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ORIGINAL ARTICLE

Monocyte fructose 1,6-bisphosphatase and cytidine deaminase enzyme activities: potential pharmacodynamic measures of calcitriol effects in cancer patients

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Abstract *Purpose*: To determine, in peripheral blood monocytes (PBM), whether the enzymatic activities of fructose 1,6-bisphosphatase (FBPase), cytidine deaminase (CDDase) and 24-hydroxylase (CYP24), enzymes regulated by calcitriol are useful pharmacodynamic (PD) measures of calcitriol effects in cancer patients. *Methods*: Cancer patients enrolled in a phase I clinical trial of calcitriol and carboplatin were studied. Baseline and calcitriol-induced changes in FBPase, CDDase and CYP24 activities were measured in PBM collected before, 6, 24, and 48 h after administration of calcitriol, prior to carboplatin, in doses ranging from 4 to 11 μg daily for 3 consecutive days (QD×3). Normal FBPase, CYP24 and CDDase activities were measured in PBM from untreated healthy volunteers. *Results*: Baseline activities in PBM from cancer patients and healthy volunteers were (median and range): 1.0 (0.0–43.5) and 4.4 (3.1–8.2) nmol/min/mg protein for FBPase (P = 0.002); 2.5 (0.9–9.3) and 0.8 (0.4–2.0) fmol/h/10^6 cells for CYP24 (P = 0.016), and 5.6 (2.5–22.3) and 6.6 (1.1–47.4) nmol/min/mg protein for CDDase ($P > 0.05$), respectively. All calcitriol doses achieved peak serum calcitriol levels > ×3 the physiological levels, increased cancer patient PBM FBPase activity to normal levels and decreased CDDase activity to undetectable levels within 48 h, with no significant change in CYP24 activity. These enzyme activity changes were not associated with hypercalcemia. *Conclusions*: Calcitriol treatment-induced increase in FBPase and decrease in CDDase activities in cancer patient PBM are potential early and sensitive non-hypercalcemia PD measures of calcitriol effects.

Keywords Calcitriol · Monocyte · Fructose 1,6-bisphosphatase · Cytidine deaminase · CYP24 · Cancer patients

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Introduction

Calcitriol (1,25-dihydroxycholecalciferol, 1,25-D_3) has potent cell growth inhibitory, pro-differentiation and apoptotic effects that might be exploited to prevent and treat human cancers [1–4]. Clinical studies have demonstrated a diminished rate of rise in the tumor marker, prostate-specific antigen (PSA), in early recurrent and androgen independent prostate cancer patients treated with calcitriol-based therapies [5–9], and a greater frequency and a more rapid PSA response in advanced prostate cancer patients who receive calcitriol + docetaxel compared to docetaxel alone [10]. These observations suggest therapeutic efficacy of calcitriol-based therapy in human prostate cancer.

Serum calcium measurements are routinely used to monitor patients on calcitriol treatment because hypercalcemia is the dose-limiting toxicity. Although it remains relevant for toxicity issues, the usefulness of hypercalcemia as a pharmacodynamic (PD) measure of calcitriol effects in cancer patient has two disadvantages: first, like many PD measures, the onset of calcitriol-induced hypercalcemia is unpredictable and delayed [11]. Second, hypercalcemia has not been dose limiting in several studies of intermittent high dose calcitriol administration either as a single agent or in combination with dexamethasone [12, 13]. An additional concern is that if non-hypercalcemic calcitriol analogs with equivalent or improved antitumor activity were developed, it would be very useful to identify non-calcium-based measures to monitor biologic effects [14–16].

We hypothesized that the identification and characterization of calcitriol-regulated enzyme activities in cells derived from cancer patients before and during the calcitriol treatment have the potential of identifying non-hypercalcemia PD measures useful for the future development of vitamin D_3-based cancer therapies. Peripheral blood monocytes (PBM) are ideal cells for these studies because they are readily accessible for repetitive sampling, easily separated from other peripheral blood mononuclear cells and are responsive to calcitriol-induced maturation and differentiation into tissue macrophages [17–20]. The PBM express a number of calcitriol-responsive enzymes, including fructose 1,6-bisphosphatase (FBPase), cytidine deaminase (CDDase) and 24-hydroxylase (CYP24) [18–27]. Changes in the activities of these enzymes are potential PD measures of calcitriol effects. The FBPase, a key gluconeogenic enzyme, catalyzes the de-phosphorylation of fructose 1,6-bisphosphate to fructose 1-phosphate and inorganic phosphate; the CDDase catalyzes the deamination of free and RNA incorporated cytidine to uridine, and the CYP24 is the major calcitriol-catabolizing enzyme [28–30]. Here, we demonstrate low FBPase and high CYP24 baseline activities in the PBM of advanced cancer patients compared to healthy volunteer PBM, and suggest that calcitriol treatment-induced increase in FBPase and decrease in CDDase activities are potential early and sensitive non-hypercalcemia PD measures of calcitriol effects in cancer patients.

Materials and methods

Reagents and chemicals

Radioactive calcitriol, dihydroxyvitamin D_3 1α,25-[26,27-^{3}H] ([^{3}H]-1,25-D_3), was purchased from Perkin Elmer Science Corporation (Boston, MA, USA). Authentic calcitriol oxidation metabolites were kindly provided by Dr. S. Reddy and Dr. M. Uskokovic. Polyclonal rabbit anti-rat FBPase, CDDase and CYP24 were kindly provided by Dr. H. Mizunuma, Dr. R. Momrapler and Dr. R. Kumar, respectively.

Study subjects

Baseline and calcitriol-induced changes in PBM FBPase, CDDase and CYP24 activity were studied in advanced solid tumor patients enrolled in a phase I study of calcitriol and carboplatin at the University of Pittsburgh Cancer Institute. Advanced solid tumor patients were eligible for this study if no standard therapy was available and if they had adequate bone marrow, kidney and liver function, as evidenced by WBC ≥ 4,000/μl, platelets ≥ 100,000/μl, serum creatinine ≤ 1.5 mg/dl, bilirubin ≤ 2 mg/dl and SGOT ≤ 75 mIU, respectively. The patients were required to have corrected serum calcium ≤ 10.5 mg/dl and no past medical history of renal stones. The protocol was approved by the Biomedical Institutional Review Board (IRB) of the University of Pittsburgh and all patients signed written informed consent before participating. Healthy volunteers were recruited from IRB approved Center of Clinical Pharmacology Registry.

Calcitriol treatment and administration schedules

This phase I study of calcitriol and carboplatin in advanced solid tumor patients was a two arm open label treatment trial: In treatment arm A (CDDD arm), carboplatin (C) was administered on day 1 and calcitriol (D) was given on day 2, 3 and 4. In treatment arm B (DDDC arm), D was given on day 1, 2 and 3 and C was administered on day 3. The calcitriol treatment induced changes in PBM enzyme activities were studied in patients enrolled on the DDDC arm. Calcitriol (Calcijex®1 μg/ml, kindly supplied by Abbott Pharmaceutical) was administered subcutaneously daily for 3 consecutive days (QD×3) every 28 days, and the dose escalation schema was 4, 6, 8 and 11 μg. At least three patients were entered at each dose level, and no intra-patient calcitriol dose escalation was allowed. Treatment was continued until disease progression or dose-limiting toxicity occurred.

Patient monitoring

Serum calcium levels were determined on day 4 of the calcitriol treatment week and weekly thereafter.

Blood samples collection and isolation of peripheral blood monocytes (PBM)

Approximately 24 ml of blood was collected in CPT-vacutainer tubes containing pre-prepared sodium citrate gel and density gradient media (Becton Dickinson & Co, Franklin Lakes, NJ, USA) immediately before (0 h) and 6 h after the day 1 calcitriol administration, and immediately before day 2 (24 h) and day 3 (48 h) calcitriol dosing in the first month of DDDC treatment. The peripheral blood mononuclear cells were separated by density gradient centrifugation according to the instructions supplied with CPT tubes. The PBM were isolated by adhesion to plastic culture dishes by 2 h incubation at 37°C in RPMI-1640 media supplemented with antibiotics, 2 mM L-glutamine and 1% fatty acid free BSA [17]. Adherent cells (PBM) showing greater than 90% viability by trypan blue exclusion test and more than 85% monocytes as determined by non-specific esterase staining were assayed for enzyme activity and protein lysates were analyzed by Western blotting. Blood samples (8 ml) for PBM FBPase, CDDase and CYP24 activities studies were obtained from healthy volunteers who received no calcitriol treatment.

PBM enzyme activity assays

The FBPase activity was measured using NADP coupled spectrophotometric assay as previously described [31]. The assay mixture (1 ml) consisted of 20 μg protein of PBM lysate, 0.5 mM NADP, 5 U/ml G6PDH and 10 U/ml glucose-6-phosphate isomerase and +/– 20 μM fructose 1, 6-bisphosphate (FBP), the substrate. The difference in absorbance change/min at 340 nm in the presence and absence of FBP was used to calculate FBPase activity. PBM FBPase activity was expressed as nmol/min/mg protein (NADPH extinction coefficient = 6,220 cm^2/mmol).

The CDDase activity was measured spectrophotometrically by monitoring the rate of cytidine deamination to uridine at 286 nm in 1 ml assay mixture containing 20 μg protein of PBM lysate [32]. The difference in absorbance change/min at 286 nm in the absence and presence of 100 μM cytidine was used to calculate the CDDase activity. The PBM CDDase activity was expressed as nmol/min/mg protein (uridine extinction coefficient = 3,000 cm^2/mmol).

The CYP24 activity was measured by incubating 1–2×10^6 freshly isolated PBM cells in 0.5 ml of RPMI 1640 (supplemented with 0.1% fatty acid free BSA, 2 mM L-glutamine and antibiotics) with 0.5 μCi (110,000 dpms) [^{3}H]- 1,25-D_3) as a substrate. Blank assays containing no cells were included in all experiments. After 2 h incubation at 37°C, calcitriol and its CYP24 metabolites were extracted by liquid/liquid partition using tetrahydrofuran and ethyl acetate [33]. Excess non-radioactive calcitriol was added to improve extraction recovery.

Extracts were dried, dissolved into 65 μl of HPLC mobile phase and CYP24 metabolites of [^{3}H]-1,25-D_3 in 50 μl of extract was separated by normal phase HPLC on a 250×4.6 cm^2 Zorbax silica column using hexane/isopropanol/methanol (84:10:6 v/v) as the mobile phase. The HPLC fractions were collected manually every 30 s, mixed with 10 ml of scintillation liquid and counted. All radioactive counts with co-eluting with known CYP24 calcitriol metabolites were pooled. The CYP24 activity, expressed as fmol/2 h/10^6 cells, was calculated using the equation:

$$A = \frac{S}{T} \times \frac{(C - B)}{N} \times \frac{65}{50}$$

Where A = CYP24 metabolites (fmol), S = calcitriol in the assay (fmol), C and B represent CYP24 metabolite radioactivity counts in the PBM containing assay extracts and no PBM (blank) assay, respectively. T = calcitriol (in dpms) and N = PBM cells (in millions) in the incubation assay mixture. The radioactivity extraction recovery in our assays ranged from 65 to 80%. Inter- and intra-assay coefficient of variation was 10–20%.

Western blot analysis

The PBM for Western blot analyses were harvested, lysates prepared, and stored at –80°C until analyzed as previously described [34]. Briefly, 30 μg protein of PBM lysate was loaded onto each lane and FBPase, CDDase and CYP24 proteins were resolved on 8, 9 and 10% SDS-PAGE, respectively. Protein bands were transferred by electro-elution and probed with the appropriate polyclonal rabbit anti-rat primary antibodies (FBPase, CDDase or CYP24 antibody). The protein bands were detected with ECL Western blotting kit according to manufacturer's instructions (Amersham Corp., Arlington Heights, IL, USA). Pretreatment Western blot densitometry measurement of FBPase, CDDase and CYP24 protein bands were normalized to 1 arbitrary unit for each individual patient. Protein loading was assessed using the β-actin protein bands.

Other analytical methods

Serum calcitriol concentrations were determined using 1,25-dihydroxyvitamin D_3-[I^{125}] RIA kits as previously

described [11]. Protein concentrations were determined by the Bio-Rad protein-binding assay using bovine serum albumin as the standard [35].

Data analysis and statistical methods

The Wilcoxon rank sum test and/or ANOVA analysis were used to compare baseline, time dependent and calcitriol-induced changes in PBM FBPase, CDDase and CYP24 activities as appropriate. Correlations were determined using the Spearman correlation coefficient (r_s). Statistical significance of data was determined by ANOVA or Wilcoxon signed rank test as appropriate. A P value of < 0.05 was considered significant. All statistical calculations were performed using NCSS statistical software (Kaysville, UT, USA).

Results

Study population characteristics

Twenty patients with the following cancers: lung ($n = 6$), colorectal ($n = 4$), brain ($n = 3$) and miscellaneous solid tumors ($n = 7$) and 28 healthy volunteers (10 FBPase, 10 CDDase and 8 CYP24 activities) were studied. The median ages of the cancer patients and healthy volunteers studied were 64.9 years (range, 32–81) and 58.6 years (range, 30–69), respectively ($P > 0.05$). The ratio of men to women for the two study population was 2:1. The median PBM yield from 24 ml of blood was 6.0×10^6 cells (range, 2.1–15.6×10^6 cells). Because of the variability in PBM yields, PBM enzyme activities and western blot analysis could not be measured in all patient samples. The number of patients studied is indicated in appropriate results sections.

Baseline PBM enzyme activities

Compared to healthy volunteers, cancer patient PBM exhibited low FBPase and increased CYP24 baseline activities; CDDase activity was not different (Table 1). Daily measurements of PBM FBPase, CDDase and CYP24 activity in three healthy volunteers for 3 consecutive days showed no significant variability (Fig. 1).

Serum calcitriol level

Peak serum calcitriol levels achieved after the subcutaneous administration of 4, 6, 8 and 11 μg calcitriol doses were at least ×3 higher than pre-baseline levels (Table 2).

Effect of calcitriol treatment on PBM FBPase activity

An increase in the PBM FBPase activity was observed within 6 h of administration of the first dose of calcitriol and was within the healthy volunteers PBM FBPase activity range 48 h after starting treatment (Fig. 2a). The increase in FBPase was observed in all eight patients with baseline PBM FBPase activity of < 2 nmol/min/mg protein; the decrease in the three patients with high baseline FBPase activity was not significant (Fig. 2b). A positive and significant correlation between baseline PBM FBPase activity and pretreatment serum calcitriol levels was observed (Fig. 2c). Western blot analysis, in three cancer patients, showed a 30–50% calcitriol treated mediated increase in PBM FBPase protein expression (Fig. 2d).

Effect of calcitriol treatment on PBM CDDase and CYP24 activities

PBM CDDase activity decreased 6 h after the calcitriol treatment and was barely detectable at 48 h (Fig. 3a). There was no relationship between calcitriol-induced changes in PBM CDDase activity and pretreatment serum calcitriol levels or PBM CDDase protein expression. No consistent or significant changes in PBM CYP24 activity were observed after the calcitriol treatment (Fig. 3b). No apparent calcitriol dose–enzyme activity response relationship was seen. No patient developed hypercalcemia, defined as serial weekly serum calcium levels post-calcitriol treatment of > 11 mg/dl.

Discussion

Our examination of the baseline calcitriol responsive enzyme activities revealed an unexpected and significant decrease in FBPase enzyme activity in PBM of cancer patients compared to healthy volunteers. The mechanisms underlying these observations are uncertain. However, two observations from this study suggest that calcitriol plays a role in the pathogenesis of low baseline FBPase activity in cancer patients: First, calcitriol treatment-induced increase in PBM FBPase activity was observed only in patients with low baseline activity and had no effect in patients with high baseline PBM FBPase activity. Second, there was a positive and a significant association between pre-treatment serum calcitriol levels and baseline PBM FBPase activities.

Since normal serum creatinine and BUN levels were a prerequisite for enrollment in the study, we suggest

Table 1 Baseline activities of calcitriol responsive PBM enzymes

Study population	PBM enzyme activities		
	FBPase (nmol/min/mg protein)	CDDase (nmol/min/mg protein)	CYP24 (fmol/h/10^6 cells)
Cancer patients	1.0 (0.0–43.5) (N = 19)	5.6 (2.5–22.3) (N = 9)	2.5 (0.9–9.3) (N = 8)
Healthy subjects	4.4 (3.1–8.2) (N = 10)	6.6 (1.1–47.4) (N = 10)	0.8 (0.4–2.0) (N = 8)
P value[a]	0.002	> 0.05	0.016

Results are presented as median and (range)

[a]Wilcoxon signed rank test

that impaired kidney function is not the reason for low serum calcitriol levels as observed in our advanced cancer patients. The low serum calcitriol levels in the advanced cancer patients has previously been attributed to impaired skin biosynthesis of calcitriol precursors secondary to limited exposure to UV and/or dietary deficiencies [36, 37]. The high baseline CYP24 activity, as we have observed in our cancer patient PBM, suggests that increased calcitriol oxidation may also play a role in the pathogenesis of low serum calcitriol levels. The reason for high baseline CYP24 activity in cancer patients PBM is unknown but cytokines, such as IL4, has been reported to modulate CYP24 activity [38, 39]. The increased baseline PBM CYP24 activity observed in this study could be attributed to tumor derived cytokines or other autocrine, endocrine and/or paracrine factors. The calcitriol treatment would be expected to have no effect if cancer patient PBM CYP24 activity is already maximally stimulated or induced.

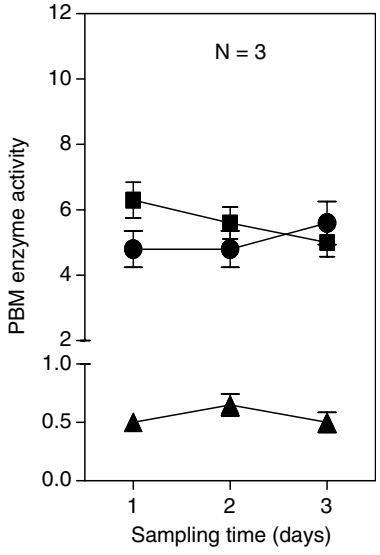

Fig. 1 PBM FBPase (*squares*), CDDase (*circles*) and CYP24 (*triangles*) activities measured daily for 3 consecutive days in three untreated healthy volunteers. Units of FBPase and CDDase activities are nmol/min/mg protein; fmol/2 h/10^6 cells for CYP24 activity

Table 2 Peak serum calcitriol levels achieved after subcutaneous calcitriol

Calcitriol dose (μg/day)	N	Peak serum calcitriol (pg/ml)
4	3	130 (102–238)
6	5	398 (288–485)
8	5	389 (312–984)
11	3	856 (250–1,434)

Results are presented as median and (range). Median (and range) baseline serum calcitriol levels for healthy volunteers and cancer patients were 48.5 (13–87) and 34.5 (13–104) pg/ml, respectively

Our results indicate that the calcitriol treatment induced significant increase in FBPase and decrease in CDDase activities in cancer patient-derived PBM. These enzyme activity changes were contrary to the in vitro results using human myeloid cells where calcitriol treatment increased CDDase activity [19, 25, 33]. One potential explanation for these in vitro and in vivo differences may be differences in the fate of calcitriol-induced maturation of the monocytes into macrophages. In cell culture, macrophages stay in the culture flask, whereas in cancer patients maturing PBM exit from circulation as tissue macrophages and thus did not contribute to enzyme activities measured in this study. The repopulation of the circulating blood by relatively immature PBM that characteristically exhibit low catabolic enzyme activities may thus account for the PBM enzyme activity profile observed in this study [18, 19, 21, 40]. An alternative explanation for the lack of significant correlation between PBM enzyme activity profile after the calcitriol treatment and protein expression could also be due to calcitriol-mediated post-translational modification of mature FBPase, CDDase and CYP24 proteins. Post-translational modification of the phosphorylation status of the ferredoxin component of CY24 complex and the FBPase protein are both known to regulate the activities of these enzymes [41–44].

Although the molecular mechanisms underlying the changes in PBM calcitriol-responsive enzyme activities we observed remains unknown, our results suggest that calcitriol treatment-induced increase in PBM FBPase

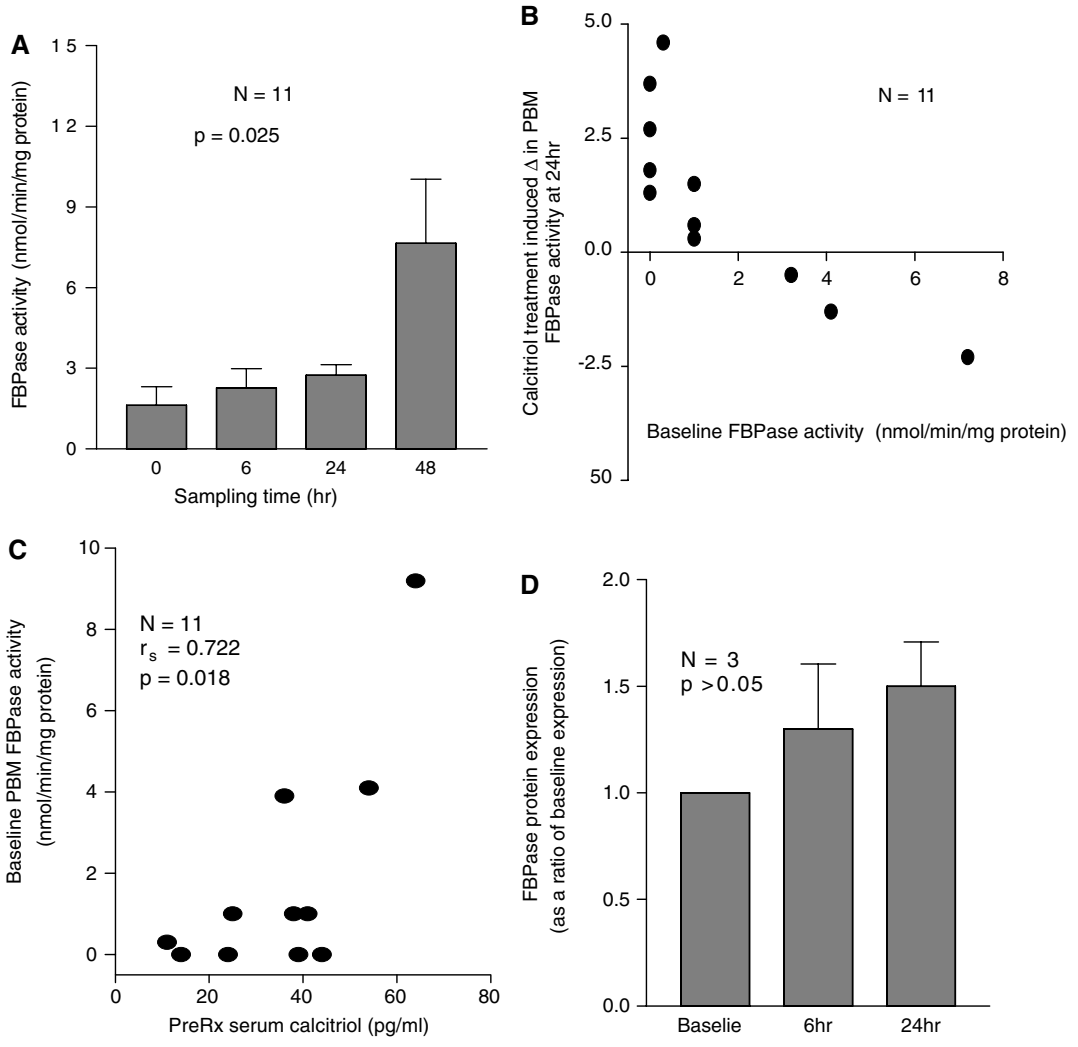

Fig. 2 PBM FBPase activities in cancer patients treated with 4, 6, 8 and 11 μg calcitriol subcutaneously daily for 3 consecutive days. Time course of calcitriol induced changes in FBPase activity (**a**). Relationship between calcitriol induced change in FBPase activity (24 h activity–baseline activity) and baseline FBPase activity: the increase in FBPase activity in eight patients with baseline PBM FBPase activity of < 2 nmol/min/mg protein was significant ($P = 0.007$); the decrease in FBPase activity in the three patients with baseline activity > 2 nmol/min/mg protein was not significant (**b**). The test for the significance of the association baseline FBPase activity values and pretreatment serum calcitriol levels using transformed log of (FBPase + 1) in order to meet normality assumption, was significant (**c**). The increase in PBM FBPase protein expression 6 and 24 h post-calcitriol treatment was not significant (**d**)

and decrease in CDDase as potential early and sensitive PD measures of calcitriol effects in advanced cancer patients. Unlike serum calcium measurements, which require administration of either multiple or high doses of calcitriol and has a delayed time of onset, the calcitriol-induced changes in PBM FBPase and CDDase activities are elicited within 6 h of administration of a single non-hypercalcemic calcitriol dose. The utility of PBM CYP24 activity as PD measure is diminished by the variability and inconsistencies in the calcitriol-induced changes in activity.

In summary, this study has documented normal CDDase, low FBPase and high CYP24 baseline enzyme activities in PBM of advanced solid tumor patients and a rapid increase and normalization of FBPase, and a decrease in CDDase activity after calcitriol treatment. These results suggest that the calcitriol-induced increase in PBM FBPase and the decrease in CDDase activities are potential early and sensitive non-hypercalcemia pharmacodynamic measures of calcitriol effects in advanced solid tumor patients.

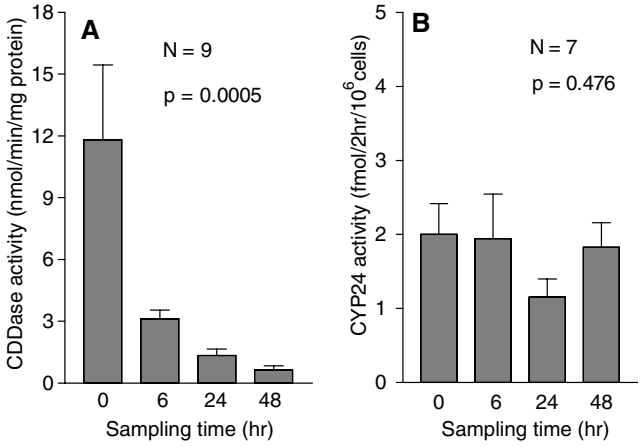

Fig. 3 Time course of changes in CDDase (**a**) and CYP24 (**b**) activities in PBM of cancer patients treated with 4, 6, 8 and 11 μg doses of subcutaneously administered calcitriol daily for 3 consecutive days. *P* values determined by one-way ANOVA. Western blot analysis of PBM CDDase and CYP24 protein expression before and 6 and 24 h after calcitriol dose were variable and not significant (data not shown)

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References

1. Colston K, Colston MJ, Feldman D (1981) 1,25-dihydroxyvitamin D_3 and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. Endocrinology 108:1083–1086
2. James SY, Mackay AG, Colston KW (1996) Effects of 1,25-dihydroxyvitamin D_3 and its analogues on the induction of apoptosis in breast cancer cells. J Steroid Biochem Mol Biol 58:395–401
3. Cross HS, Pavelka M, Slavik J, Peterlik M (1992) Growth control of human colon cancer cells by vitamin D and calcium *in vitro*. J Natl Cancer Inst 84:1355–1357
4. Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D (1994) Antiproliferative effects of 1,25-dihydroxyvitamin D_3 on primary cultures of human prostatic cells. Cancer Res 54:805–810
5. Trump DL, Potter DM, Muindi J, Brufsky A, Johnson CS (2006) Phase II trial of high dose, intermittent calcitriol (1,25 dihydroxyvitamin D3) + dexamethasone in androgen independent prostate cancer. Cancer (in press)
6. Beer TM, Lemmon D, Lowe BA, Henner WD (2003) High-dose weekly oral calcitriol in patients with a rising PSA after prostatectomy or radiation for prostate carcinoma. Cancer 97:1217–1224
7. Beer TM, Eilers KM, Garzotto M, Egorin MJ, Lowe BA, Henner WD (2003) Weekly high-dose calcitriol and docetaxel in metastatic androgen-independent prostate cancer. Clin Oncol 21:123–128
8. Woo TC, Choo R, Jamieson M, Chander S, Vieth R (2005) Pilot study: potential role of vitamin D (Cholecalciferol) in patients with PSA relapse after definitive therapy. Nutr Cancer 51:32–36
9. Gross C, Stamey T, Hancock S, Feldman D (1998) Treatment of early recurrent prostate cancer with 1,25-dihydroxyvitamin D_3 (calcitriol). J Urol 159:2035–2039
10. Beer TM, Ryan CW, Venner PM, et al (2005). Interim results from ASCENT: a double-blinded randomized study of DN-101 (high dose calcitriol) plus docetaxel vs. placebo plus docetaxel in androgen-independent prostate cancer (AIPC). Proc Am Soc Clin Oncol 23(16S):382
11. Smith DC, Johnson CS, Freeman CC, Muindi J, Wilson JW, Trump DL (1999) A phase I trial of Calcitriol (1,25-dihydroxycholecalciferal) in patients with advanced malignancy. Clin Cancer Res 5:1339–1345
12. 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
13. Muindi JR, Peng Y, Potter DM, Hershberger PA, Tauch JS, Capozzoli MJ, Egorin MJ, Johnson CS, Trump DL (2002) Pharmacokinetics of high dose calcitriol: results obtained during a phase one trial of calcitriol and paclitaxel. Clin Pharmacol Ther 72:648–659
14. Jung SJ, Lee YY, Pakkala S, deVos S, Elstner E, Norman AW (1994) 1,25$(OH)_2$-16-ene-vitamin D3 is a potent anti-leukemic agent with low potential to cause hypercalcemia. Leuk Res 56:264–267
15. Binderup L, Latini S, Binderup E, Bretting C, Calverley M, Hansen K (1991) 20-epi-vitamin D3 analogues: a novel class of potent regulators of cell growth and immune response. Biochem Pharmacol 42:1569–1575
16. Miyahara T, Harada M, Kozakai A, Matsumoto M, Hashimoto K, Inoue H, Yoda K, Nakatsu T, Kajita S, Yamazaki R, Higuchi S, Kozuka H, Nemoto N (1999) Comparison of 26,27-hexafluoro-1alpha, 25-dihydroxyvitamin D3 and 1 alpha, 25-dihydroxyvitamin D3 on the resorption of bone explants ex vivo. J Nutr Sci Vitaminol 45:239–249
17. Kumagai K, Ito K, Hinuma S, Tada T (1979) Pretreatment of petri dishes with fetal calf serum: a simple method for macrophage isolation. J Immunol Methods 29:7–25
18. Solomon DH, Raynal MC, Tejwani GA, Cayre YE (1988) Activation of the fructose 1,6-bisphosphate gene by 1,25-dihydroxyvitamin D_3 during monocytic differentiation. Proc Natl Acad Sci USA 85:6904–6908
19. Mejer J, Mortensen BT (1988) Changes in the activities of cytidine deaminase during differentiation of HL60 cells induced by 1,25-dihydroxyvitamin D_3. Leuk Res 12:405–409
20. Kuhn K, Bertling WM, Emmrich F (1993) Cloning of a functional cDNA for human cytidine deaminase (CDD) and its use as a marker of monocyte-/macrophage differentiation. Biochem Biophys Res Commun 1990:1–7
21. Mizunuma H, Tashima Y (1994) Induction and turnover of fructose 1,6-bisphosphatase in HL60 leukemia cells by calcitriol. Eur J Biochem 225:433–453

22. Kikawa Y, Takano T, Nakai A, Shigematsu Y, Saito M, Sudo M, Mizunuma H (1993) Monocytes, not lymphocytes, show increased fructose 1,6-diphosphatase activity during culture. Clin Chim Acta 215:81–88
23. Guidez F, Perignon JL, Houllier AM, Balitrand N, Abita JP (1991) Retinoic acid inhibits the expression of cytidine deaminase linked to the differentiation of the human leukemia cell line HL-60. Leukemia 5:699–703
24. Kamimura S, Gallieni M, Zhong M, Beron W, Slatopolsky E, Dusso A (1995) Microtubules mediated cellular 25-hydroxyvitamin D_3 trafficking and the genomic response to 1,25-dihydroxyvitamin D_3 in normal human monocytes. J Biol Chem 270:22160–22166
25. Kamimura S, Gallieni M, Kubodera N, Nishii Y, Brown AJ, Slatopolsky E, Dusso A (1993) Differential catabolism of 22-oxacalcitriol and 1,25-dihydroxyvitamin D3 by normal human peripheral monocytes. Endocrinology 133: 2719–2723
26. Fujisawa K, Umesono K, Kikawa Y, Shigematsu Y, Taketo A, Mayumi M, Inuzuka M (2000). Identification of a response element for vitamin D3 and retinoic acid in the promoter region of human fructose 1,6-bisphosphatase gene. J Biochem 127:373–384
27. Zierold C, Darwish HM, DeLuca HF (1995) Two vitamin D responsive elements in the rat 1,25-dihydroxyvitamin D 24-hydroxylase promoter. J Biol Chem 270:1675–1678
28. Okuda K, Usui E, Ohyama Y (1995) Recent progress in enzymology and molecular biology of enzymes involved in vitamin D metabolism. J Lipid Res 36:1641–1650
29. Omdahl JL, Morris HA, May BK (2002) Hydroxylase enzymes of the vitamin D pathway; expression function and regulation. Annu Rev Nutr 22:139–166
30. Beckman MJ, Tadikonda P, Werner E, Prahl J, Yamada S, DeLuca HF (1996) Human 25-hydroxyvitamin D3-24-hydroxylase, a multicatalytic enzyme. Biochemistry 35:8465–8472
31. Baulida J, Onetti R, Bassols A (1997) Modulation of fructose-2, 6-bisphosphate metabolism by components of the extracellular matrix in cultured cells. Interaction with epidermal growth factor. FEBS Lett 418:63–67
32. Watanabe S, Uchida T (1996) Expression of cytidine deaminase in human solid tumors and its regulation by 1 alpha,25-dihydroxyvitamin D_3. Biochim Biophys Acta 1312:99–104
33. Honda A, Nakashima N, Shida Y, Mori Y, Nagata A, Ishizuka S (1993) Modification of 1-alpha, 25-dihydroxyvitamin D_3 metabolism by introduction of 26,26,26,27,27,27-hexafluoroatoms in human promyelocytic leukemia (HL-60) cells: isolation and identification of de novel bioactive metabolite, 26,26,26,27,27,27-hexafluoro-1alpha, 23(S),25-trihdroxyvitamin D_3. Biochem J 295:509–516
34. 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
35. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
36. Schwartz GG, Hulka BS (1990) Is vitamin D deficiency a risk factor for prostate cancer (Hypothesis). Anticancer Res 10:1307–1311
37. Hanchette CL, Schwartz GG (1992) Geographic patterns of prostate cancer mortality: evidence for protective effect of ultraviolet radiation. Cancer 70:2861–2869
38. Morgan JW, Morgan DM, Lasky SR, Ford D, Kouttab N, Maizel AL (1996) Requirements for induction of vitamin D-mediated gene regulation in normal B lymphocytes. J Immunol 157:2900–2908
39. Hewison M, Zehnder D, Bland R, Stewart PM (2000) 1α-hydroxylase and the action of vitamin D. J Mol Endocrinol 25:141–148
40. Pedrozo HA, Boyan BD, Mazock J, Dean DD, Gomez R, Schwartz Z (1999) TGFβ1 regulates 25-hydroxyvitamin D_3 1alpha- and 24-hydroxylase activity in cultured growth plate chondrocytes in a maturation-dependent manner. Calcif Tissue Int 64:50–56
41. Uyeda K, Furuya E, Richards CS, Yokoyama M (1982) Fructose-2, 6-P chemistry and biological function. Mol Cell Biochem 48:97–120
42. Liu YQ, Uyade K (1996) Mechanism for fatty acid inhibition of glucose utilization in liver: role of xylulose 5-P. J Biol Chem 271:8824–8830
43. Mandla S, Tenenhouse HS (1992) Inhibition of 25-hydroxyvitamin D3-24-hydroxylase by forskolin evidence for a 3′, 5′-cyclic adenosine monophosphate-independent mechanism. Endocrinology 130:2145–2151
44. Mandel ML, Moorthy B, Ghazarian JG (1990) Reciprocal post-translational regulation of 1α and 24-hydroxylase of 25-hydroxyvitamin D_3 by phosphorylation of ferredoxin. Biochem J 266:385–392