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Failure of alphacalcidol (1α-hydroxyvitamin D_3) in treating nutritional rickets and the biochemical response to ergocalciferol☆

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ABSTRACT

It has been previously documented that alphacalcidol (1α-hydroxyvitamin D_3) is inefficient in healing rickets, partly because it results in a suboptimal rise in 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) and partly because it fails to replenish the store of 25-hydroxyvitamin D (25-OHD). However, very few studies have actually documented this outcome. The aim was to document biochemically the response to alphacalcidol and subsequently the change in response to ergocalciferol. This study was conducted at our institution from January 2005 till December 2008. We included all patients referred to our clinic with active rickets after a failed course of alphacalcidol. At baseline the median (IQR) for PTH l7.1 (4.5–35.3) pmol/L, 25-OHD 29.0 (18–66.2) nmol/L, 1,25-$(OH)_2$D 205 (158.2–311.2) pmol/L and ALP 676 (462.5–1101.7) IU/L. After 3 months treatment with ergocalciferol the concentrations changed markedly with biochemical healing: PTH 4.5 (3.9–7.5), 25-OHD 143.5 (101.5–206.5), 1,25-$(OH)_2$D 277 (221.0–572.7), ALP 369 (302.2–438.0). The results confirm the biochemical and physiological basis for using ergocalciferol (or cholecalciferol) in nutritional rickets. Unfortunately these forms are not readily available in many geographic areas. This supply problem together with marketing strategies forces physicians to make an incorrect choice of medication. Treatment with ergocalciferol was either with intramuscular stosstherapy or drops for 3 months. The former ensures compliance and is associated with higher 25-OHD and 1,25-$(OH)_2$D concentrations.

1. Introduction

Rickets remains prevalent in the Middle East despite a favorable latitude as well as abundant sunshine [1]. It is estimated that rickets in this region may be a hundredfold more common than in its western counterparts [1]. Recent studies have described cohorts of rachitic children within the United Arab Emirates [2,3]. A number of factors may account for the prevalence of vitamin D deficiency in this region, including the urbanization umbrella [4]. In addition, the Mediterranean region remains the epicenter of many cases of resistant rickets as well, possibly related to genetic predisposition and consanguinity [5,6]. Alphacalcidol's popularity in this region may in part relate to its well-known use in resistant forms of rickets. This is despite both a recent Cochrane analysis as well as a consensus statement of the American Academy of Pediatricians advocating vitamin D and calcium in the treatment and prevention of nutritional rickets [7,8].

There have been clear proscriptions against the use of alphacalcidol in nutritional rickets [9,10]. Firstly, it does not replenish the vitamin D stores and has a short half life, and secondly it fails to cause a supraphysiological increase in the 1,25-$(OH)_2$D [9,11,12]. The evidence documenting the failure of alphacalcidol or other vitamin D analogs is sparse [13]. There is also sparse evidence to the contrary supporting its use, and alphacalcidol has been used successfully in vitro [14] as well as in vivo [4,15,16] in nutritional rickets. The continued use of alphacalcidol rests on its market penetration in areas where resistant rickets is frequent, its availability in suspension form which is convenient for children and its availability to physicians. These conditions are not always met with ergocalciferol or cholecalciferol [10].

The aim of this study was to examine the plasma concentration of the major vitamin D metabolites of patients with nutritional rickets who failed to heal with alphacalcidol and their subsequent biochemical response to ergocalciferol. The secondary aim was to compare the biochemical response between ergocalciferol given orally versus stosstherapy.

2. Methods

This study was a retrospective audit of children with nutritional rickets who were referred to our hospital, Sheikh Khalifa Medical

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City (SKMC), a tertiary level 500 bed hospital in Abu Dhabi (24°N 28), which is the capital city of United Arab Emirates. The inclusion criteria were patients who met the definition of rickets (radiologically and biochemically) and who were on current therapy with alphacalcidol. All patients were referred to our clinic from peripheral clinics or physicians in private and examined and followed up by one pediatrician (JR). In many instances the referral was for suspected "resistant rickets". Patients were only included if there was a physician documented use of alphacalcidol or the parents brought the actual medication to our clinic. Exclusion criteria included renal or liver disease, use of antiepileptic medication or cases of vitamin D dependent or hypophospatemic rickets. The study was conducted from January 2005 till December 2008. Altogether, 15 patients were eligible for enrollment in the study. We excluded one who was under antiepileptic treatment, and two patients who appeared to have been successfully treated with alphacalcidol. Another patient was excluded because the documentation of alphacalcidol use was unclear. The fifth patient had his alphacalcidol stopped by his physician after documented hypercalcemia and was off therapy for 2 weeks when he visited us. The 10 patients who fulfilled our criteria were all treated with ergocalciferol at our clinic. Seven patients were treated with 2000 IU daily (Schwarz Pharma, Inc., Milwaukee, USA) for 3 months (and subsequently 400 IU daily), whereas the final three were treated with stosstherapy of 600,000 IU intramuscular as a single dose (UCB Pharma Ltd, Berkshire, UK). Stosstherapy was unavailable to us earlier. All patients were prescribed elemental calcium at a dosage of 40 mg/kg/day for 3 months as well.

Since the patients were referred to us with failed therapy of alphacalcidol and the suspicion of resistant rickets, our protocol was comprehensive and included blood tests done at baseline, at 1 month, at 2 months and rarely at 3 months. Radiological exam included upper limb and/or lower limb X-rays.

Additional data recorded from time of diagnosis included 25-OHD, 1,25-$(OH)_2$D, intact parathyroid hormone (PTH), Ca, PO_4, alkaline phosphatase (ALP), length/height and demographic details of each patient. Two-site chemiluminescent immunoassay (IMMULITE 2000) was used to measure intact PTH. Ca and PO_4 were measured by colorimetric assay (Beckman Synchron DXC800, CA, USA). Physical measurements were made by methods previously documented [2]. *Z* scores for weight and height were calculated using WHO Anthro (World Health Organization, Geneva, Switzerland) [17,18]. Approval for the study was attained from the Institutional Review Board of our hospital.

2.1. Measurement of vitamin D metabolites

25-OHD was measured at SKMC by Waters HPLC 2695 separation module with UV detection using Chromsystems kits (Munich, Germany). Briefly, 500 μL of serum (standard, controls, or specimen) and 50 μL internal standard were mixed into the labeled, light-protected reaction vial. 500 μL precipitation reagent was then added and vortex-mixed for 20 s. Reaction vials were then incubated for 10 min at 4 °C. Vials were then centrifuged for 5 min at 13,000 rpm. Immediately the supernatant was applied to a labeled sample clean up column and drawn through by centrifugation at 1500 rpm for 1 min followed by discarding the effluent. Sample clean up columns were then washed two times by using 1 mL Wash Buffer I each time by centrifuging at 1500 rpm for 1 min and discarding the effluent. Column washing was repeated by using 75 μL Wash Buffer II by centrifuging at 1500 rpm for 1 min and discarding the effluent. 200 μL elution buffer was applied to each column and the eluates were collected by centrifugation at 1500 rpm for 1 min. The eluants were collected into glass vials and then diluted with the addition of 20 μL distilled water. 50 μL of the sample was injected to the HPLC system. The prepared controls were included in every analytical series to monitor accuracy and precision within the system. The chromatographic separation was about 10 min. All the values of 25-OHD were recorded in nmol/L. This HPLC assay includes the measurement of both 25-OHD_2 and 25-OHD_3 separately and the reported values were the combined concentrations. The intra-assay coefficient of variation was 4% and the inter-assay coefficient of variation was 5.8%.

Measurement of 1,25-$(OH)_2$D was done using Diasorin RIA at Biomnis Laboratories (Lyon, France). The inter-assay coefficient of variation was 11.3% and the intra-assay coefficient of variation was 11.2%.

Table 1
Selected characteristics of rachitic infants at presentation to SKMC.

n = 10	Mean (SD)
25-OHD (nmol/L)	37.70 (25.85)
1,25-$(OH)_2$D (pmol/L)	234.88 (82.25)
Age (months)	21.20 (8.41)
Breast feeding (months)	20.33 (9.12)
% Arab ethnicity	90%
Male:female ratio	6:4
Z score height	−2.19 (1.57)
Z score weight	−1.14 (1.07)

Reference range: 25-OHD 50–200 nmol/L; 1,25-$(OH)_2$D 43–148 pmol/L.

2.2. Statistical analysis

Statistical analysis software was done with MedCalc for Windows (Version 11.0.1, Mariakerke, Belgium). Data are described as mean and standard deviation (SD) or median and interquartile range (IQR) depending on the distribution. *P* value <0.05 was regarded as statistically significant. Correlation was performed using the Pearson test.

3. Results

A total of 10 patients participated in this study. Their age range (2.5–97.5th percentile) was 11–39 months. Rapid healing (within 3 months) followed the introduction of therapeutic doses of ergocalciferol (either oral or stosstherapy). All were of Arab lineage except one patient from Southern Africa. Baseline characteristics are shown in Table 1. At the time of referral to our hospital the milk intake in six patients was provided exclusively by breastfeeding, even though all children were on weaning diets. The median (IQR) duration of alphacalcidol therapy was 45 days (27.75–225.00). The median maternal parity was 2 (IQR 1.75–3.25) children. Seven children were classified as stage 3 rickets with marked X-ray changes (rarefaction, cupping, and widened metaphysis) and elevated ALP, whereas the remaining 3 were classified as stage 2 rickets with milder changes on the X-ray (rarefaction and fraying) [19]. Five children had physical deformities with either widening at the knees or the ankles. A sixth child had more severe deformities with widening at both the knees and the ankles. There was no statistical correlation between *Z* score height and bony deformities ($P = 0.568$, Pearson's $r = 0.23$, 95% CI = −0.55 to 0.81). Table 2 depicts the biochemical response to ergocalciferol at visit 0 (baseline), visit 1 (1 month), and visit 2 (2 months). Figs. 1 and 2 depict the serial changes in 1,25-$(OH)_2$D and 25-OHD to ergocalciferol (stosstherapy versus oral drops).

4. Discussion

This study demonstrates the biochemical resolution in a group of rachitic patients who failed to improve with alphacalcidol. The patients who were subsequently treated with ergocalciferol all attained biochemical and radiological healing. This is the first case

Table 2
Biochemical response to both ergocalciferol regimens at visit 0 (baseline), visit 1 (1 month) and visit 2 (2 months).

0 = baseline visit 1 = 1 month visit 2 = 2 month visit	Vitamin D_2					
	2000 IU daily (n = 7)			Stosstherapy 600,000 IU imi (n = 3)		
	Mean	SD	Median	Mean	SD	Median
PTH 0 (pmol/L)	19.786	16.4766	16.500	50.833	34.6988	33.300
PTH 1 (pmol/L)	9.657	12.1714	3.800	15.833	2.0841	15.400
PTH 2 (pmol/L)	4.725	1.3672	4.300	7.667	3.7018	7.800
ALP 0 (IU/L)	786.571	338.7215	676.000	1103.000	371.9382	1103.000
ALP 1 (IU/L)	485.000	236.3607	415.000	724.667	470.5235	775.000
ALP 2 (IU/L)	352.000	49.0102	369.000	423.000	228.0855	454.000
PO_4 0 (mmol/L)	1.396	0.4277	1.430	0.815	0.1909	0.815
PO_4 1 (mmol/L)	1.563	0.4579	1.485	1.755	0.2051	1.755
PO_4 2 (mmol/L)	1.678	0.2065	1.725	1.777	0.1159	1.760
Ca 0 (mmol/L)	2.299	0.09990	2.300	2.245	0.09192	2.245
Ca 1 (mmol/L)	2.372	0.1427	2.385	2.265	0.04950	2.265
Ca 2 (mmol/L)	2.365	0.05447	2.380	2.377	0.09018	2.370

Reference range: PTH 1.3–9.3 pmol/L; ALP 110–302 IU/L; Ca 2.1–2.6 mmol/L, PO_4 0.78–1.53 mmol/L.

series highlighting the failure of alphacalcidol in nutritional rickets. The mean age of the patients is older than earlier cohorts described in this region [2,3], reflecting their delayed presentation to our institution.

The peak growth velocity is during the infancy period. It is therefore incumbent that suboptimal therapy be avoided. One study using either ergocalciferol or alphacalcidol in adolescents attained healing after 21 months [4]. The authors postulated that the dose of ergocalciferol of 4000 IU in adolescence may have been insufficient. Another explanation may have been that healing was delayed due to the use of alphacalcidol. Alphacalcidol (1 α-hydroxyvitamin D_3) is an analog of the active metabolite of vitamin D, 1,25-$(OH)_2$D and is rapidly converted in vivo to 1,25-$(OH)_2$D [16]. At 2 months (Figs. 1 and 2), stosstherapy resulted in a high concentration of 25-OHD (storage form of vitamin D) as well as a supraphysiologic increase in 1,25-$(OH)_2$D (which is the physiologically active component of vitamin D responsible for bone healing). In our group of patients, such a response was expectedly absent at baseline, while the patients were on current alphacalcidol therapy [9]. Attempting to attain supraphysiologic healing doses with alphacalcidol would risk hypercalcemia as well as nephrocalcinosis [9].

Because of the attendant hypophosphatemia (due to elevated PTH's phosphaturic action), and delayed healing, many of our study patients were referred for apparent "resistant rickets" or hypophosphatemic rickets. The clues to nutritional deficiency were prolonged breast feeding in women with a high prevalence of vitamin deficiency [3], the elevated PTH concentration at baseline and the rapid increase in PO_4 at visit 1. The 25-OHD concentration was higher than rachitic infants in the UAE described previously [2,3]. This would be expected since sunlight exposure may have been recommended by their referring physicians. A more likely explanation in the face of an elevated 1,25-$(OH)_2$D concentration is that these children have associated calcium deficiency as well, similar to the pattern previously described in African children [20,21]. The diet in many Middle Eastern countries is low in calcium and high in phytate, resulting in a high phytate:calcium ratio [22]. Consumption of carbonated drinks and fruit juices which replaces milk is another possible factor. Since breast feeding provides a highly adsorbable source of calcium intake, in this cohort of patients it is possible that a weaning diet may have resulted in restrictive calcium intake by replacing breast feeding. It is likely that this cohort of children reflects a continuum of vitamin D deficiency as well as calcium deficiency [22,23].

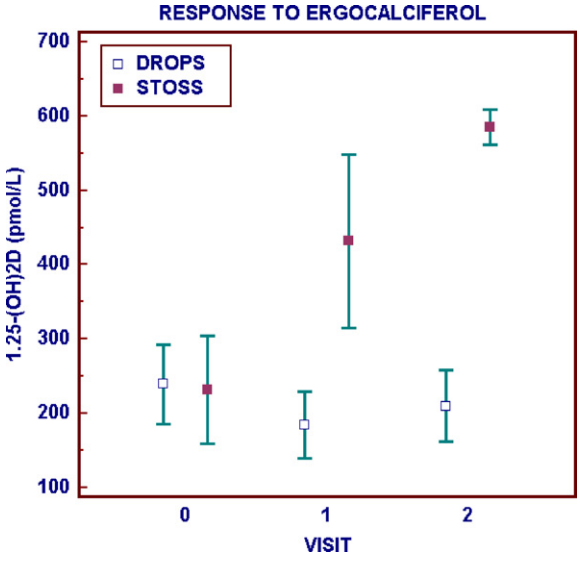

Fig. 1. The figure represents 1,25-$(OH)_2$D concentration of all patients at visit 0 (baseline), visit 1 (1 month) and visit 2 (2 months) and compares the response of ergocalciferol orally to stosstherapy. The dots show the mean and the bars one standard error of the mean.

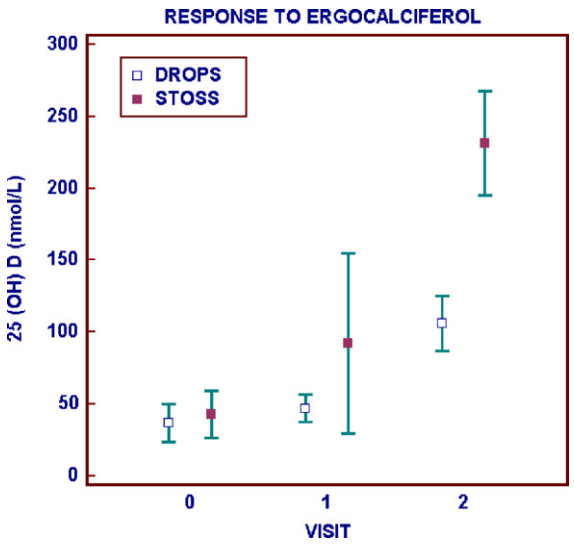

Fig. 2. The figure represents the 25-OHD concentration of all patients at visit 0 (baseline), visit 1 (1 month) and visit 2 (2 months) and compares the response of ergocalciferol orally to stosstherapy. The dots show the mean and bars one standard error of the mean.

The study has a number of limitations. The sample size was small after the exclusions. The improvements seen could therefore theoretically be ascribed to random variation (regression to the mean). This is unlikely as all patients treated with ergocalciferol responded to therapy within 3 months. The biochemical findings of the patients fit the expected pharmacological response to alphacalcidol which has a short half life and is not expected to significantly raise the 1,25-$(OH)_2$D concentration (Table 1 and Fig. 1; baseline visit 0). The 1,25-$(OH)_2$D concentration in patients treated with oral ergocalciferol did not raise at visits 1 and 2 (Fig. 1). However, other studies have shown that the peak response to oral vitamin D occurs within the first week [11,12,24]. Data was not captured during this period in our patients but this peak most likely occurred. The smaller than expected increase in 25-OHD concentrations with oral vitamin D may also be explained by the lesser potency or lesser stability of ergocalciferol (vitamin D_2) compared to cholecalciferol (vitamin D_3) despite healing. The success of ergocalciferol could also be ascribed to better compliance. This would be expected with stosstherapy, however, seven of the ten patients were treated successfully with oral vitamin D for 3 months.

The pervasive practice of alphacalcidol (or other analogs of vitamin D) in treating nutritional rickets, is to be condemned as it is largely ineffective, an inappropriate treatment and not in keeping with a recent consensus statement of the American Academy of Pediatricians [8]. The semantic confusion of medical practitioners, the public and pharmaceuticals between nutritional vitamin D and its analogs has been another source of incorrect prescription practices [25]. There is an urgent and important need to assess regionally through cross-sectional surveys the extent of the misuse and abuse of vitamin D analogs in treating and preventing nutritional rickets and vitamin D deficiency. Our use of alphacalcidol in nutritional rickets is limited to patients with symptomatic hypocalcemia (tetany, convulsions, and cardiomyopathy) only.

Conflict of interest

None.

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