Bone 56 (2013) 271–275

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Calculated free and bioavailable vitamin D metabolite concentrations in vitamin D-deficient hip fracture patients after supplementation with cholecalciferol and ergocalciferol

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ARTICLE INFO

Article history:
Received 31 January 2013
Revised 22 May 2013
Accepted 8 June 2013
Available online 20 June 2013

Edited by: Rene Rizzoli

Keywords:
Hip fracture
Cholecalciferol
Ergocalciferol
Calculated free vitamin D concentrations
Vitamin D-binding protein

ABSTRACT

We previously showed that oral cholecalciferol and ergocalciferol have comparable effects in decreasing circulating parathyroid hormone (PTH), despite a greater increase in total serum 25-hydroxyvitamin D (25OHD) concentration with cholecalciferol supplementation. However, the effects of cholecalciferol and ergocalciferol on total serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), vitamin D-binding protein (DBP), free 25OHD and free $1,25(OH)_2D$ concentrations have not been previously studied.

We randomized 95 hip fracture patients (aged 83 ± 8 years) with vitamin D deficiency (serum 25OHD <50 nmol/L) to oral supplementation with either cholecalciferol 1000 IU/day (n = 47) or ergocalciferol 1000 IU/day (n = 48) for three months. All were given matching placebos of the alternative treatment to maintain blinding. We measured serum 25OHD (high-pressure liquid chromatography), $1,25(OH)_2D$ (Diasorin radioimmunoassay), DBP (immunonephelometry), ionized calcium (Bayer 800 ion-selective electrode) and albumin (bromocresol green) concentrations before and after treatment. We calculated free and bioavailable concentrations of the vitamin D metabolites using albumin and DBP, and calculated free vitamin D metabolite indices as the ratios between the molar concentrations of the vitamin D metabolites and DBP.

Seventy participants (74%) completed the study with paired samples for analysis. Total serum $1,25(OH)_2D$ did not change significantly with either treatment ($p > 0.05$, post-treatment vs baseline). Both treatments were associated with comparable increases in DBP (cholecalciferol: +18%, ergocalciferol: +16%, $p = 0.32$ between groups), albumin (cholecalciferol: +31%, ergocalciferol: +21%, $p = 0.29$ between groups) and calculated free 25OHD (cholecalciferol: +46%, ergocalciferol: +36%, $p = 0.08$), with comparable decreases in free $1,25(OH)_2D$ (cholecalciferol: −17%, ergocalciferol: −19%, $p = 0.32$ between groups). In the treatment-adherent subgroup the increase in ionized calcium was marginally greater with cholecalciferol compared with ergocalciferol (cholecalciferol: +8%, ergocalciferol: +5%, $p = 0.03$ between groups). There were no significant differences between the treatments in their effects on the calculated bioavailable concentrations or free indices of the vitamin D metabolites ($p > 0.05$ between groups).

In vitamin D-deficient hip fracture patients, oral supplementation with cholecalciferol and ergocalciferol had no effect on total serum $1,25(OH)_2D$, and comparable effects on DBP and free vitamin D metabolite concentrations. This is despite cholecalciferol having greater effects than ergocalciferol in increasing total 25OHD, and in increasing ionized calcium in treatment-adherent subjects. These findings may explain why cholecalciferol and ergocalciferol supplementation result in similar magnitudes of PTH reduction, but implicate potential differences in other vitamin D metabolites, such as $24,25(OH)_2D$, that could explain their different effects on ionized calcium.

Introduction

Low 25-hydroxyvitamin D (25OHD) concentrations are associated with hip fracture [1]. We and others have shown that oral supplementation with cholecalciferol results in a greater increase in total serum 25OHD concentration than an equivalent dose of ergocalciferol [2–6]. However, despite this differential effect on total 25OHD, we

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http://dx.doi.org/10.1016/j.bone.2013.06.012

and others found no significant difference between the calciferol formulations in their effect in lowering parathyroid hormone (PTH) [2,7].

Since 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) also regulates PTH, the discordant effects of cholecalciferol and ergocalciferol therapy on total 25OHD and PTH could be explained by differences in the catalytic rate of 1-α-hydroxylation by CYP27B1 of the respective metabolites, or differences in substrate inhibition [8]. Most circulating 25OHD and $1,25(OH)_2D$ is bound with high affinity to vitamin D-binding protein (DBP), and only a small fraction remains unbound [9]. It is therefore possible that calciferol supplementation might have different effects on free or bioactive serum 25OHD and $1,25(OH)_2D$ compared with total concentrations. Accordingly, if only the free or bioavailable fraction is available for cellular processing, the free or bioavailable vitamin D metabolite concentrations rather than their total serum concentrations could determine the biological effect. Indirect animal experimental data [10] and observational data from human studies [11–13] suggest that free concentrations of 25OHD and/or $1,25(OH)_2D$ may be more physiologically important in calcium homeostasis and provide a more useful index of vitamin D bioactivity than total serum concentrations.

The aim of the present study was to examine the effects of cholecalciferol and ergocalciferol supplementation on total serum $1,25(OH)_2D$, calculated free 25OHD and calculated free $1,25(OH)_2D$ concentrations in a group of vitamin D-deficient hip fracture patients.

Material and methods

Study design

Details of the study's experimental design and participants have been published previously [2]. We recruited vitamin D-deficient (serum 25OHD <50 nmol/L) hip fracture patients from two teaching hospitals in the metropolitan area of Perth, Western Australia over a two-year period. Eligible subjects were randomized, double-blind, to treatment with either cholecalciferol 1000 IU/day (Vigantoletten, Merck, Germany) or ergocalciferol 1000 IU/day (Ostelin, Boots, Australia) for three months. All participants were also given placebos matching the alternative treatment to maintain blinding of treatment allocation. The study was approved by the Royal Perth Hospital Ethics Committee, and all patients gave written informed consent to participate.

Biochemical measurements

Serum samples collected at baseline and at the end of treatment were stored at −70 °C for measurement of 25OHD, $1,25(OH)_2D$ and DBP. We measured 25OHD using high-performance liquid chromatography (HPLC) (inter-assay coefficient of variation (CV) < 12%) [14]. Comparison of this HPLC method with LC–MSMS yielded good interassay agreement with regression equations of $y = 0.95x + 1.94$ for 25-hydroxycholecalciferol and $y = 1.04x + 0.52$ for 25-hydroxyergocalciferol. This HPLC method was monitored using both internal and external quality assurance, the latter involving enrolment in an international quality assurance program (DEQAS). We measured $1,25(OH)_2D$ by DiaSorin radioimmunoassay following C18 column extraction (inter-assay CV 11%) [15]. DBP was measured by immunonephelometry (Dade Behring) (inter-assay CV < 6%), and albumin was measured by dye binding reaction to bromocresol green (inter-assay CV < 5%). We measured ionized calcium by direct ion-selective electrode analysis on the Bayer 800 analyzer (interassay CV < 2%). We calculated free and bioavailable (free plus albumin-bound) concentrations of 25OHD and $1,25(OH)_2D$ using DBP and albumin concentrations, according to published equations [13]. We also calculated free indices of 25OHD and $1,25(OH)_2D$ as the ratios between the molar concentrations of the vitamin D metabolites and DBP [12,16].

Statistical analyses

Skewed data were logarithmically transformed for parametric analysis. Baseline values of the treatment groups were compared using Student's t-tests. Within-group treatment effects were assessed using paired t tests. General linear modeling was used to compare treatment effects on post-intervention values, adjusted for baseline. P values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS 14.0 (Chicago, IL, USA). An estimated sample size of at least 37 subjects per treatment group was required to detect a 50% between-group difference in treatment effect at $\alpha = 0.05$ with 80% power. Treatment adherence was assessed by tablet/capsule count and questionnaire at the end of the study, and the main data analyses were repeated for the subgroup of patients who took ≥80% of their study treatment.

Results

Of the 95 patients (mean age 83 ± 8 years) randomized to treatment with cholecalciferol (n = 47) and ergocalciferol (n = 48), five patients withdrew their consent, 10 died prior to study completion, and 70 (74%) completed the study with paired samples for analysis (cholecalciferol: n = 36, ergocalciferol: n = 34). Of these, 37 (53%) had satisfactory treatment adherence, defined as ≥80% consumption of study treatment (cholecalciferol: n = 17, ergocalciferol: n = 20).

At randomization, total, free and bioavailable 25OHD and $1,25(OH)_2D$, ionized calcium, DBP and albumin concentrations were not significantly different between the treatment groups ($p > 0.05$) (Table 1). The effects of the calciferol treatments are summarized in Table 2. Although cholecalciferol supplementation was associated with a greater increase in total 25OHD compared with ergocalciferol (cholecalciferol: +88%, ergocalciferol: +57%, $p = 0.01$ between groups), total serum $1,25(OH)_2D$ concentrations did not change significantly in either group ($p > 0.05$, post-treatment vs baseline). Both calciferol formulations were associated with comparable increases in DBP (cholecalciferol: +18%, ergocalciferol: +16%, $p = 0.32$ between groups) and albumin (cholecalciferol: +31%, ergocalciferol: +21%, $p = 0.29$ between groups), as well as comparable decreases in calculated free $1,25(OH)_2D$ (cholecalciferol: −17%, ergocalciferol: −19%, $p = 0.32$ between groups). While there was a trend towards a greater increase in calculated free 25OHD with cholecalciferol therapy (cholecalciferol: +46%, ergocalciferol: +36%, $p = 0.08$ between groups), this did not meet statistical significance, and the trend was not distinct in the treatment-adherent subgroup analysis

Table 1
Baseline total, calculated free and bioavailable 25OHD and $1,25(OH)_2D$, vitamin D binding protein, albumin and ionized calcium concentrations at randomization.

	Cholecalciferol (n = 47)	Ergocalciferol (n = 48)	p value
Total 25OHD (nmol/L)	38 (33–44)	34 (31–38)	0.26
Total $1,25(OH)_2D$ (pmol/L)	80 (69–93)	71 (58–86)	0.31
Vitamin D binding protein (umol/L)	4.4 (4.2–4.7)	4.3 (4.0–4.6)	0.52
Serum albumin (g/L)	32 ± 4	32 ± 5	0.95
Free 25OHD (pmol/L)	11 (10–13)	11 (10–12)	0.43
Bioavailable 25OHD (nmol/L)	3.3 (2.8–3.8)	3.1(2.8–3.4)	0.46
Free 25OHD index ($\times 10^{-3}$)	8.6 (7.5–9.9)	8.1 (7.4–9.0)	0.48
Free $1,25(OH)_2D$ (pmol/L)	0.40 (0.34–0.47)	0.36 (0.30–0.44)	0.46
Bioavailable $1,25(OH)_2D$ (pmol/L)	10.9 (9.2–12.8)	9.9 (8.1–12.0)	0.45
Free $1,25(OH)_2D$ index ($\times 10^{-6}$)	18.2 (15.5–21.2)	16.5 (13.7–19.9)	0.45
Ionized calcium (mmol/L)	1.15 ± 0.06	1.14 ± 0.06	0.33

Data are geometric mean (95% CI) and mean ± SD.