Safety of vitamin D₃ in adults with multiple sclerosis¹–³

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ABSTRACT

Background: Vitamin D₃ may have therapeutic potential in several diseases, including multiple sclerosis. High doses of vitamin D₃ may be required for therapeutic efficacy, and yet tolerability—in the present context, defined as the serum concentration of 25-hydroxyvitamin D [25(OH)D] that does not cause hypercalcemia—remains poorly characterized.

Objective: The objective of the study was to characterize the calcemic response to specific serum 25(OH)D concentrations.

Design: In a 28-wk protocol, 12 patients in an active phase of multiple sclerosis were given 1200 mg elemental Ca/d along with progressively increasing doses of vitamin D₃: from 700 to 7000 μg/wk (from 28 000 to 280 000 IU/wk).

Results: Mean (± SD) serum concentrations of 25(OH)D initially were 78 ± 35 nmol/L and rose to 386 ± 157 nmol/L (P < 0.001). Serum calcium concentrations and the urinary ratio of calcium to creatinine neither increased in mean values nor exceeded reference values for any participant (2.1–2.6 mmol/L and <1.0, respectively). Liver enzymes, serum creatine, electrolytes, serum protein, and parathyroid hormone did not change according to Bonferroni repeated-measures statistics, although parathyroid hormone did decline significantly according to the paired t test. Disease progression and activity were not affected, but the number of gadolinium-enhancing lesions per patient (assessed with a nuclear magnetic brain scan) decreased from the initial mean of 1.75 to the end-of-study mean of 0.83 (P = 0.03).

Conclusions: Patients’ serum 25(OH)D concentrations reached twice the top of the physiologic range without eliciting hypercalcemia or hypercalciuria. The data support the feasibility of pharmacologic doses of vitamin D₃ for clinical research, and they provide objective evidence that vitamin D₃ intake beyond the current upper limit is safe by a large margin. Am J Clin Nutr 2007;86:645–51.

KEY WORDS Vitamin D, safety, 25-hydroxyvitamin D, 25(OH)D, multiple sclerosis

INTRODUCTION

The safety of vitamin D remains contentious, especially in the United Kingdom, where the guidance level (the publicly stated safe limit) is exceptionally conservative at 25 μg/d (1000 IU/d) (1, 2). In Canada and the United States, the upper limit of intake (UL) for vitamin D is 50 μg/d (2000 IU/d) (3). These values were obtained by determining an intake that functioned as the no-observable-adverse-effects-level (NOAEL) and then adjusting this level downward by dividing the NOAEL by an uncertainty factor (UF) (4). Evidence from studies conducted since the establishment of the UL value suggests that it is much too low.

Intakes of 100 μg/d (4000 IU/d) (5) and 250 μg/d (10 000 IU/d) (6) have been shown to be safe. In fact, fracture prevention studies suggest that the desirable serum 25-hydroxyvitamin D [25(OH)D] concentration exceeds 75 nmol/L (7–9). To attain and sustain these concentrations throughout the year, many adults require vitamin D intakes of ≥20–25 μg/d (800–1000 IU/d) (10, 11).

There is much interest in the role of vitamin D₃ in many aspects of health and disease. The rationale for vitamin D₃ treatment in multiple sclerosis (MS) is that metabolites of vitamin D₃ function as paracrine immune modulators (12), decreasing the proliferation of proinflammatory T lymphocytes and decreasing the production of cytokines, both of which contribute to the pathogenesis of MS (13–15). In experimental allergic encephalomyelitis (EAE), the mouse model of MS, treatment with 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] (the active form of vitamin D) prevented EAE in asymptomatic mice and lessened the severity of disease in mice with active EAE (16–18). In patients with congestive heart failure, vitamin D₃ treatment (50 μg/d or 2000 IU/d) affected cytokine profiles (19) in a way that would be desirable for patients with MS. The seasonal fluctuation in the number of gadolinium-enhancing lesions determined by magnetic resonance imaging (MRI) tend to be fewest at the times when serum 25(OH)D concentrations are highest (20, 21). Taken together, the data suggest that vitamin D₃ may play a role in the regulation of clinical disease activity.

The therapeutic use of pharmacologic doses of vitamin D₃ for MS or for any other disease requires tolerability studies, but they remain lacking (2, 22–24). To this end, we conducted a phase I trial to characterize the tolerability of the serum 25(OH)D concentrations achieved through administration of pharmacologic doses of vitamin D₃ to patients with MS.

The primary purpose of this study was to show the tolerability of high serum concentrations of 25(OH)D for future efficacy studies of vitamin D₃ treatment in MS. The known toxicity of vitamin D relates solely to calcium metabolism. As a group,
patients with MS do not have a primary abnormality in bone and mineral homeostasis.

SUBJECTS AND METHODS

Subjects

Between December 2003 and January 2005, we enrolled 12 patients with clinically definite relapsing remitting (RR) MS or secondary progressive (SP) MS as determined by the criteria of McDonald et al (25). All subjects were patients at the MS Clinic at St Michael’s Hospital (Toronto, Canada). Inclusion criteria included Expanded Disability Status Scale (EDSS) scores of 0 to 7 and ≥1 gadolinium-enhancing lesion found by MRI of the brain. Subjects were allowed to continue using 1 of the 4 MS disease-modifying drugs (Avonex; Biogen Idec, Cambridge, MA; Rebif; Serono, Rockland, MA; Betaseron; Berlex, Montville, NJ; or Copaxone; Teva, North Wales, PA) if already receiving this therapy. Exclusion criteria included a history of renal stones or dysfunction, cardiac disease, and comorbid granulomatous disease (including sarcoidosis, tuberculosis, silicosis, chronic or active fungal infections, or lymphoma).

Written informed consent was obtained from each subject. The St Michael’s Hospital Research Ethics Board approved this study.

Outcome measures

Once screened for the presence on MRI of a gadolinium-enhancing lesion, patients with positive scans underwent baseline neurologic examination with EDSS scoring (a measure of disability in MS in which 8 functional systems are scored) and ambulation index [(AI) a 25-foot timed walk and measure of disability in MS] scoring. We screened 24 subjects to identify 12 patients with active disease. These tests, including MRI, were repeated at trial completion.

Serum biochemical analysis was conducted at screening and at each study visit for the following: calcium, 25(OH)D, parathyroid hormone (PTH), and renal function (creatinine). As an adjunct to safety testing, we periodically measured electrolytes and liver function enzymes [ie, amylase, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP)]. For every urine test, one random urine sample was obtained on the Synchron LX-20 analyzer (Beckman, Fullerton, CA) was used to measure serum 25(OH)D concentrations. Other serum and urine biochemical analysis were measured on a diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA) were used for the measurement of the molar extinction coefficient of 18 300 AU/cm path length. Tricalcium phosphate powder (Rhodia, Cranbury, NJ) was provided for subjects to mix with water. We used SPSS software (version 12.0; SPSS Inc, Chicago, IL) for statistical analysis and graphic presentation of results. Descriptive statistics, paired t testing, and Wilcoxon’s sign-ranked comparisons were used to analyze the results. For repeated measurements [eg, serum 25(OH)D at baseline compared with that at visits 1–7], paired t testing was used in conjunction with Holm’s...
adjusted Bonferroni for significance testing. Mean ± SD values are given.

RESULTS

The demographic characteristics of the patients in this study at baseline are shown in Table 2. Baseline and end-of-study mean serum 25(OH)D, calcium, PTH, and creatinine concentrations and urinary Ca:Cr are shown in Table 3. Paired *t* testing at every follow-up time point indicated no significant change in serum calcium concentrations from pretreatment values during the 28-wk protocol of escalating doses of vitamin D₃. None of the patients developed hypercalcemia. Serum calcium concentrations remained within the reference range (2.1–2.6 mmol/L). Likewise there was no significant change in urinary Ca:Cr. The urinary Ca:Cr did not exceed 1.0 for any participant over the course of the dose-escalation schedule (Figure 1). In one subject, the urinary Ca:Cr reached 1.0 on 2 separate occasions, at baseline and again at the final visit. In both instances, the patient was brought back a week later to repeat the urinary Ca:Cr measurement; both times, the high ratio had resolved. Serum 25(OH)D concentrations were significantly increased from the mean baseline values of 78.2 ± 35.3 nmol/L to 385.5 ± 157.0 nmol/L at trial completion (*P* < 0.001) (Figure 2). PTH concentrations at trial completion were lower than those at baseline (Figure 3), but the difference was not statistically significant according to the statistical method appropriate for post hoc comparisons among repeated measures. As should be expected, baseline and final PTH values were significantly different according to the paired *t* test (*P* = 0.02). Serum creatinine concentrations, a reflection of kidney function, remained stable and within the reference ranges throughout the trial in all participants.

Serum protein, electrolytes, urea, and liver function enzymes were also measured (see Table S1 under “Supplemental data” in the current online issue at www.ajcn.org). All remained within clinical reference ranges, and there were no significant differences by paired *t* test between baseline values and values after the last dose of vitamin D₃ (7000 μg/wk or 280 000 IU/wk).

No adverse clinical effects were seen in any patient throughout the 28-wk trial. MRI studies of the brain with and without gadolinium contrast were obtained for each participant at the beginning and end of the study to ensure that the pharmacologic doses of vitamin D₃ together with calcium did not result in measurable changes consistent with a worsening of disease activity in the form of new or enlarged gadolinium-enhancing lesions (See Table S2 under “Supplementary data” in the current online issue at www.ajcn.org). At baseline, each MRI scan showed ≥1 gadolinium-enhancing lesion as an inclusion criterion (median: 1; range: 1–6). The median number of gadolinium-enhancing MRI lesions, new and enlarged, remained unchanged after 28 wk of therapy (median: 1; range: 0–2). In 4 patients with gadolinium-enhancing lesions at baseline, these had resolved entirely by study completion. The remaining 8 patients had gadolinium-enhancing lesions on MRI, but the number of lesions per patient had declined. The mean number of gadolinium-enhancing lesions in the 12 study subjects was significantly lower at trial completion (0.83 ± 0.72) than at baseline (1.75 ± 1.42) (*P* = 0.03, Wilcoxon’s signed-ranks test).

Relapse activity was monitored for the 12 mo before enrollment, during the 28-wk trial period, and for up to 4 mo after study completion (see Table S2 under “Supplemental data” in the current online issue at www.ajcn.org). Eight patients experienced a total of 11 relapses in the year before the beginning of the trial. Five participants experienced a total of 9 relapses during the 28-wk study period; 4 of those 9 relapses occurred in 1 patient. Relapses were treated as deemed appropriate by the neurologist. In one case, pulse steroid therapy was used. Seven participants did not experience any relapse events during the study period or follow-up (total, 10 mo). There was no statistically significant difference between annualized relapse rates at baseline and completion of the study.

Disease progression was monitored through measurements of the EDSS and AI at baseline and study completion (see Table S2 under “Supplemental data” in the current online issue at www.ajcn.org).

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Demographic characteristics of subjects at study enrollment ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (<em>n</em> = 5)</td>
<td>Female (<em>n</em> = 7)</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>38.0 ± 6.0 ²</td>
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<tr>
<td>Mean EDSS</td>
<td>4.3 ± 2.7</td>
</tr>
<tr>
<td>RRMS (<em>n</em>)</td>
<td>4</td>
</tr>
<tr>
<td>SPMS (<em>n</em>)</td>
<td>1</td>
</tr>
<tr>
<td>DMD (<em>n</em>)</td>
<td>2</td>
</tr>
</tbody>
</table>

¹ EDSS, expanded disability status scale; RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; DMD, disease-modifying drug.

² x ± SD (all such values).

<p>| TABLE 3 | Effect of the full protocol of vitamin D₃ supplementation on biochemical measures: comparison of baseline values and values after a 28-wk vitamin D₃ treatment (100–1000 μg/d) ² |
| --- | --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Serum 25(OH)D</th>
<th>Serum calcium</th>
<th>Urinary calcium: creatinine</th>
<th>PTH</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>nmol/L</td>
<td>nmol/L</td>
<td>pmol/L</td>
<td>pmol/L</td>
<td>μmol/L</td>
</tr>
<tr>
<td>Baseline</td>
<td>12</td>
<td>78.2 ± 35.3 ²</td>
<td>2.36 ± 0.09 ²</td>
<td>0.42 ± 0.31 ²</td>
<td>2.75 ± 1.54 ²</td>
</tr>
<tr>
<td>Trial completion</td>
<td>12</td>
<td>385.5 ± 157.0 ⁰</td>
<td>2.23 ± 0.43 ³</td>
<td>0.47 ± 0.28 ³</td>
<td>1.81 ± 1.15 ³</td>
</tr>
<tr>
<td>Reference values ³</td>
<td>&lt;250</td>
<td>2.1–2.6</td>
<td>&lt;1.0</td>
<td>1.3–5.4</td>
<td>50–110</td>
</tr>
</tbody>
</table>

¹ 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone. Values in a column with different superscript letters are significantly different, *P* < 0.001 (paired *t* test with Holm’s adjusted Bonferroni correction).

² x ± SD (all such values).

³ The normal distribution as obtained in the clinical laboratory at St Michael’s Hospital (Toronto, Canada).
EDSS remained unchanged for 4 patients. There were no significant changes in either EDSS or AI.

**DISCUSSION**

The present protocol was designed to test the tolerability of the specific 25(OH)D concentrations attained—and not the long-term effects of the vitamin D₃ doses used. Even though vitamin D₃ per se is cleared from the circulation within 2 or 3 d, its effect on serum 25(OH)D concentration exhibits a half-life that is on the order of 2 mo, which makes the complete attainment of a plateau in the 25(OH)D concentration impractical for a study of this nature (29, 30).

Concerns about the safety of vitamin D and calcium have been raised recently, because the Women's Health Initiative study showed a 17% greater hazard ratio for kidney stones in women randomly assigned to receive calcium and vitamin D than in those receiving placebo (31). The dose of vitamin D used for that trial was 10 µg/d (400 IU/d), which was too small to produce a convincing change in serum 25(OH)D. Furthermore, the mean background intake of calcium in that study was 1100 mg/d, onto which the intervention added 1200 mg Ca/d. In the Women’s Health Initiative study, the increase in risk of kidney stones was attributable to calcium intakes near the UL of 2500 mg/d, and not to the vitamin D. In fact, across more modest calcium intakes, calcium and vitamin D are associated with a lower risk of kidney stone formation (32).

The circulating 25(OH)D concentrations that have convincingly manifested toxicity as hypercalcemia and increased urinary calcium exceed 700 nmol/L (33–35). For the patients in the present study who have MS and no underlying disorder of bone and mineral metabolism, serum 25(OH)D concentrations averaged 386 nmol/L, and there was no detrimental sign of calcium metabolism. Although it is well known that vitamin D increases...
supplementation tended to lower the serum 1,25(OH)_{2}D concentration. We did not measure 1,25(OH)_{2}D in the present study because the focus was on parameters of clinical tolerability.

As a Phase I trial, this study was not powered to detect changes in clinical outcomes in the patients. The evidence for tolerability to high intakes of vitamin D_{3} is not relevant to MS patients only. Clinical testing of the subjects in the present study found no statistically significant increase in annualized relapse rate, disability score, or ambulatory index. We also established that high intakes of vitamin D_{3} do not lead to an increase in gadolinium-enhancing lesions on MRI brain studies. In fact, the overall number of lesions per patient decreased significantly from baseline to the end of the trial. In the context of MS, the requirement of an active lesion at baseline for inclusion in this study could in part explain the reduction in the number of gadolinium-enhancing lesions on MRI and the lack of worsening of the relapse rate (not an inclusion criterion). Regression to the mean, because of the inclusion criteria, could account for the desirable clinical change during the study. EDSS scores would not naturally change over the course of a short-term study such as this; noise is the best explanation for all of the changes (either worsening or improvement) in EDSS that were seen.

Our rationale for studying higher doses of vitamin D_{3} is to improve the paracrine production of 1,25(OH)_{2}D. The mechanisms by which 25(OH)D could affect brain and immune function have been shown in laboratory studies. 25-hydroxyvitamin D-1-α-hydroxylase has been found in the cerebrospinal fluid (42). The activity of extrarenal 1-α-hydroxylase follows first-order reaction kinetics in vivo, so that a greater supply of substrate should increase the production of 1,25(OH)_{2}D (43). Vitamin D receptor (VDR) has been found in the central nervous system (44, 45). 1,25(OH)_{2}D stimulates the production of neurotrophins (46) and suppresses neurotoxicity (47). Therefore, an adequate supply of substrate for paracrine production and the use of 1,25(OH)_{2}D in the central nervous system may improve immune regulation in an autoimmune disease such as MS.

The present findings should facilitate other investigations with higher doses of vitamin D_{3}. Furthermore, the present data justify a revision of the UL, or the guidance level for vitamin D (2). There is no evidence that adults with MS are different from healthy adults with respect to their tolerance of vitamin D; their disease is due to an inflammatory response. Serum 25(OH)D concentrations represent the combined contributions of cutaneous synthesis and oral ingestion of dietary sources of vitamin D. Within 15 min of full-body exposure at midday during the summer, white adults can produce vitamin D equivalent to an intake of 250 μg (10 000 IU) (30). A recent publication provided no evidence of toxicity resulting from such intakes (48), nor has a serum 25(OH)D concentration that is toxic been determined, although it is believed to be in excess of 250 nmol/L. It is therefore reasonable to expect that oral intakes of vitamin D_{3} that produce serum concentrations of 25(OH)D such as those achieved with sun exposure will not cause hypercalcemia or hypercalcuria. That is what we have shown in this small population of patients whose 25(OH)D concentration at baseline already was relatively high. Our objective was not to determine the long-term safety of the vitamin D_{3} doses per se but to assess the safety of the resulting serum 25(OH)D concentrations. Longer treatment with 7000 μg/wk (280 000 IU/wk) will produce higher concentrations of 25(OH)D than were observed here. If we apply the estimate of Heaney et al (6) that a 1-μg/d increase in intake
raises 25(OH)D by 0.7 nmol/L, then the average final increase in 25(OH)D observed here—ie, 308 nmol/L—represents a plateau of 25(OH)D concentration equivalent to that resulting from the long-term intake of 440 g (1000 IU)/d. In summary, we have shown that serum concentrations of 25(OH)D in the range of 400 nmol/L can be attained without causing hypercalcemia or hypercalcuria, and they do not cause adverse clinical or paraclinical effects. These findings are encouraging for larger-scale clinical trials in MS and in other medical conditions that may respond to vitamin D. The widespread use of vitamin D supplements [25 μg (1000 IU)/d] has been advised as a simple way to improve many aspects of public health (7, 10). Because the guidance level for vitamin D in the United Kingdom remains at 25 μg/d (1000 IU/d), the British public may not be able to benefit from that advice. The present study provides an objective confirmation that the recent proposal by Hathcock et al is appropriate—ie, a UL of 250 μg/d (10 000 IU/d) for vitamin D intake can be justified (48).

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