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Tissue distribution and activity studies of 1,24-dihydroxyvitamin D_2, a metabolite of vitamin D_2 with low calcemic activity in vivo

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Abstract

The active vitamin D compound 1α,24(*S*)-dihydroxyvitamin D_2 ($1,24(OH)_2D_2$) is under development as a therapy for disorders including cancer and secondary hyperparathyroidism. $1,24(OH)_2D_2$ is a potent inhibitor of cell proliferation in vitro and, relative to calcitriol ($1,25(OH)_2D_3$), has reduced calcemic activity in vivo. To examine the mechanisms underlying this reduced calcemic activity, we studied the tissue distribution in rats of radiolabeled $1,24(OH)_2D_2$ or $1,25(OH)_2D_3$ over 24 h. Serum levels of $1,24(OH)_2D_2$ were lower than those of $1,25(OH)_2D_3$ at all time points; however, tissue levels of radiolabeled compounds followed different patterns. In duodenum and kidney, $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ rose to similar levels at early time points; $1,24(OH)_2D_2$ levels then declined more rapidly. In bone marrow, $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ were present at similar levels at all time points. In liver, $1,24(OH)_2D_2$ levels were two-fold higher than $1,25(OH)_2D_3$ at 1 h post-injection, declining to similar levels by 8 h. In vitamin D-deficient rats, doses of $1,24(OH)_2D_2$ 30-fold higher than $1,25(OH)_2D_3$ were required to produce equal stimulation of intestinal calcium absorption. In the same deficient animals, $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ were nearly equipotent at stimulating bone calcium mobilization. In cultured bone cells, $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ were equipotent at stimulating osteoclast formation and bone resorption. In summary, the reduced calcemic activity of $1,24(OH)_2D_2$ may result from altered pharmacokinetics relative to $1,25(OH)_2D_3$, resulting in relatively rapid decreases in $1,24(OH)_2D_2$ levels and activity in target organs such as intestine. Further studies will be necessary to confirm these findings and to confirm the clinical utility of $1,24(OH)_2D_2$.

Keywords: Vitamin D; 1,24-Dihydroxyvitamin D_2; Calcium; Metabolism; Intestine; Osteoclast

1. Introduction

Vitamin D analogs are used therapeutically in the US for the treatment of secondary hyperparathyroidism of kidney disease [1,2] and for the topical treatment of psoriasis [3]. In other countries, vitamin D analogs are also used in the treatment of additional disorders including osteoporosis [4]. Vitamin D analogs have shown promising activity against other disorders, including cancer, in both cell culture [5–7] and animal studies [8–10], but clinical usage of vitamin D compounds at high doses in cancer patients has been limited by hypercalcemic side effects [11,12]. Older vitamin D analogs used in the treatment of secondary hyperparathyroidism of kidney disease, such as $1,25(OH)_2D_3$, may exacerbate problems with tissue calcification that occur with high frequency in renal patients [1,2]. Newer vitamin D analogs are being developed with the aim of maximizing clinical utility while minimizing unwanted hypercalcemic side effects.

The active vitamin D compound $1,24(OH)_2D_2$ has potent antiproliferative activity [13] and reduced calcemic activity in vivo relative to $1,25(OH)_2D_3$ [14] and is under development as a drug for the treatment of disorders

Abbreviations: $1,24(OH)_2D_2$, 1α,24(*S*)-dihydroxyvitamin D_2; $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3, calcitriol; CYP24, 25-hydroxyvitamin D-24-hydroxylase; DBP, Vitamin D-binding protein; α-MEM, α-Minimal Essential Medium; OCT, 22-oxacalcitriol; TRAP, tartrate-resistant acid phosphatase

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including secondary hyperparathyroidism and cancer. $1,24(OH)_2D_2$ is a naturally occurring compound [15,16] that may contribute to the reduced calcemic activity of vitamin D_2 and the synthetic vitamin D_2 analog doxercalciferol (1α-hydroxyvitamin D_2) [17]. Studies with $1,24(OH)_2D_2$ have demonstrated high affinity for the vitamin D receptor (VDR) [17], high activity in gene expression assays [17], and potent antiproliferative activity in vitro against a number of cell types [13,18], yet in vivo $1,24(OH)_2D_2$ is at least an order of magnitude less calcemic than $1,25(OH)_2D_3$ [14].

The mechanisms underlying the reduced calcemic activity of $1,24(OH)_2D_2$ in vivo are unclear, and studies of other vitamin D compounds suggest several possibilities. Paricalcitol (19-nor-1,25-dihydroxyvitamin D_2), a vitamin D analog with reduced calcemic activity in rats, has pharmacokinetic properties similar to $1,25(OH)_2D_3$ [19] yet possesses reduced effects on bone [20] and intestine [19] relative to $1,25(OH)_2D_3$, possibly through altered effects on receptor stabilization [21] or target gene expression [22,23]. Other analogs with low calcemic activity, such as 22-oxacalcitriol (OCT), have altered pharmacokinetic properties, including reduced serum levels, because of reduced affinity for the serum transport protein, DBP. The modified pharmacokinetic properties of OCT result in reduced effects on calcium-regulatory target organs and reduced calcemic activity [24–26]. Like OCT, $1,24(OH)_2D_2$ has reduced affinity for DBP relative to calcitriol [17], with correspondingly altered pharmacokinetics and lower serum levels (20–25% of calcitriol) [14]. However, the magnitude of the reduction in calcemic activity of $1,24(OH)_2D_2$ (10–30-fold) is much more than can be accounted for by this reduction in serum levels. Other mechanisms may therefore also be involved; possibilities include reduced target organ levels [25] or decreased efficacy in target organs [20]. To investigate these possibilities, we examined the tissue distribution of radiolabeled $1,24(OH)_2D_2$ and radiolabeled $1,25(OH)_2D_3$ in normal rats over 24 h. To assess efficacy in target organs, $1,24(OH)_2D_2$ or $1,25(OH)_2D_3$ were administered to vitamin D-deficient rats and calcium mobilization in intestine and bone were quantitated. To assess the direct effects of $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ on bone, the compounds were added to cultures of mouse bone marrow and effects on osteoclastogenesis and activation of osteoclastic bone resorption were determined.

2. Materials and methods

2.1. Materials

Radiolabeled $1,24(OH)_2D_2$ (9,11-[^{3}H], specific activity 60 Ci/mmol) was generated at the National Tritium Labeling Facility using carrier-free tritium gas and further purified on high-pressure liquid chromatography (HPLC) prior to use. Radiolabeled $1,25(OH)_2D_3$ (26,26,26,27,27,27-[^{3}H], specific activity 180 Ci/mmol) was purchased from Amersham. Rats were purchased from Harlan Sprague–Dawley. Fetal bovine serum and α-MEM were purchased from HyClone.

2.2. Tissue distribution studies

Normal Sprague–Dawley male rats (250 g) received either [^{3}H]$1,25(OH)_2D_3$ or [^{3}H]$1,24(OH)_2D_2$ (1 nmol/kg, 1 μCi) via subcutaneous injection, following previously used methodology [14]. Animals were euthanized under anesthesia by exsanguination at timed intervals (1, 2, 4, 8, 16 or 24 h) after dose administration (four rats per time point). The amount of tritiated compound in the blood was determined directly by liquid scintillation counting. Though not assessed in the current study, previous work suggests that >95% of radioactivity is present as the parent compound up to 24 h after dose administration (unpublished observations). Bone marrow from one femur, the mucosa of the first 8 cm of small intestine (duodenum), one-half of one kidney, and liver were dissolved in tissue solubilizer (BTS-450, Beckman Instruments, Fullerton, CA) and the tritium quantitated by liquid scintillation counting.

2.3. Calcium absorption and mobilization experiments in vitamin D-deficient rats

Male Sprague–Dawley rats were purchased as weanlings and raised for 4 weeks on a vitamin D-deficient low-calcium (0.02%) diet, followed by 2 weeks on a vitamin D-deficient diet containing 0.4% calcium. Two days prior to treatment the rats were placed back on the vitamin D-deficient low-calcium diet. On the day of treatment, rats were injected intraperitoneally with vehicle (100 μl propylene glycol), $1,24(OH)_2D_2$ or $1,25(OH)_2D_3$ (0.1, 0.25 or 1.0 nmol/kg), following previous methodology [25,26]. Twenty-four hours after injection, intestinal calcium transport was measured in situ using the isolated duodenal loop method [25]. Bone calcium mobilization was estimated by the increase in plasma calcium.

2.4. In vitro bone mobilization

The mouse bone marrow culture system of Suda [27] was used to compare the osteoclastogenic and bone resorbing activities of $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$. In brief, bone marrow was flushed from the femur and tibia of 4–6-week-old Swiss–Webster mice and suspended in α-MEM with 10% fetal bovine serum (FBS), washed twice, and plated in 24-well plates or 100 mm dishes. The cells were cultured for 5 days in medium containing vitamin D compounds (0.1, 1.0 or 10 nM $1,24(OH)_2D_2$ or $1,25(OH)_2D_3$). During this period, osteoclast progenitors in the cultures differentiated to mature osteoclasts in the

presence of active vitamin D compounds. The cells were fed on days 3 and 5 by replacing half of the medium with fresh medium containing the vitamin D compounds. Cells in the 24-well plates were stained for tartrate-resistant acid phosphatase (TRAP) to identify the number of osteoclasts. In the absence of vitamin D compounds, few TRAP-positive cells are observed [28]. Bone marrow cultures grown in 100 mm dishes were scraped free, washed with α-MEM with 10% FBS three times, and plated onto dentine slices pre-equilibrated to the culture medium. The vitamin D compounds, at the doses described earlier, were added on day 0 and replaced on day 3. After 5 days, the dentine slices were washed with 1% sodium dodecyl sulphate (SDS) to remove cellular debris, fixed with 2.5% glutaraldehyde, dehydrated through a series of increasingly concentrated ethanol solutions, and air dried. The dentine slices were then sputter-coated with gold and examined with a Hitachi H-400 scanning electron microscope. Bone resorption was quantified by placing grids with 42 μm spaces over photos of the bone slices and measuring the percentage of spaces containing resorption pits. No pits were formed in the absence of vitamin D compounds. Thus, the ability of vitamin D compounds to activate these two processes, osteoclast maturation and bone resorption, could be assessed independently.

2.5. Statistics

Statistical calculations for Fig. 1 (tissue distribution of radiolabeled $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$) and Fig. 3 (in vitro bone mobilization) compared each time point of

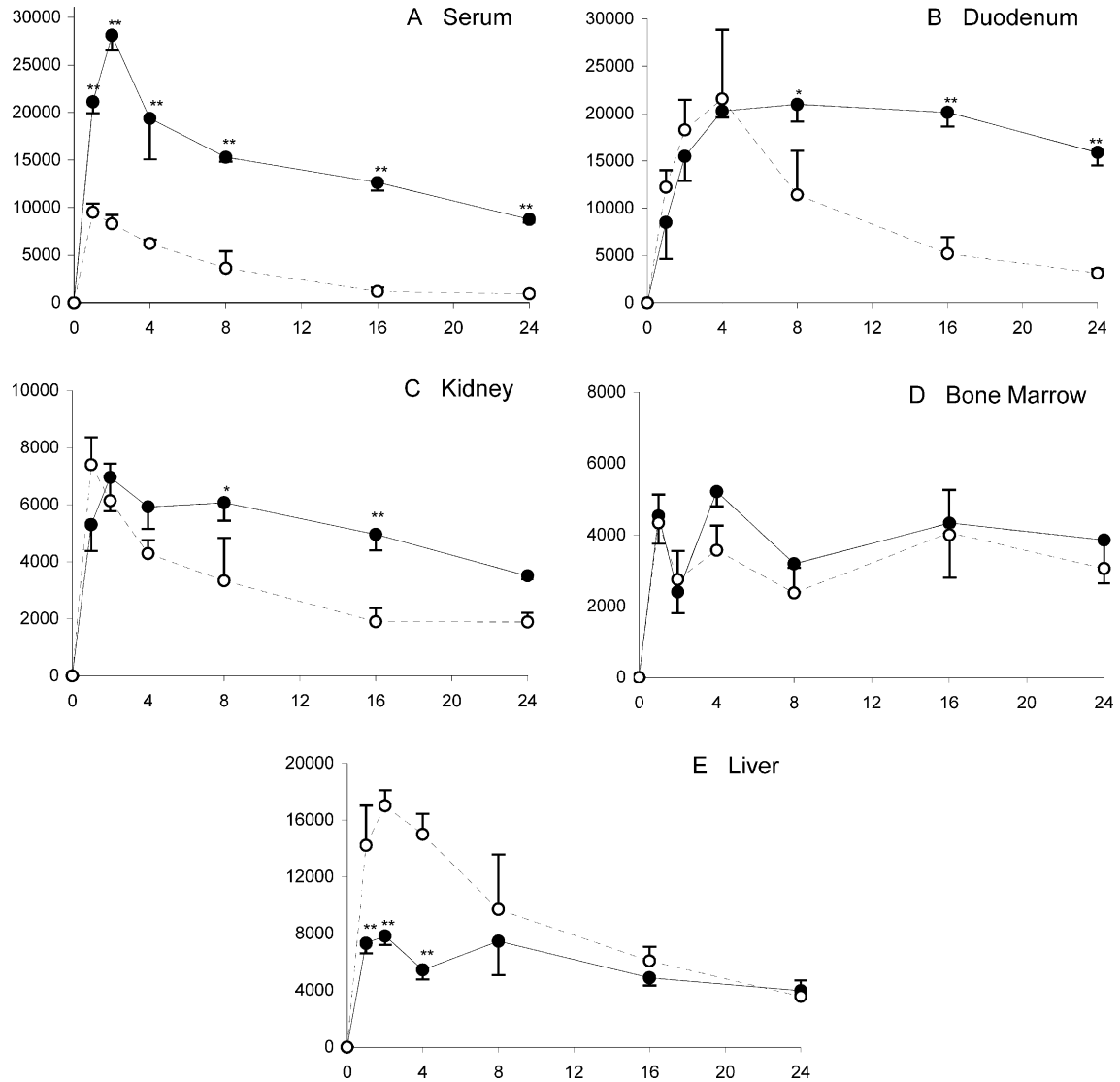

Fig. 1. Tissue distribution of radiolabeled $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$. Rats were injected with tritiated compounds as described in Section 2. At the indicated times, animals were killed and radioactivity in target organs quantitated by liquid scintillation counting. All Y-axis values are in DPM/g tissue, except for serum which is DPM/mL. Data are expressed as mean ± S.E.M. (N = 3–4). (●) $1,25(OH)_2D_3$; (○) $1,24(OH)_2D_2$. Significantly different from $1,24(OH)_2D_2$ at the same time point ($^{*}P < 0.05$); significantly different from $1,24(OH)_2D_2$ at the same time point ($^{**}P < 0.001$).

Table 1
Tissue distribution of radiolabeled $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$

Time (h)	Kidney		Liver		Serum		Duodenum		Bone Marrow	
	$1,24(OH)_2D_2$	$1,25(OH)_2D_3$	$1,24(OH)_2D_2$	$1,25(OH)_2D_3$	$1,24(OH)_2D_2$	$1,25(OH)_2D_3$	$1,24(OH)_2D_2$	$1,25(OH)_2D_3$	$1,24(OH)_2D_2$	$1,25(OH)_2D_3$
1	404 ± 53	289 ± 50	776 ± 152	400 ± 38	518 ± 50	1153 ± 66	667 ± 97	464 ± 211	237 ± 43	247 ± 43
2	335 ± 71	380 ± 65	929 ± 59	428 ± 34	453 ± 50	1534 ± 86	997 ± 172	845 ± 140	150 ± 43	131 ± 33
4	234 ± 26	323 ± 42	818 ± 79	298 ± 37	339 ± 22	1057 ± 234	1175 ± 399	1106 ± 36	195 ± 38	284 ± 23
8	182 ± 82	331 ± 35	530 ± 211	407 ± 130	198 ± 96	834 ± 26	624 ± 253	1144 ± 99	129 ± 39	174 ± 43
16	104 ± 26	271 ± 31	331 ± 55	267 ± 29	64 ± 24	689 ± 45	284 ± 95	1099 ± 82	221 ± 66	236 ± 83
24	103 ± 17	191 ± 7	195 ± 62	217 ± 14	51 ± 1	478 ± 18	171 ± 25	868 ± 74	167 ± 45	210 ± 66

All values are in ng/g tissue, except for serum which is ng/mL. Data are expressed as mean ± S.E.M. (N = 3–4).

$1,24(OH)_2D_2$ with the corresponding time point for $1,25(OH)_2D_3$ using one-way analysis of variance with Bonferroni's adjustment. Statistical calculations for Fig. 2 (calcium mobilization in vitamin D-deficient rats) were carried out using one-way analysis of variance with Dunnett's multiple comparison procedure for comparison to the control group; each dose of $1,24(OH)_2D_2$ was compared to the to the corresponding dose of $1,25(OH)_2D_3$ using one-way analysis of variance with Fisher's least-significant difference test.

3. Results

The results of the tissue distribution study are shown in Fig. 1 and tabulated in Table 1. Serum levels of $1,24(OH)_2D_2$ peaked at 1 h after dose administration and declined thereafter (Fig. 1A). As expected, serum levels of $1,24(OH)_2D_2$ were several-fold lower than those of $1,25(OH)_2D_3$ at any given time point (Table 1). This result is consistent with that seen previously in rats dosed with unlabeled compound whose serum metabolite levels were determined by receptor binding analysis [14].

In contrast, the tissue levels of the radiolabeled compounds did not directly reflect the serum levels. Tissue levels of $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ in duodenal mucosa (Fig. 1B) were similar for the first 4 h after dose administration, after which the tissue levels of $1,24(OH)_2D_2$ declined rapidly while the levels of $1,25(OH)_2D_3$ remained relatively elevated out to 24 h.

Results in kidney were similar to those in duodenum (Fig. 1C). Kidney levels of $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ were similar for the first 2 h after dose administration, after which levels of $1,24(OH)_2D_2$ declined to levels significantly below those of $1,25(OH)_2D_3$.

Tissue levels of radiolabeled compound in bone marrow also did not reflect serum levels (Fig. 1D). Over the entire 24 h period examined, levels of $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ in bone marrow were approximately equal. The apparent decrease in levels of both compounds at 2 h post-dosing, followed by the elevation at 4 h, may be an artifact of the relatively low amount of radiolabel recovered in this tissue. In any event, the tissue levels of $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ at 16 and 24 h were not significantly different and did not reflect the differing serum levels of these compounds.

The pattern of tissue levels of radiolabeled compound in liver was the inverse of that seen in serum (Fig. 1E) in that, at early time points, the level of $1,24(OH)_2D_2$ was considerably higher than that of $1,25(OH)_2D_3$. Levels of both

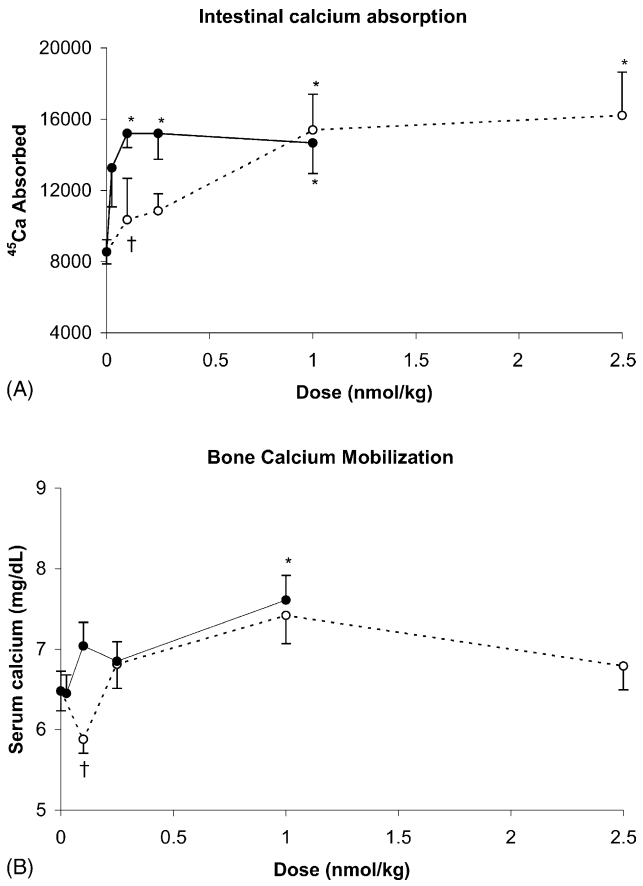

Fig. 2. Calcium mobilization in vitamin D-deficient rats. (A) Regulation of intestinal calcium absorption. Vitamin D-deficient rats were injected with the indicated doses of $1,24(OH)_2D_2$ or $1,25(OH)_2D_3$. Twenty-four hours after injection, intestinal calcium transport was measured by the isolated duodenal loop method. All data are expressed as mean ± S.E.M. (N = 4). (B) Regulation of bone calcium mobilization. Serum was obtained and total calcium was determined. All data are expressed as mean ± S.E.M. (N = 4). (●) $1,25(OH)_2D_3$; (○) $1,24(OH)_2D_2$. Significantly different from control ($^*P < 0.05$); significantly different from corresponding dose of $1,25(OH)_2D_3$ ($^\dagger P < 0.05$).

compounds converged to similar concentrations by 8 h after dose administration.

The activities on intestinal calcium transport and bone calcium mobilization of $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ were determined in vitamin D-deficient rats. Doses of $1,24(OH)_2D_2$ 30-fold higher than $1,25(OH)_2D_3$ were required to increase intestinal calcium transport to the same degree (Fig. 2A). Conversely, $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ were approximately equipotent at increasing calcium release from bone (Fig. 2B). At low doses (0.1 nmol/kg), $1,24(OH)_2D_2$ may have had a reduced effect on bone compared to $1,25(OH)_2D_3$, but at doses of 0.3 nmol/kg and above no significant differences on bone calcium mobilization were apparent between $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$.

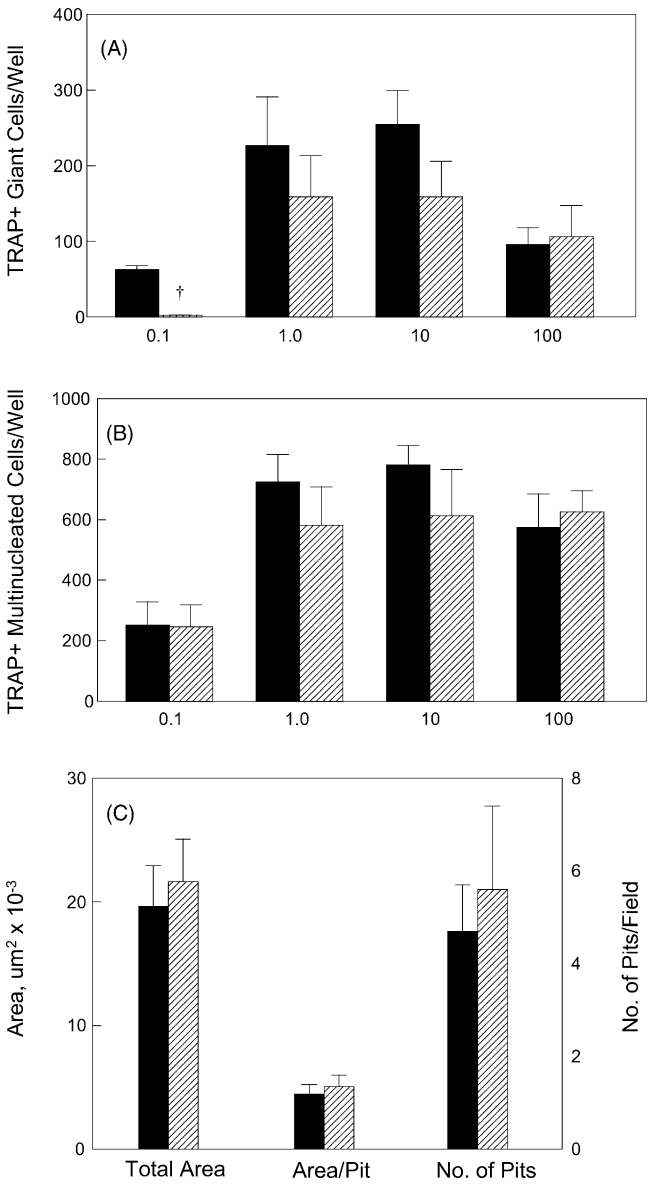

Fig. 3. In vitro bone mobilization. (A) Formation of giant cells. Differentiation of osteoclast precursors by $1,24(OH)_2D_2$ or $1,25(OH)_2D_3$. Procedure was as described in Section 2. (B) Formation of multinucleated cells. No multinucleated cell formation was observed in untreated cultures. (C) Bone resorption. Bone resorption was determined by electron microscopic analysis. All data are expressed as mean ± S.E.M. (N = 4). (Black bars) $1,25(OH)_2D_3$; (hatched bars) $1,24(OH)_2D_2$. Significantly different from corresponding dose of $1,25(OH)_2D_3$ ($^{\dagger}P < 0.05$).

In vitro studies examined the direct effects $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ on bone cells. Incubation of mouse marrow cultures with either $1,24(OH)_2D_2$ or $1,25(OH)_2D_3$ at several doses showed that both compounds were equally effective at higher doses at inducing the formation of TRAP+ multinucleated cells, and at inducing the formation of resorption pits on dentine slices (Fig. 3B and C). At the lowest dose (10^{-10} M), $1,24(OH)_2D_2$ appeared to be less effective than $1,25(OH)_2D_3$ at induction of giant cell formation (Fig. 3A), but no differences in giant cell formation rate were apparent at higher doses.

4. Discussion

The present study investigated some of the possible mechanisms underlying the reduced calcium-mobilizing activity of $1,24(OH)_2D_2$ compared to $1,25(OH)_2D_3$. Tissue distribution and activity studies were used to examine the concentration and activity of each compound in target organs. Consistent with previous studies, serum levels of radiolabeled $1,24(OH)_2D_2$ were lower than those of $1,25(OH)_2D_3$ at all time points (Fig. 1A) [14]. The absolute amounts of $1,25(OH)_2D_3$ and $1,24(OH)_2D_2$ in serum after 24 h (478 and 51 pg/mL; Table 1) were also consistent with previous studies ([14], Table 5), allowing for differences in dose. Activity measurements for intestinal calcium absorption and bone calcium release following $1,25(OH)_2D_3$ administration were also in the range expected based on previous studies, again allowing for differences in dose [19].

Interestingly, the concentration of $1,24(OH)_2D_2$ or $1,25(OH)_2D_3$ in a target organ appears to correlate with its activity in that tissue. After 24 h, the level of $1,24(OH)_2D_2$ in duodenum was considerably lower than $1,25(OH)_2D_3$ (Fig. 1B), and $1,24(OH)_2D_2$ was much less active on intestinal calcium transport than $1,25(OH)_2D_3$ (Fig. 2A). In contrast, $1,24(OH)_2D_2$ levels in bone were equal to $1,25(OH)_2D_3$ at all time points, and $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ had equal activity at stimulating bone calcium release. $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ were also equipotent at stimulating osteoclast formation and bone resorption in cell culture (Fig. 3), consistent with previous data on the equal potency of these compounds in vitro [13,18].

The rapid decline in duodenal levels of $1,24(OH)_2D_2$ relative to $1,25(OH)_2D_3$, starting 4 h after dose administration (Fig. 1B), is of unknown etiology. This decline may have been due to rapid catabolism of $1,24(OH)_2D_2$ by the enzyme CYP24 [29,30], which is upregulated in intestine within 4 h of dose administration [31]. However, $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ have been shown to be

catabolized at the same rate in keratinocyte-derived cells [13], which have high levels of CYP24 activity. Another possibility, therefore, is that $1,25(OH)_2D_3$ may be relatively protected from CYP24-mediated catabolism in intestinal cells, perhaps through binding to an intracellular protein [32]. Alternatively, $1,25(OH)_2D_3$ levels in duodenum might have been replenished via uptake of $1,25(OH)_2D_3$ from serum; uptake of $1,24(OH)_2D_2$ into duodenum would have been relatively difficult at later time points due to its lower serum concentration. In any case, the data suggest that reduced tissue levels of $1,24(OH)_2D_2$ may be a mechanism underlying the reduced effect of this compound on intestinal calcium absorption. This hypothesis is consistent with the finding that OCT levels in intestine declined rapidly after initial time points, and that this reduced level correlated with reduced calcium transport [25].

Further support for a pharmacokinetic mechanism underlying the transient effects of OCT or $1,24(OH)_2D_2$ on the intestine is the finding that infusion of OCT by osmotic minipump gave a sustained induction of intestinal calcium transport which was quickly reversed following removal of the pump [25,26]. This rapid clearance of OCT and the short-lived effects on the intestine and bone are believed to arise from the very low DBP affinity of this analog. The moderately lower DBP affinity of $1,24(OH)_2D_2$ likely also contributes to its faster disappearance from intestine. Intestinal calcium transport is upregulated principally by vitamin D metabolites [33], and reduction of intestinal calcium absorption by a low calcium diet has been shown to greatly reduce the toxicity of vitamin D compounds [34]. Taken together, these findings support the hypothesis that reduced effects on intestinal calcium transport by $1,24(OH)_2D_2$ may contribute significantly to its reduced calcemic activity.

An intriguing aspect of the present study is the inconsistency between compound levels in serum and target organs. At early time points, $1,24(OH)_2D_2$ levels in target organs (kidney, intestine) were equal to or greater than $1,25(OH)_2D_3$ levels, yet serum levels of $1,24(OH)_2D_2$ were several-fold lower. This observation agrees with previous work on OCT and $1,25(OH)_2D_3$ in animals; OCT serum levels were one-tenth those of $1,25(OH)_2D_3$, yet OCT tissue levels matched or exceeded those of $1,25(OH)_2D_3$ at early time points (1–3 h post-dose) [35,36]. It appears, therefore, that serum levels of a compound such as $1,24(OH)_2D_2$ are a poor surrogate for tissue levels, and that low serum levels do not reliably indicate that such a compound will be present at low levels in target tissues, especially soon after dose administration.

To be consistent with previous studies, radiolabeled $1,24(OH)_2D_2$ and $1,25(OH)_2D_3$ were administered subcutaneously in the tissue distribution study [14], and were administered intraperitoneally in the activity study [25,26]. The tissue distribution and activity data agreed well with the previous studies on which they were based, providing reassurance about the integrity of the study data. However, it is possible that the difference in delivery methods might have affected compound distribution and made it difficult to correlate the two datasets. Reassuringly, intraperitoneal administration of $1,25(OH)_2D_3$ [19] has been shown to produce a serum pharmacokinetic profile very similar to that seen in the present study using subcutaneous administration, with perhaps slightly earlier peak serum levels. Thus, the differing delivery methods probably did not have a major impact on compound distribution or on the validity of extrapolating between the tissue distribution and activity data.

The dose of $1,25(OH)_2D_3$ in the tissue distribution study exceeded the dose required to maximize intestinal calcium absorption. Of note, this dose (1 nmol/kg) was barely sufficient to maximize intestinal calcium absorption for $1,24(OH)_2D_2$ and is therefore in a relevant range for that compound. However, we cannot rule out the possibility that the tissue distributions of $1,25(OH)_2D_3$ or $1,24(OH)_2D_2$ at lower doses might have followed different patterns from those observed, or that target tissue levels of $1,25(OH)_2D_3$ or $1,24(OH)_2D_2$ at lower doses might have diverged even further than in the present study. Further studies will be necessary to examine these possibilities.

Although not addressed in the current study, other factors may also contribute to the reduced calcemic activity of $1,24(OH)_2D_2$, especially following chronic administration. Repeated dosing of animals with $1,24(OH)_2D_2$ produced suppression of endogenous $1,25(OH)_2D_3$ [14] to levels below that seen with other analogs [37], presumably through inhibition of 1-hydroxylase activity. It is possible that, as with paricalcitol, this reduction of endogenous $1,25(OH)_2D_3$ could lead to reduced vitamin D receptor levels in target tissues, reducing responsiveness to vitamin D [21]. Additional mechanisms that contribute to reduced calcemic activity, such as suppression of PTH levels and accompanying reduced effects on bone [26], have been noted for OCT but have not yet been examined for $1,24(OH)_2D_2$.

In summary, the present study suggests that target tissue levels of $1,24(OH)_2D_2$ correlate with activity, and that rapid reduction in tissue levels of $1,24(OH)_2D_2$ in target organs, particularly intestine, may contribute significantly to the reduced calcemic effects of $1,24(OH)_2D_2$. Further studies will be necessary to confirm the mechanism underlying the reduced calcemic activity of $1,24(OH)_2D_2$ and ultimately extend the use of this compound into the clinic.

Acknowledgements

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References

[1] Brown AJ, Dusso AS, Slatopolsky E. Vitamin D analogues for secondary hyperparathyroidism. Nephrol Dial Transplant 2002;17 (Suppl 10):10–9.

[2] Maung HM, Elangovan L, Frazao JM, Bower JD, Kelley BJ, Acchiardo SR, et al. Efficacy and side effects of intermittent intravenous and oral doxercalciferol (1alpha-hydroxyvitamin D(2)) in dialysis patients with secondary hyperparathyroidism: a sequential comparison. Am J Kidney Dis 2001;37:532–43.

[3] Fogh K, Kragballe K. Recent developments in Vitamin D analogs. Curr Pharm Des 2000;6:961–72.

[4] Kanis JA, McCloskey EV, de Takats D, Bernard J, Zhang DM. Treatment of osteoporosis with Vitamin D. Osteoporos Int 1997;7: s140–6.

[5] Colston KW, Chander SK, Mackay AG, Coombes RC. Effects of synthetic Vitamin D analogues on breast cancer cell proliferation in vivo and in vitro. Biochem Pharmacol 1992;44:693–702.

[6] Jensen SS, Madsen MW, Lukas J, Bartek J, Binderup L. Sensitivity to growth suppression by 1alpha,25-dihydroxyvitamin D-3 among MCF-7 clones correlates with Vitamin D receptor protein induction. J Steroid Biochem Mol Biol 2002;81 (2):123–33.

[7] Bauer JA, Thompson TA, Church DR, Ariazi EA, Wilding G. Growth inhibition and differentiation in human prostate carcinoma cells induced by the Vitamin D analog 1alpha,24-dihydroxyvitamin D_2. Prostate 2003;55:159–67.

[8] Vanweelden K, Flanagan L, Binderup L, Tenniswood M, Welsh J. Apoptotic regression of MCF-7 xenografts in nude mice treated with the Vitamin D_3 analog EB1089. Endocrinology 1998;139: 2102–10.

[9] Grostern RJ, Bryar PJ, Zimbric ML, Darjatmoko SR, Lissauer BJ, Lindstrom MJ, et al. Toxicity and dose–response studies of 1alpha-hydroxyvitamin D_2 in a retinoblastoma xenograft model. Arch Ophthalmol 2002;120 (5):607–12.

[10] Vegesna V, O'Kelly J, Said J, Uskokovic M, Binderup L, Koeffler HP. Ability of potent Vitamin D_3 analogs to inhibit growth of prostate cancer cells in vivo. Anticancer Res 2003;23:283–9.

[11] Osborn JL, Schwartz GG, Smith DC, Bahnson R, Day R, Trump DL. Phase II trial of oral 1,25-dihydroxyvitamin D (calcitriol) in hormone refractory prostate cancer. Urol Oncol 1995;1:195–8.

[12] Smith DC, Johnson CS, Freeman CC, Muindi J, Wilson JW, Trump DL. A phase I trial of calcitriol (1,25-dihydroxycholecalciferol) in patients with advanced malignancy. Clin Cancer Res 1999;5:1339–45.

[13] Jones G, Byford V, Makin HL, Kremer R, Rice RH, deGraffenried LA, et al. Anti-proliferative activity and target cell catabolism of the Vitamin D analog 1alpha,24(*S*)-$(OH)_2D_2$ in normal and immortalized human epidermal cells. Biochem Pharmacol 1996;52:133–40.

[14] Knutson JC, LeVan LW, Valliere CR, Bishop CW. Pharmacokinetics and systemic effect on calcium homeostasis of 1alpha,24-dihydroxyvitamin D_2 in rats. Comparison with 1alpha,25-dihydroxyvitamin D_2, calcitriol, and calcipotriol. Biochem Pharmacol 1997;53:829–37.

[15] Mawer EB, Jones G, Davies M, Still PE, Byford V, Schroeder NJ, et al. Unique 24-hydroxylated metabolites represent a significant pathway of metabolism of Vitamin D_2 in humans: 24-hydroxyvitamin D_2 and 1,24-dihydroxyvitamin D_2 detectable in human serum. J Clin Endocrinol Metab 1998;83:2156–66.

[16] Horst RL, Koszewski NJ, Reinhardt TA. 1α-Hydroxylation of 24-hydroxyvitamin D_2 represents a minor physiological pathway for the activation of Vitamin D_2 in mammals. Biochemistry 1990;29:578–82.

[17] Strugnell S, Byford V, Makin HLJ, Moriarty RM, Gilardi R, LeVan LW, et al. 1α,24(*S*)-Dihydroxyvitamin D_2: a biologically active product of 1α-hydroxyvitamin D_2 made in the human hepatoma, Hep3B. Biochem J 1995;310:233–41.

[18] Levy Y, Knutson JC, Bishop C, Shany S. The novel analog 1,24(*S*)-dihydroxyvitamin D_2 is as equipotent as 1,25-dihydroxyvitamin D_3 in growth regulation of cancer cell lines. Anticancer Res 1998;18:1769–76.

[19] Brown AJ, Finch J, Takahashi F, Slatopolsky E. Calcemic activity of 19-nor-1,25(OH)(2)D(2) decreases with duration of treatment. J Am Soc Nephrol 2000;11:2088–94.

[20] Holliday LS, Gluck SL, Slatopolsky E, Brown AJ. 1,25-Dihydroxy-19-nor-Vitamin D(2), a Vitamin D analog with reduced bone resorbing activity in vitro. J Am Soc Nephrol 2000;11:1857–64.

[21] Takahashi F, Finch JL, Denda M, Dusso AS, Brown AJ, Slatopolsky E. A new analog of 1,25-$(OH)_2D_3$, 19-nor-1,25-$(OH)_2D_2$, suppresses serum PTH and parathyroid gland growth in uremic rats without elevation of intestinal Vitamin D receptor content. Am J Kidney Dis 1997;30:105–12.

[22] Issa LL, Leong GM, Sutherland RL, Eisman JA. Vitamin D analogue-specific recruitment of Vitamin D receptor coactivators. J Bone Miner Res 2002;17 (5):879–90.

[23] Brown AJ, Finch J, Slatopolsky E. Differential effects of 19-nor-1,25-dihydroxyvitamin D_2 and 1,25-dihydroxyvitamin D_3 on intestinal calcium and phosphate transport. J Lab Clin Med 2002;139 (5):279–84.

[24] Hirata M, Katsumata K, Endo K, Fukushima N, Ohkawa H, Fukagawa M. In subtotally nephrectomized rats 22-oxacalcitriol suppresses parathyroid hormone with less risk of cardiovascular calcification or deterioration of residual renal function than 1,25(OH)(2) Vitamin D(3). Nephrol Dial Transplant 2003;18: 1770–6.

[25] Brown AJ, Finch J, Gridff M, Ritter C, Kubodera N, Nishii Y, et al. The mechanism for the disparate actions of calcitriol and 22-oxacalcitriol in the intestine. Endocrinology 1993;133:1158–64.

[26] Finch JL, Brown AJ, Mori T, Nishii Y, Slatopolsky E. Suppression of PTH and decreased action on bone are partially responsible for the low calcemic activity of 22-oxacalcitriol relative to 1,25-$(OH)_2D_3$. J Bone Miner Res 1992;7:835–9.

[27] Takahashi N, Yamana H, Yoshiki S, Roodman GD, Mundy GR, Jones SJ, et al. Osteoclast-like cell formation and its regulation by osteotropic hormones in mouse bone marrow cultures. Endocrinology 1988;122:1373–82.

[28] Takahashi N, Yamana H, Yoshiki S, Roodman GD, Mundy GR, Jones SJ, et al. Osteoclast-like cell formation and its regulation by osteotropic hormones in mouse bone marrow cultures. Endocrinology 1988;122:1373–82.

[29] Beckman MJ, Tadikonda P, Werner E, Prahl J, Yamada S, DeLuca HF. Human 25-hydroxyvitamin D_3-24-hydroxylase, a multicatalytic enzyme. Biochemistry 1996;35:8465–72.

[30] Sakaki T, Sawada N, Nonaka Y, Ohyama Y, Inouye K. Metabolic studies using recombinant *Escherichia coli* cells producing rat mitochondrial CYP24 CYP24 can convert 1alpha,25-dihydroxyvitamin D-3 to calcitroic acid. Eur J Biochem 1999;262 (1):43–8.

[31] Shinki T, Jin CH, Nishimura A, Nagai Y, Ohyama Y, Noshiro M, et al. Parathyroid hormone inhibits 25-hydroxyvitamin D_3-24-hydroxylase mRNA expression stimulated by 1alpha,25-dihydroxyvitamin D_3 in rat kidney but not in intestine. J Biol Chem 1992;267:13757–62.

[32] Wu SX, Ren SY, Chen H, Chun RF, Gacad MA, Adams JS. Intracellular Vitamin D binding proteins: novel facilitators of Vitamin D-directed transactivation. Mol Endocrinol 2000;14 (9): 1387–97.

[33] Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of Vitamin D. Physiol Rev 1998;78:1193–231.

[34] Mortensen JT, Brinck P, Binderup L. Toxicity of Vitamin D analogues in rats fed diets with standard or low calcium contents. Pharmacol Toxicol 1993;72:124–7.

[35] Okano T, Tsugawa N, Masuda S, Takeuchi A, Kobayashi T, Nishii Y. Protein-binding properties of 22-oxa-1α,25-dihydroxyvitamin D_3, a

synthetic analogue of 1α,25-dihydroxyvitamin D_3. J Nutr Sci Vitaminol 1989;35:529–33.

[36] Kobayashi T, Tsugawa N, Okano T, Masuda S, Takeuchi A, Kubodera N, et al. The binding properties, with blood proteins, and tissue distribution of 22-oxa-1alpha,25-dihydroxyvitamin D_3, a noncalcemic analogue of 1alpha,25-dihydroxyvitamin D_3, in rats. J Biochem Tokyo 1994;115:373–80.

[37] Horst R, Prapong S, Reinhardt T, Koszewski N, Knutson J, Bishop C. Comparison of the relative effects of 1,24-dihydroxyvitamin D(2) [1,24-(OH)(2)D(2)], 1,24-dihydroxyvitamin D(3) [1,24-(OH)(2)D(3)], and 1,25-dihydroxyvitamin D(3) [1,25-(OH)(2)D(3)] on selected Vitamin D-regulated events in the rat. Biochem Pharmacol 2000;60:701–8.