Pharmacokinetics of doxercalciferol, a new vitamin D analogue that lowers parathyroid hormone

Robert A. Upton¹, Joyce C. Knutson², Charles W. Bishop² and Leon W. LeVan²

¹Department of Biopharmaceutical Sciences, University of California, San Francisco, CA and ²Bone Care International, Inc., Middleton, WI, USA

Abstract

**Background.** This is the first detailed pharmacokinetic report published on the administration of doxercalciferol \([1\alpha\text{OH}]\text{D}_2\) recently introduced to treat secondary hyperparathyroidism.

**Methods.** \([1\alpha\text{OH}]\text{D}_2\) was administered in a range of single and multiple doses to volunteers with and without normal renal and/or hepatic function. Subsequent serial blood samples were assayed by HPLC/radioimmunoassay for the metabolite \([1,25\text{(OH)}_2\text{D}_2]\), the major active species.

**Results.** Bioavailability of \([1,25\text{(OH)}_2\text{D}_2]\) from a single 5 mg \([1\alpha\text{OH}]\text{D}_2\) oral-capsule dose was estimated to be normally ~42% of that from a 5 mg intravenous injection. Steady-state serum concentrations of \([1,25\text{(OH)}_2\text{D}_2]\) were attainable within 8 day, and fluctuated ~2.5-fold from peak to trough when oral \([1\alpha\text{OH}]\text{D}_2\) doses were taken every second day, and the terminal half-life was 34 ± 14 h. Mean steady-state serum concentrations rose less than proportionally (from 20 to 45 pg ml⁻¹) on increasing oral \([1\alpha\text{OH}]\text{D}_2\) doses from 5 to 15 mg every 48 h. Renal patients showed 39 ± 37% increase in serum \([1,25\text{(OH)}_2\text{D}_2]\) concentration during 3–4 h haemodialysis sessions, but no other difference in steady-state pharmacokinetics was found between these or hepatically impaired patients and normal subjects.

**Conclusions.** Given the sensitivity limits of current assays, the pharmacokinetics of this and other vitamin-D compounds is best elucidated from steady-state studies. The pharmacokinetics of \([1,25\text{(OH)}_2\text{D}_2]\), \([1\alpha\text{OH}]\text{D}_2\) and \([1\alpha\text{OH}]\text{D}_3\) appear to be similar to that of \([1,25\text{(OH)}_2\text{D}_3]\) with a few exceptions. Deviation of \([1,25\text{(OH)}_2\text{D}_2]\) pharmacokinetics from linearity appears to be marginal enough to be clinically manageable with adequate precaution.

**Keywords:** doxercalciferol; hepatic disease; pharmacokinetics; renal disease; steady state; vitamin D₂

Introduction

Both oral and intravenous (i.v.) formulations of doxercalciferol \([1\alpha\text{hydroxyvitamin D}_2\) or \([1\alpha\text{OH}]\text{D}_3\) have recently been approved by the US Food and Drug Administration for the reduction of intact parathyroid hormone (iPTH) in haemodialysis patients suffering from secondary hyperparathyroidism. Renal disease prevents 1-hydroxylation of vitamin D in kidney mitochondria, which, together with 25-hydroxylation in the liver, is essential to activate vitamins D₃ and D₂ for their normal roles in suppression of iPTH synthesis and promotion of intestinal absorption of dietary calcium, renal tubular reabsorption of calcium and skeletal remodelling [1]. Thus, to control hyperparathyroidism, renal patients have been treated with calcitriol \([1,25\text{(OH)}_2\text{D}_3]\), but, whether calcitriol dosing has been i.v. or oral, the desired iPTH lowering has been associated with occasional hypercalcaemia and hyperphosphataemia [2].

Alphacalcidol \([1\alpha\text{hydroxyvitamin D}_3\) or \([1\alpha\text{OH}]\text{D}_3]\) has also been used for secondary hyperparathyroidism and for osteoporosis, primarily in Europe and Japan. Because alphacalcidol is hydroxylated at carbon 25 only upon reaching the liver [3], it was hoped that oral doses would avoid dihydroxylation of vitamin D₃ before its own absorption, exerting a direct and local effect that promotes calcium absorption from the gastrointestinal tract [4]. However, the extent to which treatment with alphacalcidol, either by oral or i.v. dosage, does avoid the hypercalcaemia associated with calcitriol is still the subject of scientific debate [5,6].
Based on similar rationale, doxercalciferol, the D₂ analogue of alphacalcidol, was also developed to effectively reduce iPTH without causing frequent hypercalcaemia or hyperphosphataemia [7] and it is now in wide clinical use to treat secondary hyperparathyroidism. To date, however, little detailed pharmacokinetic information has been available on doxercalciferol and clinically relevant pharmacokinetics is not straightforward to predict, given that the active species is not doxercalciferol per se, but its metabolite, 1,25-dihydroxyvitamin D₂ [1,25(OH)₂D₂]. In order to optimize use of doxercalciferol, therefore, it is important to characterize its pharmacokinetics, not only in normal subjects but also in the populations to be treated (e.g. renal disease and osteopenia patients) and, further, in hepatically impaired patients, in whom both the formation and elimination of 1,25(OH)₂D₂ might be compromised.

This report covers four studies, each describing the pharmacokinetics of 1,25(OH)₂D₂ arising from doses of the prodrug 1α(OH)D₂. Study A was designed to investigate the bioavailability and pharmacokinetics of 1,25(OH)₂D₂ from single oral and i.v. doses of 1α(OH)D₂ to post-menopausal women with osteopenia and the potential dose-dependence of such pharmacokinetics. Using multiple-dose oral regimens, study B investigated steady-state pharmacokinetics and its potential dose dependence in normal young men and women, study C the impact of renal disease and haemodialysis and study D the impact of varying degrees of hepatic disease.

Subjects and methods

All four studies were approved by an Institutional Review Board in compliance with US law and all subjects gave informed consent. All studies were open label and all non-placebo doses were of the precursor drug, 1α(OH)D₂. No subject participated in more than one study. The 1α(OH)D₂ doses employed in the studies reflected usual clinical regimens that, at the time of commencing study A, were to start patients on 5 μg/day, but which, by the time the other studies were performed, had been refined to starting patients on 10 μg three times per week. The i.v. doses were as 0.5 ml of a 10 μg/ml sterile solution in ethanol and oral doses comprised a number of soft-gelatin capsules each containing 1 μg (study A) or 2.5 μg (studies B–D) of 1α(OH)D₂ and also fractionated coconut oil, gelatin, glycerine, titanium dioxide, FD&C red no. 40 or D&C yellow no. 10, ethanol (2.5 μg capsules) and butylated hydroxyanisole. Plasma samples were assayed in study A, serum samples in studies B–D.

Study A

An oral placebo dose in 22 osteopenic women with normal kidney and liver function was followed by a three-treatment [5 μg i.v. and 2 and 5 μg oral doses of 1α(OH)D₂] crossover design, with 1 week intervening between each of the four doses. The six possible sequences of the three drug treatments were each randomly assigned to four patients but two patients withdrew for personal reasons (one on the 5 μg oral first and i.v. last, one on the 5 μg oral first and 2 μg oral last).

Study B

This was a two-treatment crossover study in which, for each treatment [5 or 15 μg oral 1α(OH)D₂ doses], 24 normal subjects (12 per gender) each received a dose every 48 h for 5 doses. The two treatments were separated by at least 14 days, with six women and six men chosen randomly to receive one of the treatments first and the remaining subjects the other first. All subjects were in normal health according to physical examination and standard blood screens. The women (four African-American, eight Caucasian) were 20–35 years old and 39–109 kg; the men (three African-American, nine Caucasian) were also 20–35 years, but 59–118 kg. Doses were given ~1 h or less before breakfast. For each regimen, blood samples were taken on three occasions at least 24 h apart during the week preceding the first dose, just before each of the remaining doses and 2, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96 and 120 h after the final dose.

Study C

Fourteen haemodialysis patients each received a 10 μg oral 1α(OH)D₂ dose every 48 h for five doses, but 48 h following the 5th dose, after three patients had developed hypercalcaeemia and were withdrawn, the dose for the remaining 11 patients was lowered to 5 μg every 48 h for a further five doses. These patients (four women, 45–73 years, 52–83 kg; seven men, 29–69 years, 61–82 kg; all Caucasian other than one Hispanic woman and one African-American man) had a serum creatinine concentration of >6 mg/dl (9.4 ± 3.0 mg/dl) and had been on haemodialysis three times per week for >1 month. During the study, dialysis was at two 2 day and one 3 day intervals per week. Doses were given immediately after dialysis and at the same time of day on dosing days without dialysis. Each regimen was commenced such that its last dose was taken after a dialysis preceded by the two 2 day interdialysis intervals. Patients underwent 1 week free of vitamin-D supplementation prior to receiving the first study dose. Blood samples were taken immediately before that dose and before and after dialysis on the 8th day of dosing (the first dialysis after a 3 day interdialysis interval), just before the 8th, 9th and 10th (final) doses and 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168 and 240 h after the 10th dose.

Study D

Thirteen patients with hepatic impairment and four normal subjects received a 10 μg oral 1α(OH)D₂ dose every 48 h for eight doses. The patients were subcategorized as having mild hepatic impairment if they scored 5–6 points, moderate impairment 7–9 points and severe impairment 10–15 points, by the Pugh modification of the Child–Turcotte hepatic impairment criterion [8]. Accordingly, there were three women (40–65 years, 64–82 kg) and five men (43–70 years, 67–100 kg) with mild hepatic disease, two men (49–60 years, 89–98 kg) with moderate disease and one woman (41 years, 64 kg) and two men (40–58 years, 89–105 kg) with severe
from an unweighted least-squares linear fit utilizing the last three or (if, in studies B–D, it gave a fit with a higher correlation coefficient squared) four points on the curve before they declined below double the baseline concentration in that subject. Concentrations were entered into the fit after subtracting that baseline concentration.

AUC (the area under the plasma or serum concentration–time curve) was calculated as AUC extrapolated from single doses and as AUC interdose from multiple doses. Firstly, AUC unextrapolated was estimated, from the time of the (last) dose to the time of the assayable sample taken closest to 48 h, using the trapezoidal and logarithmic-trapezoidal equations, respectively, for periods of increasing (or stationary) and decreasing concentrations. To obtain AUC extrapolated, AUC unextrapolated from single doses was extrapolated to infinite time by adding (C_{last} - C_{baseline})/t_{z} and to obtain AUC interdose, AUC extrapolated from multiple doses was adjusted for interdose intervals not exactly as scheduled by adding \([C_{sced48} - C_{actual48}] \ln(C_{sced48}/C_{actual48})\) where \(C_{sced48}\) is the concentration in the sample scheduled for 48 h but really collected at \(t_{actual48}\) and where \(C_{sced48} = (C_{baseline}) \times e^{-\frac{t_{z}}{2} \ln\left(\frac{C_{actual48}}{C_{baseline}}\right)}\). (Throughout studies B–D, this adjustment for inexact interdose intervals accounted for <1.3% of AUC.) The resultant area yielded AUC extrapolated and AUC interdose after it was corrected for innate 1,25(OH)_{2}D_{2} not dose-generated, by subtracting \(AUC_{(0-48)h}\) for the placebo dose in study A and the baseline concentration multiplied by 48 h in the other studies.

Bioavailability of test relative to reference treatment was estimated as \((AUC_{test}/AUC_{reference}) \times (Dose_{reference}/Dose_{test})\). Peak plasma or serum concentration was taken as the concentration in the sample with the highest measured concentration minus the baseline in that subject and peak time was the time of that sample. For studies B–D, the trough concentration and time of trough were estimated analogously from the sample with the least concentration within 48 h after the last dose of each treatment. Where, for some hepatically impaired subjects, the baseline concentration estimate appeared greater than the least observed concentration, the trough concentration was taken as zero. Peak-to-trough swing refers to the peak minus trough concentrations.

Statistical analysis

Data are shown as means ± SD. Comparisons were made by analysis of variance (ANOVA) based on the type III sums of squares using SAS software (SAS Institute, Cary, NC, USA). All parameters were corrected for dose size (and, in study B, for the subject’s body weight) before logarithmic (natural) transformation and then analysis, except that peak and trough times were analysed uncorrected and untransformed. For studies A and B, ANOVA analysed for treatment and period as within-subject factors and sequence and (in study B) gender as between-subjects factors. For studies C and D, one-factor (subject group) ANOVAs were performed, with, for study D, a test for linear trend according to severity of disease—from normal, to mildly impaired, to moderately, to severely. Additionally, for studies B–D, pre-dose concentrations and the concentration 48 h after the last dose were all compared by ANOVA analysing for subject and day-of-treatment as factors and for linear trend in the days of trough. Multi-sample ANOVAs, if found significant, were followed by a Tukey pairwise-comparison procedure. For studies A and B, bioequivalence of treatments was also investigated by

Pharmacokinetic analysis

Each subject’s innate steady-state ‘baseline’ concentration of dose-unrelated 1,25(OH)_{2}D_{2} was estimated in study A as \(AUC_{(0-48)h}/48\) h from the placebo dose (AUC is described below). In studies C and D, the concentration in the serum sample taken before commencing the first regimen was used and in study B the mean of the three such concentrations. In study C, six patients had a baseline 1,25(OH)_{2}D_{2} below assay sensitivity, so their baseline concentration was taken as half the limit of assay sensitivity on the day of assay.

Half-life was estimated as \(\ln(2)/\lambda_{z}\) where \(\lambda_{z}\) the terminal rate-constant, was minus the terminal slope of the plasma or serum concentration (C) vs time (t) curve (semilogarithmic) after each treatment in each subject. This slope was estimated
the Schuirmann ‘two one-sided (z = 0.05) confidence intervals (CIs) test’ that yielded a 90% CI for the ratio, between test and standard treatments, of each parameter processed by ANOVA as its logarithmic transform [9].

Results

Study A

Baseline plasma concentrations were 4.27 ± 1.61 pg/ml. Per µg of dose given, the 5 µg i.v. 1α(OH)D2 treatment in study A generated a larger 1,25(OH)2D2 AUC unextrapolated than either the 2 µg oral or 5 µg oral doses (305 ± 152 vs 143 ± 73 and 108 ± 42 h × pg/ml per µg dose, respectively), a larger AUC extrapolated (661 ± 228 vs 554 ± 951 and 268 ± 213 h × pg/ml per µg dose) and a larger peak concentration (10.2 ± 6.3 vs 5.00 ± 2.65 and 3.48 ± 1.45 pg/ml per µg dose) (Figure 1, Table 1). Thus, bioavailability of 1,25(OH)2D2 from orally administered 1α(OH)D2 appears to have been less than half that from intravenously administered 1α(OH)D2 (41 ± 18% from 5-µg doses if based upon AUC unextrapolated, 43 ± 35% if based upon AUC extrapolated) and the absolute bioavailability even lower if conversion of 1α(OH)D2 to 1,25(OH)2D2 was less than complete after even i.v. doses.

Peak concentration (per µg dose) was also larger after the 2 µg than after the 5 µg oral dose, but no other significant differences between the two oral doses were found, nor any between any of the three treatments in their times of peak concentration. Nevertheless, bioequivalence could not be established according to the two one-sided (Schuirmann) CIs test, between the two oral treatments in respect of any of the parameters expressed per µg of dose given (i.e. for the 2 µg oral dose, none of these parameters was found, with 95% confidence, to be both >80% and <125% of that for the 5 µg oral dose). None of the parameters evaluated showed a significant period or sequence effect.

Study B

Baseline concentrations were 2.72 ± 1.18 pg/ml. No significant difference or linear trend with study day was distinguishable between trough serum concentrations 2 days prior to, immediately before and one normal interdose interval (48 h) after the last dose, indicating attainment of steady state. Figure 2 shows concentrations during an interdose interval at steady state and Figure 3 (logarithmic) concentrations for a full 120 h after the last dose. Half-life was estimated to be 34 ± 14 h.

Table 1. Pharmacokinetic parameters in plasma compared among single doses of 2 and 5 µg oral capsules and a 5 µg i.v. injection (study A; means ± SD, n = 22a)

<table>
<thead>
<tr>
<th></th>
<th>Oral 2 µg</th>
<th>Oral 5 µg</th>
<th>Intravenous 5 µg</th>
<th>Statistical P-value for a treatment effect (and pairwise contrasts)</th>
<th>90% CI for ratio 2 and 5 µg oral doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC unextrapolated</td>
<td>143 ± 73</td>
<td>108 ± 42</td>
<td>305 ± 152</td>
<td>0.0001</td>
<td>1.01–1.49</td>
</tr>
<tr>
<td>(h × pg/ml per µg dose)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC extrapolateda</td>
<td>554 ± 951</td>
<td>268 ± 213</td>
<td>661 ± 228</td>
<td>0.0001</td>
<td>1.04–2.11</td>
</tr>
<tr>
<td>(h × pg/ml per µg dose)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak concentration</td>
<td>5.00 ± 2.65</td>
<td>3.48 ± 1.45</td>
<td>10.2 ± 6.3</td>
<td>0.0001</td>
<td>1.14–1.65</td>
</tr>
<tr>
<td>(pg/ml per µg dose)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of peak concentration (h)</td>
<td>11.0 ± 4.4</td>
<td>11.1 ± 5.0</td>
<td>8.00 ± 5.89</td>
<td>0.0751</td>
<td></td>
</tr>
</tbody>
</table>

a n = 20 for AUC extrapolated from 2 µg oral and 5 µg i.v. doses, due to inability to extrapolate three of the AUCs because of negative terminal rate-constant estimates and after omission of AUC extrapolated for subject 12’s 5 µg i.v. dose (16 409 h × pg/ml per µg dose), which is >16-fold the next highest AUC for that treatment type. When subject 12’s AUC is not omitted, AUC extrapolated for 5 µg i.v. = 1411 ± 3444 h × pg/ml per µg dose (P = 0.0001) and the 90% CI for the ratio of AUC extrapolated from the 2 µg oral dose compared with that from the 5 µg oral dose is 0.98–2.23.
been averaged across subjects at each sampling time. Corrected concentrations, expressed per 48 h by normal young adults during Study B. Baseline-AUC (h$^2$ vs $\mu$g dose) was smaller AUC (145 $vs$ 191 h $\times$ pg/ml per $\mu$g dose) than did the 5 $\mu$g regimen (Table 2). Indeed, according to the two one-sided (Schuirmann) CIs test, each of these parameters was found, with 95% confidence, to be $>65\%$ (69%, 67% and 65%, respectively) of that during a 5 $\mu$g regimen, but also, with 95% confidence, to be $<92\%$ (92%, 92% and 91%) of that during the 5 $\mu$g regimen. Trough concentration was not found to be different between regimens, but estimation of baseline-corrected trough concentrations is less certain (since, even before baseline correction, trough concentrations did not always appear to be greatly above baseline, particularly during the 5 $\mu$g regimen) and, accordingly, the Schuirmann CI appeared wider (68–142%). No difference was found between regimens in the time of peak concentration either.

None of the parameters evaluated showed a significant period or sequence effect; nor was there any significant difference between end-of-washout concentrations from each treatment sequence and either those from the other sequence or baseline concentrations. Nor was any gender effect found, nor a significant interaction between period and gender nor between treatment and gender, although a significant interaction between sequence and gender was found for peak time ($P=0.023$)—for men the peak time was similar between those receiving the 5 $\mu$g dose and those the 15 $\mu$g dose first (11.8 $vs$ 11.1 $\pm$ 5.4 h), but the peak time for women was unexpectedly different between these two sequences (7.92 $\pm$ 3.3 $vs$ 15.4 $\pm$ 5.0 h).

**Study C**

Baseline concentrations were 2.18 $\pm$ 2.13 pg/ml. On day 8, post-dialysis concentrations were found to be greater than those pre-dialysis by 39 $\pm$ 37% ($P=0.003$), doubling in two of the patients (from 63.4 to 126 pg/ml and from 35.3 to 75.0 pg/ml), but none of the steady-state pharmacokinetic parameters for renal patients was found different from that for normal subjects on the same dose in study B (Table 3).

After 10 days of this 18 day study, when three patients with initially normal iPTH developed serum calcium $>11.2$ mg/dl and were withdrawn, the

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**Table 2.** Pharmacokinetic parameters in serum compared between regimens of 5 and 15 $\mu$g oral capsules given every 48 h (study B; means $\pm$ SD, $n=24^a$)

<table>
<thead>
<tr>
<th></th>
<th>5 $\mu$g every 48 h</th>
<th>15 $\mu$g every 48 h</th>
<th>Statistical $P$-value for a treatment effect</th>
<th>90% CI for ratio between 15 and 5 $\mu$g doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (h $\times$ pg/ml per $\mu$g dose)</td>
<td>191 $\pm$ 107</td>
<td>145 $\pm$ 75</td>
<td>0.012</td>
<td>0.69–0.92</td>
</tr>
<tr>
<td>Peak concentration (pg/ml per $\mu$g dose)</td>
<td>5.98 $\pm$ 3.53</td>
<td>4.47 $\pm$ 2.42</td>
<td>0.017</td>
<td>0.67–0.92</td>
</tr>
<tr>
<td>Time of peak concentration (h)</td>
<td>11.6 $\pm$ 5.7</td>
<td>11.5 $\pm$ 5.5</td>
<td>0.973</td>
<td></td>
</tr>
<tr>
<td>Trough concentration$^a$ (pg/ml per $\mu$g dose)</td>
<td>2.32 $\pm$ 1.80</td>
<td>1.76 $\pm$ 1.14</td>
<td>0.939</td>
<td>0.68–1.4</td>
</tr>
<tr>
<td>Peak-to-trough swing (pg/ml per $\mu$g dose)</td>
<td>3.77 $\pm$ 2.55</td>
<td>2.72 $\pm$ 1.53</td>
<td>0.013</td>
<td>0.65–0.91</td>
</tr>
</tbody>
</table>

$^a$n = 23 for the trough concentration for the 5 $\mu$g regimen, due to a negative estimate when subject 24’s trough concentration was corrected for baseline contribution.
48-hourly dose in the remaining subjects was halved from 10 to 5 μg, leaving only 8 days for attainment of steady state. Since it takes 3.3 half-lives to reach (90% of) steady state, only subjects with half-lives of ≤58 h (= 8 × 24/3.3 h) might be expected to have attained concentrations within 10% of steady state and half-life estimates in this study (61 ± 42 h) ranged from 23 to 102 h, except for one of 159 h. Yet, one-compartment kinetics predicts half-lives between 58 and 159 h would have given rise to concentrations only 4–12% higher than true steady state (patients with longer half-lives achieved a lower percentage of the 10 μg steady-state concentration, but were then slower to come down to the 5 μg steady state). Indeed, no significant difference was found between the trough 2 days before and that immediately before the last dose, indicating attainment of steady state by that time and, as seen in Table 3, very similar pharmacokinetic estimates were obtained, whether from all 11 patients or from only the seven renal patients with half-lives of ≤58 h (AUC: 1232 ± 731 vs 1165 ± 629 h × pg/ml). Moreover, for the seven subjects with half-lives <58 h, just as for all 11 subjects, none of these estimates was found significantly different from that for normal subjects (all with half-lives <58 h).

**Study D**

Baseline concentrations were, respectively, 6.70 ± 3.02, 8.15 ± 2.19, 5.77 ± 3.61 and 7.33 ± 2.14 pg/ml in the patients with mild, moderate, severe and no hepatic impairment. No significant difference or linear trend with study day was distinguishable (both for all patients and for each disease-state group) between trough concentrations 2 days prior to, immediately before and one normal interdose interval (48 h) after the last dose, indicating attainment of steady state. No significant differences or trends in the steady-state parameters measured were identified statistically between the varying degrees of hepatic disease (Table 4). Each of the moderately impaired patients, however, showed an AUC that appeared remarkably low (682 ± 504, 738 ± 646 and 711 ± 502 vs 85 ± 57 h × pg/ml, respectively, for mild, severe and no impairment vs moderate impairment), but there were only two moderately impaired patients and their AUCs were so marginally (47% and 5%) above baseline as to be difficult to measure precisely. For AUC, ANOVA found \( P = 0.067 \).

**Discussion**

**Limitations of single-dose vitamin-D studies and advantages of steady-state studies**

In study A, the terminal half-life of 1,25(OH)\(_2\)D\(_2\) varied in osteopenic post-menopausal women from around 1 day to substantially longer. Yet, absent prior knowledge of this and due to assay-sensitivity considerations, the plasma-sampling schedule in study A had been

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**Table 3. Pharmacokinetic parameters (means ± SD) in serum compared between renal-disease patients (study C) and subjects of normal health (study B)**

<table>
<thead>
<tr>
<th></th>
<th>All renal-disease patients (n = 11)</th>
<th>Those renal patients with a ( t_{1/2} ) allowing steady state to be reached (( t_{1/2} = &lt; 58 ) h) (n = 7)</th>
<th>Normal subjects, when receiving 5 μg doses in study B (n = 24)</th>
<th>Statistical P-value for all renal patients vs normals (and for renal patients with ( t_{1/2} = &lt; 58 ) h vs normals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (h × pg/ml)</td>
<td>1232 ± 731</td>
<td>1165 ± 629</td>
<td>957 ± 533</td>
<td>0.254 (0.439)</td>
</tr>
<tr>
<td>Peak concentration (pg/ml)</td>
<td>38.0 ± 21.1</td>
<td>38.4 ± 21.4</td>
<td>29.9 ± 17.7</td>
<td>0.295 (0.395)</td>
</tr>
<tr>
<td>Time of peak concentration (h)</td>
<td>12.4 ± 2.8</td>
<td>12.0 ± 3.3</td>
<td>11.6 ± 5.7</td>
<td>0.662 (0.849)</td>
</tr>
<tr>
<td>Trough concentration (pg/ml)</td>
<td>13.6 ± 10.0</td>
<td>11.4 ± 6.8</td>
<td>11.6 ± 9.0</td>
<td>0.375 (0.641)</td>
</tr>
<tr>
<td>Peak-to-trough swing (pg/ml)</td>
<td>24.5 ± 13.6</td>
<td>27.0 ± 15.1</td>
<td>18.9 ± 12.8</td>
<td>0.322 (0.256)</td>
</tr>
</tbody>
</table>

\( n = 23 \) for the trough concentration for normal subjects, due to a negative estimate when normal subject 24’s trough concentration was corrected for baseline contribution.

**Table 4. Pharmacokinetic parameters (means ± SD) in serum compared among patients with varying degrees of hepatic impairment and subjects of normal health (study D)**

<table>
<thead>
<tr>
<th></th>
<th>Mild hepatic disease (n = 8)</th>
<th>Moderate hepatic disease (n = 2)</th>
<th>Severe hepatic disease (n = 3)</th>
<th>Normal subjects (n = 4)</th>
<th>Statistical P-value for a subject-group difference (and for a trend among subject-groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (h × pg/ml)</td>
<td>682 ± 504</td>
<td>85 ± 87</td>
<td>738 ± 646</td>
<td>711 ± 502</td>
<td>0.067 (0.277)</td>
</tr>
<tr>
<td>Peak concentration (pg/ml)</td>
<td>20.5 ± 12.9</td>
<td>7.1 ± 0.4</td>
<td>21.9 ± 17.8</td>
<td>22.3 ± 14.7</td>
<td>0.417 (0.334)</td>
</tr>
<tr>
<td>Time of peak concentration (h)</td>
<td>9.25 ± 3.01</td>
<td>12.0 ± 5.7</td>
<td>13.3 ± 9.5</td>
<td>11.8 ± 9.0</td>
<td>0.771 (0.631)</td>
</tr>
<tr>
<td>Trough concentration (pg/ml)</td>
<td>7.71 ± 7.14</td>
<td>0.00 ± 0.00</td>
<td>8.03 ± 9.62</td>
<td>6.42 ± 5.63</td>
<td>0.786* (0.762*)</td>
</tr>
<tr>
<td>Peak-to-trough swing (pg/ml)</td>
<td>13.2 ± 6.3</td>
<td>9.00 ± 2.26</td>
<td>13.2 ± 8.0</td>
<td>15.9 ± 10.0</td>
<td>0.846 (0.522)</td>
</tr>
</tbody>
</table>

*Both subjects with moderate hepatic disease and one from each of the other three groups had baseline-corrected trough concentrations of 0. All such subjects were omitted from the ANOVA which required log transformation. Accordingly, the statistical \( P \)-value shown reflects these data as missing and a comparison between three groups only, i.e. without the group with moderate disease.
designed to last only 48 h, of which the first 4-28 h (8 ± 6 h i.v., 11 ± 5 h oral) had elapsed before attainment of the peak concentration, with the terminal phase starting even later. A sampling schedule lasting no more than one or even two half-lives after establishment of the terminal log-linear phase limits the statistical confidence of estimation of the terminal rate-constant (or half-life) and, thus, also of clearance and bioavailability estimation based upon AUC extrapolated using that terminal rate-constant [10].

This is a limitation that study A shares with virtually all previously published reports on vitamin-D phosphokineti cs, but which studies B–D, described here, largely escape due to their steady-state design. For steady-state concentrations, the AUC that relates to bioavailability and clearance is the interdose AUC, whose estimation does not require extrapolation and is, thus, not sensitive to terminal rate-constant estimates. In any case, estimation of even the terminal rate-constant itself is also assisted by the larger concentrations usual after steady-state doses than after a single dose. Furthermore, there is more direct assurance that concentrations actually observed at steady state, particularly if they happen to arise from non-linear mechanisms (as found here), will reflect concentrations eventuating during multi-dose clinical use than there is from concentrations projected from single-dose studies.

**Achieving steady state**

In order to gain a more reliable pharmacokinetic description in the face of the sensitivity limitations of current assays, a multiple-dosing approach was implemented in studies subsequent to study A. A comparison of trough concentrations in study B after 6, 8 and 10 days had elapsed, indicates that steady-state conditions can be reasonably approximated with 8 days of dosing, consistent with the half-life estimates observed. Since it takes 3.3 half-lives to reach 90% of steady-state concentrations, only subjects with a half-life > 58 h would not have reached 90% of steady-state levels by 8 days. In study B, of the 42 half-lives that could be estimated from terminal slopes fit with correlation coefficients > 0.75 (32 ± 11 and 37 ± 16 h after the 5 and 15 μg regimens, respectively; n = 21), only one had an estimate (95 h) > 58 h.

**Terminal half-life**

Half-lives were usually substantially longer than 24 h (34 ± 14 h in normal young adults in study B). Since, additionally in study A, the time of peak 1,25(OH)2D2 concentrations after i.v. 1α(OH)D2 injections (8.0 ± 5.9 h) was far from instantaneous (and not significantly different after oral doses; 11.1 ± 5.0 h), it is possible that the terminal half-life for 1,25(OH)2D2 might be relatively long because it reflects a rate-limiting formation of this species from the 1α(OH)D2 dosed entity, rather than the elimination of 1,25(OH)2D2 itself (the terminal phase reflects the slowest process [11]). Indeed, this may be analogous to the case with the vitamin-D3 compounds, since both the terminal half-life and peak time of 1,25(OH)2D3 after oral dosing with 1,25(OH)2D3 have been reported to be only ≤50% of those of 1,25(OH)2D2 after oral dosing with 1α(OH)D2 (half-life: 13 ± 2 vs 36 ± 11 h [12], 28 ± 9 vs 47 ± 10 h [13]; peak time: 2.3 ± 0.1 vs 6.5 ± 3.1 h [13], 4.3 ± 1.3 vs 11 ± 3.1 h [14]). (The D3 half-life estimates were, however, reported from studies with a limited number of blood samples collected and/or with assayable samples only until 24 h after dosing.) Normally, 25-hydroxylation of dietary vitamin D2 and D3 is observed to occur largely before 1-hydroxylation [1,15]. Therapies employing alphacalcidol and doxercalciferol, which are already hydroxylated at the 1-position, reverse the innate sequence and the uncovering of a normally bypassed rate-limiting conversion might be the result. Clinically, this might prove more of an advantage than a disadvantage (as discussed below).

**Deviation from pharmacokinetic linearity**

Study B shows that serum concentrations rise less than proportionally on raising the dose from 5 μg every 48 h to 15 μg every 48 h. AUC during the 15 μg regimen was only 85.7 ± 33.5%, peak concentration only 85.7 ± 35.4% and the peak-trough concentration difference only 83.9 ± 34.4% of those that would have been predicted from the 5 μg regimen, had pharmacokinetics been linear (and after taking into account endogenous background concentrations). Peak concentration behaved similarly in study A. The similar percentage by which AUC, peak concentration and the peak-to-trough concentration swing deviate from linear expectations is consistent with saturation in one or more process responsible for metabolic conversion of 1α(OH)D2 to 1,25(OH)2D2, but other mechanisms are also possible (e.g. saturable gastrointestinal transport, saturable plasma-protein binding and saturable renal reabsorption). This marginal non-linearity in 1,25(OH)2D2 formation and/or disposition kinetics might explain in part why the metabolite 1,24-dihydroxyvitamin D2, a vitamin D of interest because of its reportedly low calcaemic activity [16], appears at only ~10% or less of the concentrations of 1,25(OH)2D2 after standard doses of vitamin D2, but in increasing proportions after higher doses of vitamin D2 [17,18].

**Comparison of vitamin-D2 and -D3 pharmacokinetics**

The terminal half-life of 1,25(OH)2D2 was about 36 h in normal subjects taking multiple oral 1α(OH)D2 doses (study B). By comparison, 1,25(OH)2D3 terminal half-lives of, respectively, 36 ± 11 and 48 ± 4 h were observed by Kimura [12] and Brandi [13] and their colleagues in haemodialysis patients taking 1α(OH)D3 orally and in normal subjects receiving 1α(OH)D3 either orally or intravenously. Additionally, Joffe et al. [19] reported that the half-life after i.v. 1α(OH)D3 doses in peritoneal-dialysis patients appeared to be several
days on average, although in the eight subjects studied the estimate covered a 25-fold range. The accuracy of estimation of 1,25(OH)2D3 half-lives as large as 36 h or more is, however, uncertain in all three of the D3 studies as each is hampered either by no samples being collected after 24 h following a single dose or by such samples not being significantly above innate baseline levels (peak times were typically 7 h or substantially longer). Further, this uncertainty is transmitted into estimates of extrapolated AUC, of which 50% or substantially more might reside in the extrapolation after 24 h [19], but with such extrapolation relying on the uncertain half-life estimate.

Using data up until 24 h, however, Joffe et al. [19] found a 1,25(OH)2D3 AUC of 1316 ± 237 h x pg/ml after a single 5.6 ± 1.7 μg (80 ng/kg) i.v. 1α(OH)D3 dose in five men and three women on peritoneal dialysis and a mean serum concentration of 62 ± 13 pg/ml at 4 h, the highest after this dose. This is comparable with study A above in which the 1,25(OH)2D2 AUC to 28 h (the sample nearest 24 h) was 1130 ± 550 h x pg/ml after a single 5 μg (~70 ng/kg) i.v. 1α(OH)D2 dose in 22 osteopenic but otherwise normal women and the mean concentration at 4 h was 53 ± 32 pg/ml, also the highest after this dose. Brandi et al. [13] also showed a comparable 1,25(OH)2D3 AUC to 72 h (approximating AUC to 24 h if concentrations after 24 h were essentially background as reported) of 1366 ± 757 h x pg/ml and a mean plasma concentration of ~50 pg/ml 4 h after an i.v. dose of 4 μg (~70 ng/kg) to six normal women. Thus, the data available suggest serum concentrations of a magnitude similar between 1,25(OH)2D2 and 1,25(OH)2D3, at least in the first 24 h after administering similar doses of their respective 1α-monohydroxylated prodrugs.

In study A, less than half of orally dosed 1α(OH)D2 appeared to reach the systemic circulation as 1,25(OH)2D2. By comparison, Joffe et al. [19] found that oral doses of 1α(OH)D3 given to peritoneal-dialysis patients gave rise to a mean 1,25(OH)2D3 unextrapolated AUC 65% of that from i.v. 1α(OH)D3 doses of the same strength. The data of Brandi et al. [13] show this figure to be 80% in normal subjects and, further, that the unextrapolated AUC from either oral or i.v. 1α(OH)D3 doses was only ~40% of that from 1,25(OH)2D3 given as such intravenously. This would indicate that probably as much, or more, of the active species is lost in the conversion of 1α(OH)D3 to 1,25(OH)2D3 than in oral absorption of the former. Whether this proves to be so depends upon whether the relationships observed between unextrapolated AUCs reflect those that exist between extrapolated AUCs, which are a much closer index of bioavailability, but which could not be confidently estimated from single-dose data.

Renally impaired patients on haemodialysis

Haemodialysed renal patients receiving 1α(OH)D2, were not found different from normal subjects in their steady state 1,25(OH)2D2 pharmacokinetic parameters, but had substantial increases in serum concentration during a dialysis session. As the previous dose was 3 days beforehand, this increase and its rapidity is indicative of a decrease in volume of distribution. Yet a loss of so much fluid during dialysis as to account directly for a doubling of concentration in two of the patients is unlikely [20]. In renal disease, increased volumes of distribution of drugs with normally significant plasma-protein binding (1,25(OH)2D2 > 99%; Bone Care International, WI, USA, unpublished data) are not unusual [10] and are largely the result of competition for binding proteins from endogenous substances normally, but no longer, cleared by a healthy kidney. Such substances tend to be also dialysable and, if they are lost during a dialysis session, drug binding can increase, volumes of distribution decrease and drug concentrations increase. Protein-binding studies would clarify this question and also whether the lack of difference apparent in total serum concentrations between renal patients (after dialysis) and normal subjects is true also for concentrations of unbound drug, since the latter are usually more directly predictive of effect. A lack of significant impact of renal disease on the pharmacokinetics of unbound 1,25(OH)2D2 from doses of 1α(OH)D2 would, however, be unsurprising, given that 25-hydroxylation is mediated by hepatic enzymes, not renal [3], and assuming that elimination of the large hydrophobic 1,25(OH)2D2 molecule is also predominantly hepatic (and is relatively low extraction).

Hepatically impaired patients

The metabolic pattern described above predicts a direct effect by hepatic disease on the pharmacokinetics of 1,25(OH)2D2 arising from 1α(OH)D2 dosing. Yet, in the face of the within-group variability seen even in normal subjects (10-fold range in AUC from the 5 μg study B regimen, 184–2118 h x pg/ml; 957 ± 533 h x pg/ml), it seems that more subjects than the two to eight per subgroup in study D would be needed to answer this question conclusively. Nevertheless, it is noteworthy that not only normal subjects and mildly impaired patients, but also severely impaired patients, yielded apparently very similar mean steady-state concentrations (14.8 ± 10.5, 14.2 ± 10.5 and 15.4 ± 13.5 pg/ml) in study D, while it was the two moderately impaired patients who yielded markedly low concentrations (3.1 and 0.5 pg/ml). This apparent divergence in the moderate but not severe group might be chance, but might, however, instead arise because 1,25(OH)2D2 concentrations reflect an interplay between the effects of the level of hepatic function not only on elimination of 1,25(OH)2D2, but also on its production from 1α(OH)D2. Any influence of hepatic disease on the 1,25(OH)2D2 pharmacokinetics might, therefore, not be simple either to ascertain or to characterize and might depend on a more specific diagnosis than just ‘hepatic disease’.
Clinical ramifications

Steady-state concentrations of 1,25(OH)₂D₃ appear to be attainable in most normal and renal-failure patients within about 1 week or a little longer after commencing dosage with 1α(OH)D₃, which, given the use of this agent, should not prove clinically burdensome. A half-life of >1 day allows dosing with this agent as infrequently as every day or even three times per week, with more modest interdose fluctuations in concentrations than achieved with vitamin-D treatments exhibiting less half-life. While there is some non-linearity in the pharmacokinetics of 1,25(OH)₂D₃, causing concentrations to rise less than proportionally with doses of 1α(OH)D₃, an overestimate of only 15% in mean steady-state concentrations when tripling the dose from 5 μg every 48 h to 15 μg every 48 h does appear to be of low enough magnitude to be clinically manageable with adequate precaution (particularly since this is a drug where the current recommendation is continual monitoring and dose titration). Moreover, this deviation from linear pharmacokinetics does not itself appear to require separate dosing-monitoring considerations for men and women to any extent that was detectable. It is not known whether analogous non-linearity arises from alphacalcidol [1α(OH)D₃] dosage also.

There appears to be no difference in 1,25(OH)₂D₃ pharmacokinetics in haemodialysis patients which would dictate a dosing schedule different from that of patients with normal renal function. However, such an assessment would be better based on measurements of unbound concentration in plasma, as it itself appear to require separate dosing-monitoring considerations for men and women to any extent that was detectable. It is not known whether analogous non-linearity arises from alphacalcidol [1α(OH)D₃] dosage also.

Conflict of interest statement. R.A.U. and C.W.B. are paid consultants of Bone Care International Inc., the developer of doxercalciferol; J.C.K. and L.W.L. are employees and C.W.B. was an employee of Bone Care International. C.W.B. holds stock in Bone Care International.

References