serum calcitriol PK, we have compared the plots of the serum calcitriol concentration versus time of patients treated with i.v. calcitriol at 57, 74, and 96 μg/week with dexamethasone (this study) and without dexamethasone from our previous study [11]. Dexamethasone had no effect on serum calcitriol pharmacokinetics (Fig. 4). The tolerability of higher calcitriol doses when administered in combination with dexamethasone is thus mediated via non-pharmacokinetic mechanisms.

In summary, this phase I and pharmacokinetic study has demonstrated that weekly intravenous calcitriol administered in combination with dexamethasone and gefitinib is safe and well tolerated. The combination was not associated with any significant clinical activity, consistent with our prior findings from a phase I clinical trial of calcitriol and gefitinib [11]. Therefore, further evaluation of this combination in efficacy clinical trials is not justified.

The co-administration of dexamethasone with calcitriol increased the MTD of i.v. calcitriol from 74 to 125 μg/week, a dose that consistently achieved serum calcitriol PK parameters associated with antitumor activity. The clinical evaluation of the MTD of calcitriol and dexamethasone in combination with platinum compounds, taxanes, and gemcitabine may be justified based on favorable preclinical synergy with these cytotoxics [7, 26, 27].

Acknowledgments This work was supported by Grants CA67267, CA85142, CA63502, and CA95045 from the NIH and career development award MRSG-04-270-01 to Marwan G. Fakih from the American Cancer Society.

References

1. Tsuchiya H, Morishita H, Tomita K, Ueda Y, Tanaka M (1993) Differentiating and antitumor activities of 1α, 25-dihydroxyvitamin D3 in vitro and 1 α-hydroxyvitamin D3 in vivo on human osteosarcoma. J Orthop Res 11:122–130
2. Testa U, Masciulli R, Tritarelli E, Pustorino R, Mariani G, Martucci R, Barberi T, Camagna A, Valtieri M, Peschle C (1993) Transforming growth factor-ß potentiates vitamin D3-induced terminal monocytic differentiation of human leukemic cell lines. J Immunol 150:2418–2430

3. Eisman JA, Barkla DH, Tutton PJ (1987) Suppression of in vivo growth of human cancer solid tumor xenografts by 1, 25-dihydroxyvitamin D3. Cancer Res 47:21–25
4. Chung I, Wong MK, Flynn G, Yu WD, Johnson CS, Trump DL (2006) Differential antiproliferative effects of calcitriol on tumor-derived and matrigel-derived endothelial cells. Cancer Res 66:8565–8573
5. Hershberger PA, Modzelewski RA, Shurin ZR, Rueger RM, Trump DL, Johnson CS (1999) 1, 25-dihydroxycholecalciferal (1, 25–D3) inhibits the growth of squamous cell carcinoma and down-modulates p21 (waf1/Cip1) in vitro and in vivo. Cancer Res 59:2644–2649
6. Bernardi RJ, Trump DL, Yu WD, McGuire TF, Hershberger PA, Johnson CS (2001) Combination of 1α, 25-dihydroxyvitamin D3 with dexamethasone enhances cell cycle arrest and apoptosis: role of nuclear receptor cross-talk and Erk/Akt signaling. Clin Cancer Res 7:4164–4173
7. Hershberger PA, McGuire TF, Yu WD, Zuhowski EG, Schellens JH, Egorin MJ et al (2002) Cisplatin potentiates 1, 25-dihydroxyvitamin D3-induced apoptosis in association with increased mitogen-activated protein kinase kinase kinase 1 (MEKK-1) expression. Mol Cancer Ther 10:821–829
8. Wang X, Studzinski GP (2001) Activation of extracellular signal-regulated kinases (ERKs) defines the first phase of 1, 25-dihydroxyvitamin D3-induced differentiation of HL60 cells. J Cell Biochem 80:471–482
9. Tong WM, Kállay E, Hofer H, Hulla W, Manhardt T, Peterlik M, Cross HS (1998) Growth regulation of human colon cancer cells by epidermal growth factor and 1, 25-dihydroxyvitamin D3 is mediated by mutual modulation of receptor expression. Eur J Cancer 34:2119–2125
10. Bernardi R, Johnson C, Trump D (2002) ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor (EGDR-TKI), enhances the antiproliferative effects of calcitriol (1, 25-dihydroxyvitamin D3) in squamous cell carcinoma: effects on ERK and Akt [abstract 3883]. Proc Am Assoc Cancer Res 43:783
11. Fakih MG, Trump DL, Muindi JR, Black JD, Bernardi RJ, Creaven PJ, Schwartz J, Brattain MG, Hutson A, French R, Johnson CS (2007) A phase I pharmacokinetic and pharmacodynamic study of intravenous calcitriol in combination with oral gefitinib in patients with advanced solid tumors. Clin Cancer Res 13:1216–1223
12. Davidson TG (2001) Conventional treatment of hypercalcemia of malignancy. Am J Health Syst Pharm 58(Suppl 3):S8–S15
13. Jibani M, Hodges NH (1985) Prolonged hypercalcemia after exposure to vitamin D3. Br Med J 290:748–749
14. Klein RG, Arnaud SB, Gallagher JC, Deluca HF, Riggs BL (1977) Intestinal calcium absorption in exogenous hypercortisonism. Role of 25-hydroxyvitamin D and corticosteroid dose. J Clin Invest 60:253–259
15. Suzuki Y, Ichikawa Y, Saito E, Homma M (1983) Importance of increased urinary calcium excretion in the development of secondary hyperparathyroidism of patients under glucocorticoid therapy. Metabolism 32:151–156
16. Yu WD, McElwain MC, Modzelewski RA, Russell DM, Smith DC, Trump DL, Johnson CS (1998) Enhancement of 1, 25-dihydroxyvitamin D3-mediated antitumor activity with dexamethasone. J Natl Cancer Inst 90:134–141
17. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92:205–216
18. Smith DC, Johnson CS, Freeman CC, Muindi J, Wilson JW, Trump DL (1999) A phase I trial of calcitriol (1, 25-dihydroxycholecalciferol) in patients with advanced malignancy. Clin Cancer Res 5:1339–1345
19. Kane KF, Langman MJ, Williams GR (1996) Antiproliferative responses to two human colon cancer cell lines to vitamin D3 are differently modified by 9-*cis*-retinoic acid. Cancer Res 56:623–632
20. Akhter J, Goerdel M, Morris DL (1996) Vitamin D3 analogue (EB 1089) inhibits in vitro cellular proliferation of human colon cancer cells. Br J Surg 83:229–230
21. Muindi JR, Peng Y, Potter DM, Hershberger PA, Tauch JS, Capozzoli MJ et al (2002) Pharmacokinetics of high dose calcitriol: results obtained during a phase one trial of calcitriol and paclitaxel. Cancer Pharmacol Ther 72:648–659
22. Beer TM, Munar M, Henner WD (2001) A phase I trial of pulse calcitriol in patients with refractory malignancies: pulse dosing permits substantial dose escalation. Cancer 91:2431–2439
23. Beer TM, Javle M, Lam GN, Henner WD, Wong A, Trump DL (2005) Pharmacokinetics and tolerability of a single dose of DN-101, a new formulation of calcitriol, in patients with cancer. Clin Cancer Res 11:7794–7799
24. Beer TM, Javle MM, Ryan CW, Garzotto M, Lam GN, Wong A, Henner WD, Johnson CS, Trump DL (2007) Phase I study of weekly DN-101, a new formulation of calcitriol, in patients with cancer. Cancer Chemother Pharmacol 59:581–587
25. Muindi JR, Modzelewski RA, Peng Y, Trump DL, Johnson CS (2004) Pharmacokinetics of 1α, 25-dihydroxyvitamin D3 in normal mice after systemic exposure to effective and safe antitumor doses. Oncology 66:62–66
26. Ma Y, Yu WD, Hershberger PA, Flynn G, Kong RX, Trump DL, Johnson CS (2008) 1alpha, 25-Dihydroxyvitamin D3 potentiates cisplatin antitumor activity by p73 induction in a squamous cell carcinoma model. Mol Cancer Ther 7:3047–3055
27. Hershberger PA, Yu WD, Modzelewski RA, Rueger RM, Johnson CS, Trump DL (2001) Calcitriol (1, 25-dihydroxycholecalciferol) enhances paclitaxel antitumor activity in vitro and in vivo and accelerates paclitaxel-induced apoptosis. Clin Cancer Res 7:1043–1051