Journal of Steroid Biochemistry & Molecular Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Review

Modified-release oral calcifediol corrects vitamin D insufficiency with minimal CYP24A1 upregulation

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ARTICLE INFO

Article history:
Received 29 August 2014
Received in revised form 19 November 2014
Accepted 21 November 2014
Available online xxx

Keywords:
Calcifediol
Chronic kidney disease
Vitamin D insufficiency
Secondary hyperparathyroidism
CYP24A1
Modified-release
Clinical study
Rat

ABSTRACT

Vitamin D insufficiency is prevalent in chronic kidney disease (CKD) and associated with secondary hyperparathyroidism (SHPT) and increased risk of bone and vascular disease. Unfortunately, supplementation of stage 3 or 4CKD patients with currently recommended vitamin D_2 or D_3 therapies does not reliably restore serum total 25-hydroxyvitamin D to adequacy ($\geq$30 ng/mL) or effectively control SHPT. Preclinical and clinical studies were conducted to evaluate whether the effectiveness of vitamin D repletion therapies depends, at least in part, on the rate of repletion. A modified-release (MR) oral formulation of calcifediol (25-hydroxyvitamin D_3) was developed which raised serum 25-hydroxyvitamin D_3 and calcitriol levels gradually. Single doses of either bolus intravenous (IV) or oral MR 25-hydroxyvitamin D_3 were administered to vitamin D deficient rats. Bolus IV 25-hydroxyvitamin D_3 produced rapid increases in serum 25-hydroxyvitamin D_3, calcitriol and FGF23, along with significant induction of CYP24A1 in both kidney and parathyroid gland. In contrast, oral MR 25-hydroxyvitamin D_3 produced gradual increases in serum 25-hydroxyvitamin D_3 and calcitriol and achieved similar exposure, yet neither CYP24A1 nor FGF23 were induced. A 10-fold greater exposure to bolus IV than oral MR 25-hydroxyvitamin D_3 was required to similarly lower intact parathyroid hormone (iPTH). Single doses of oral MR (450 or 900 μg) or bolus IV (450 μg) 25-hydroxyvitamin D_3 were administered to patients with stage 3 or 4CKD, SHPT and vitamin D insufficiency. Changes in serum 25-hydroxyvitamin D_3 and calcitriol and in plasma iPTH were determined at multiple time-points over the following 42 days. IV 25-hydroxyvitamin D_3 produced abrupt and pronounced increases in serum 25-hydroxyvitamin D_3 and calcitriol, but little change in plasma iPTH. As in animals, these surges triggered increased vitamin D catabolism, as evidenced by elevated production of 24,25-hydroxyvitamin D_3. In contrast, MR 25-hydroxyvitamin D_3 raised serum 25-hydroxyvitamin D_3 and calcitriol gradually, and meaningfully lowered plasma iPTH levels. Taken together, these studies indicate that rapid increases in 25-hydroxyvitamin D_3 trigger CYP24A1 and FGF23 induction, limiting effective exposure to calcitriol and iPTH reduction in SHPT. They also support further investigation of gradual vitamin D repletion for improved clinical effectiveness.

This article is part of a Special Issue entitled "17th Vitamin D Workshop".

Contents

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http://dx.doi.org/10.1016/j.jsbmb.2014.11.022
0960-0760/

1. Introduction

Q4 Vitamin D insufficiency is associated with chronic kidney disease (CKD) and gives rise to secondary hyperparathyroidism (SHPT) which can lead to loss of bone density and elevated rates of fracture in renal patients [1]. Vitamin D therapies are therefore widely used in the management of chronic kidney disease (CKD). Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) supplementation is the standard of care for correcting vitamin D insufficiency in CKD [2], while vitamin D hormones (calcitriol and other synthetic hormones) are used to control SHPT [3]. Both of these therapeutic approaches have significant limitations.

Vitamins D_2 and D_3 (collectively "vitamin D") are absorbed less readily than more polar vitamin D compounds [4], and the degree of absorption can vary considerably between patients [5]. Once absorbed, vitamin D must undergo two sequential hydroxylations to be active: first at carbon 25 by CYP2R1 or CYP27A1 to form 25-hydroxyvitamin D, and then at carbon 1 by CYP27B1 to form 1,25-dihydroxyvitamin D [6]. Hepatic 25-hydroxylation varies widely in efficiency and, together with variable absorption, complicates the determination of optimal dose [7,8]. Significant percentages of CKD patients receiving vitamin D supplements do not attain targeted levels of serum 25-hydroxyvitamin D [9,10]. Recommended repletion [11] comprises intermittent high dose regimens which may trigger accelerated vitamin D catabolism [12]. A comprehensive review of the topic concluded that vitamin D supplementation is generally ineffective in clinical management of CKD patients [13].

Vitamin D hormones induce the desired clinical responses in target tissues, such as increased intestinal calcium uptake and suppression of iPTH production, by directly activating the vitamin D receptor [14]. Production of 1,25-dihydroxyvitamin D by renal CYP27B1 is controlled by feedback inhibition, thereby protecting tissues from overexposure. However, vitamin D hormone therapy is not subject to feedback regulation and can readily cause oversuppression of iPTH, hypercalcemia and hyperphosphatemia, leading to adynamic bone disease and vascular calcification [15]. Hormones also accelerate vitamin D catabolism and raise target tissue resistance by inducing CYP24A1 [16] which can mitigate the desired therapeutic responses and exacerbate vitamin D insufficiency.

The limitations of current vitamin D supplementation and hormone replacement therapies have led us to re-examine 25-hydroxyvitamin D_3 (calcifediol) as a potentially effective intervention for restoring adequate serum levels of 25-hydroxyvitamin D and safely controlling SHPT. Calcifediol is more readily absorbed than vitamin D [17,18] and requires only 1-hydroxylation for activation, which remains under physiological feedback regulation. We investigated whether gradual delivery of calcifediol, using a modified-release (MR) formulation for oral administration, would minimize CYP24A1 upregulation, thereby improving its effectiveness. The nonclinical and clinical studies described herein compared MR and bolus intravenous (IV) calcifediol with regard to effects on serum levels of vitamin D metabolites, plasma iPTH, serum FGF-23, and tissue expression of the catabolic enzyme CYP24A1.

2. Materials, methods and results

2.1. Non-clinical studies

2.1.1. Animals

Adult male Sprague Dawley rats (6–8 weeks of age) from Hilltop Lab Animals Inc., (Scottdale, PA, USA) were maintained on a vitamin D deficient diet for 8 weeks after which detectable serum 25-hydroxyvitamin D was negligible. Two groups of twenty-five rats were administered a single 0.4 mL IV injection of either calcifediol (4.5 μg) or vehicle (30:50:20, v/v/v propylene glycol: saline:ethanol). Two additional groups of 25 rats were administered by gavage hard shell gelatin capsules containing an MR formulation containing calcifediol (4.5 μg) or the MR formulation alone (comprising a wax matrix). The MR formulation progressively released calcifediol over a 12-hour period during *in vitro* dissolution testing. Serum or plasma were collected post-dose at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h.

2.1.2. Plasma iPTH

Determined with the rat iPTH ELISA kit (Immutopics, San Clemente, CA, USA).

2.1.3. Serum FGF23

Measured using an FGF23 ELISA kit (Kainos Laboratories, Tokyo, Japan).

2.1.4. CYP24A1, CYP27B1 and PTH mRNA

Kidney and parathyroid gland tissue samples were excised and frozen in RNAlater® and were processed using an automated hard tissue homogenizer. RNA was isolated using TRIzol® Reagent (Invitrogen). The ThermoScript™ RT-PCR System kit (Invitrogen) was used to create cDNA from 10 μg of RNA. The TaqMan® probes specific for rat Cyp24A1 (Cat. # Rn01423141_g1), Cyp27B1 (Rn00678309_g1), PTH (Rn00566882_m1) and GAPDH

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(Rn99999916_s1) were designed and manufactured by Applied Biosystems Inc., (AB - Foster City, CA). Quantitative real-time PCR was performed using an ABI Prism 7000 sequence detection system (Applied Biosystems (ABI), Foster City, CA, USA) using Taqman Universal PCR Master Mix (ABI #4304437). The relative expression value was calculated by the comparative C_T method using GAPDH as endogenous control. Data were normalized such that the level of expression in control rats was equal to 1.0.

2.1.5. Vitamin D metabolites

Serum samples were spiked with [26,27-2H6] 25(OH)D_3 or [25,26-2H6] 1α,25$(OH)_2D_3$ to serve as internal standards and extracted using Accubond II ODS-C_{18} 100 mg, 1 mL SPE cartridges (Agilent Technologies, Palo Alto, CA, USA). The collected fractions were dried under nitrogen, and residues were reconstituted in 50 μL of methanol/H_2O (80/20; v/v) and analyzed using LC–MS/MS (Waters Alliance HPLC-Waters Quattro Ultima Mass Spectrometer, Milford, MA, USA).

2.1.6. Statistical analysis

ANOVA (one- or two-way) and Bonferroni Multiple Comparison post-test were used to determine statistical significance set at $p < 0.05$.

2.2. Results from non-clinical studies

A single bolus IV dose of calcifediol (4.5 μg) increased serum calcifediol levels to approximately 320 ng/mL within 5 min (Fig. 1A). Thereafter, calcifediol levels dropped to 110 ng/mL by 30 min and to 96 ng/mL by 24 h. A single oral dose of MR calcifediol (4.5 μg) produced a detectable rise in serum calcifediol at 3 h post-dose, which peaked 2 h later at 16 ng/mL and dropped to 10 ng/mL by 24 h. No changes in serum calcifediol were noted in animals treated with vehicles.

Bolus IV calcifediol produced a rapid increase in serum calcitriol from baseline (which was below the limit of quantitation) to 1.1 ng/mL by 4 h (Fig. 1B). Serum calcitriol returned toward baseline by 24 h. MR calcifediol produced detectable increases in calcitriol (>0.1 ng/mL) as early as 1 h post-dose and levels rose gradually to 0.6 ng/mL by 24 h. No significant changes in serum calcium or phosphorus were observed for either treatment group over the 24-hour post-dose period (data not shown).

Pharmacodynamic changes associated with the observed increases in serum calcifediol and calcitriol are shown in Fig. 2A through D. Bolus IV calcifediol rapidly induced CYP24A1 expression in the kidney which reached a 40-fold increase by 8 h post-dose. In contrast, MR calcifediol produced detectable increases in kidney CYP24A1 expression after 4 h which peaked at only 6-fold above baseline by 12 h. No changes in CYP24A1 expression were observed in vehicle-treated animals.

Serum FGF23 levels increased significantly only in animals receiving bolus IV calcifediol (Fig. 2B) and remained higher 24 h post-dose. Kidney CYP27B1 mRNA transcript levels were rapidly and completely suppressed with IV calcifediol treatment by 8 h and remained suppressed at 24 h (Fig. 2C). In contrast, neither MR calcifediol nor vehicle treatment caused significant changes in serum FGF23 or CYP27B1 expression.

A rapid and prominent surge in CYP24A1 expression was observed in parathyroid gland tissue obtained from animals treated with bolus IV calcifediol which peaked at 4 h post-dose at a level 13-fold higher than baseline (Fig. 2D). In contrast, parathyroid gland CYP24A1 expression rose more gradually in animals treated with MR calcifediol, peaking at 12 h post-dose at a level 5-fold higher than baseline. Plasma iPTH was equally suppressed in both treatment groups at 24 h post-dose (Fig. 3).

2.3. Clinical studies

2.3.1. Subjects

Twenty-nine (29) subjects with stage 3 or 4CKD, SHPT and vitamin D insufficiency (defined as serum total 25-hydroxyvitamin D below 30 ng/mL) were randomized to one of three treatment groups.

2.3.2. Treatment

Subjects were orally administered a single oral dose of MR calcifediol (either 450 μg or 900 μg) or a single bolus IV injection of calcifediol (448 μg). For the oral doses, 5 or 10 capsules (90 μg each) were administered after an overnight fast with water (maximum 12 ounces) within 15 m. The MR capsules used in these clinical studies were similar to those used in the non-clinical studies, also comprising a wax matrix to effect the more gradual release of calcifediol. *In vitro* dissolution testing showed that the MR capsules progressively released calcifediol over a 12-hour

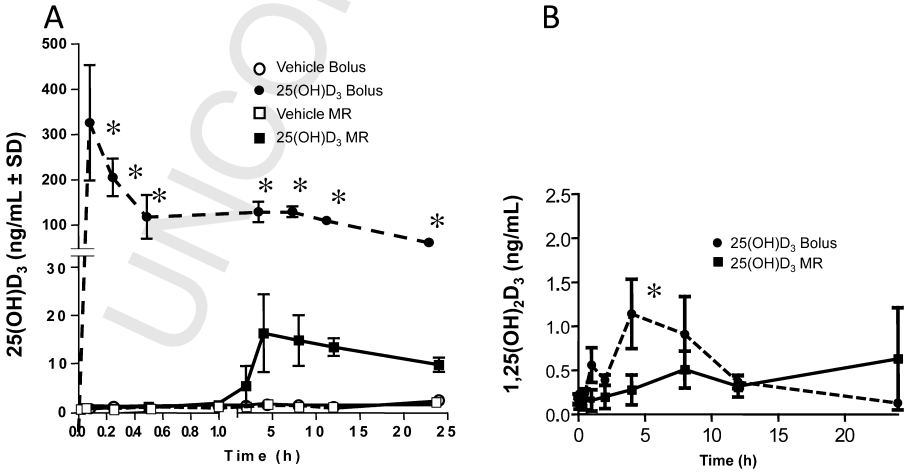

Fig. 1. Effect of bolus IV or oral MR calcifediol administration on serum calcifediol and calcitriol levels in rats. Male rats were maintained on a vitamin D deficient diet for 8 weeks and then divided into 4 treatment groups. Each group received a single 4.5 μg dose of bolus IV calcifediol (solid circles) or oral MR calcifediol (solid squares) or the corresponding vehicles (open circles and squares). Serum levels of calcifediol (A) and calcitriol (B) were measured at the indicated time points post-dose. Error bars indicate standard deviation (SD). Asterisks denotes significant differences between IV and MR treatment groups ($P < 0.05$).

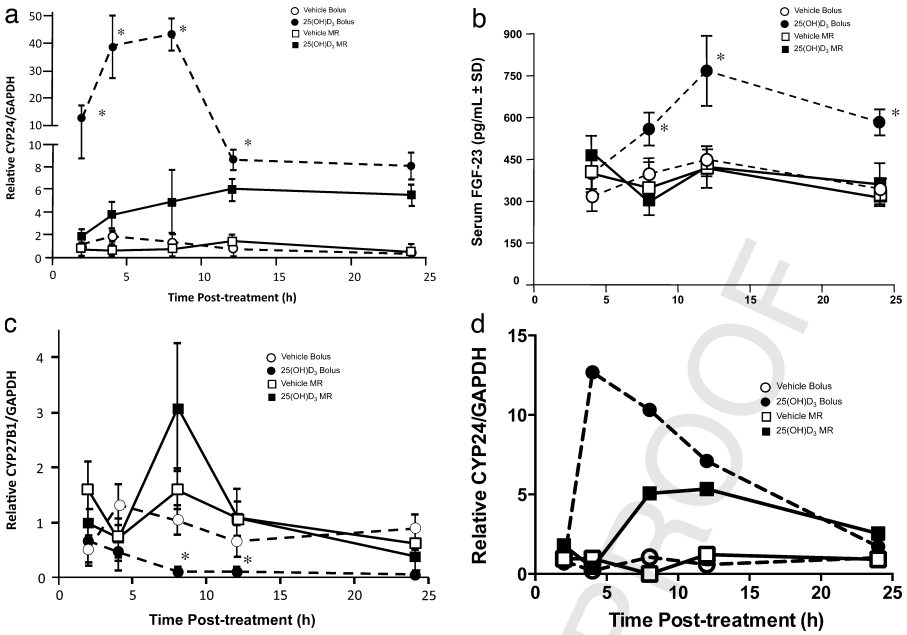

Fig. 2. Effect of bolus IV or oral MR calcifediol administration on mediators of vitamin D metabolism in rats. Expression of kidney CYP24A1 transcripts (A), serum intact FGF23 (B), kidney CYP27b1 transcripts (C) and parathyroid gland CYP24A1 transcripts (D) were measured in vitamin D deficient rats sacrificed at the indicated time points following treatment with a single 4.5 μg dose of bolus IV calcifediol (solid circles) or oral MR calcifediol (solid squares) or the corresponding IV or MR vehicles (open circles and squares). Asterisks denote significant differences between IV and MR treatment groups ($P < 0.05$).

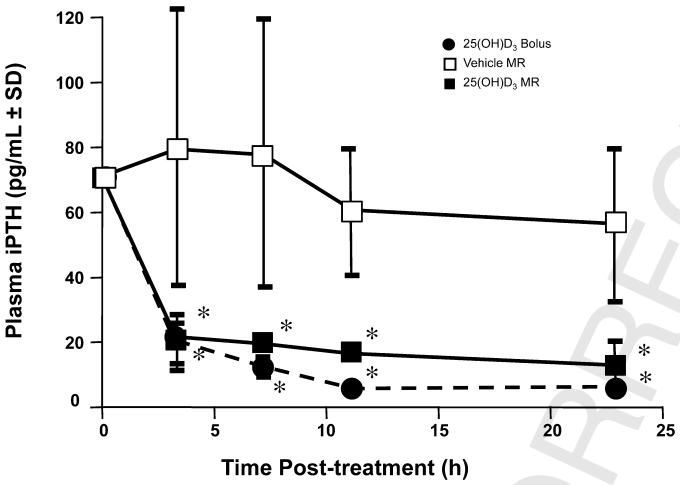

Fig. 3. Effect of bolus IV or oral MR calcifediol administration on plasma iPTH levels in rats. Plasma iPTH levels were determined in vitamin D deficient rats treated with bolus IV calcifediol (solid circles), MR capsules (solid squares) or the corresponding MR vehicle capsules (open squares). Baseline iPTH level corresponds to mean iPTH levels obtained in vehicle control treated animals over the course of the treatment period. Data for the IV vehicle were equivalent to the MR vehicle and were omitted for improved clarity. Both IV and MR iPTH treatment groups were significantly different from their correspnding vehicle controls at all time points post-treatment as denoted by asterisks ($P < 0.05$).

period (data not shown). For IV dosing, 0.56 mL (448 μg) of calcifediol formulated in propylene glycol:saline:ethanol (30:50:20, v/v/v), was injected within 1 min into a peripheral vein. The strength of the dosing formulations were verified prior to and after administration.

2.3.3. Sample analysis

Blood samples were collected at 18, 12 and 6 h pre-dose to establish baseline values. For the oral dose groups, post-dose blood samples were collected at 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, and 48 h, and at 4, 7, 14, 21, 28, and 42 days. For the IV group, post-dose samples were collected at 5, 10, 15, and 30 min, at 1, 2, 4, 6, 8, 12, 24, and 48 h, and at 4, 7, 14, 21, 28, and 42 days. Blood samples were shipped to Spectra Clinical Research (Rockleigh, New Jersey) for all analyses except determinations of serum calcifediol and 24,25-dihydroxyvitamin D_3, for which samples were forwarded to inVentiv (Québec, QC, Canada) for analysis by high performance liquid chromatographic method with tandem mass spectrometry detection (HPLC–MS/MS). Spectra determined the level of 1,25-dihydroxyvitamin D in serum using an Immunodiagnostic Systems Ltd. (IDS) Enzyme Immuno Assay (EIA) kit.

2.3.4. Statistical analysis

Differences between treatment groups were analyzed by a one- or two-sided *t*-test, as appropriate, with statistical significance set at $p < 0.05$.

2.4. Results of clinical studies

2.4.1. Serum calcifediol

The effects of single bolus IV versus oral MR administration of calcifediol on baseline-adjusted serum calcifediol levels are shown in Fig. 4A for 0–96 h. Mean baseline concentrations were 23.7 ng/mL for the 448-μg IV group and 18.3 and 18.7 ng/mL for the MR 450 μg and 900 μg groups, respectively. Peak mean calcifediol concentrations were observed at 0.5 h after bolus IV dosing versus 13.1 and 13.6 h post-dose for oral MR dosing at 450 μg and 900 μg, respectively. Exposure to calcifediol, based on observed area-under-the-curve (AUC) and maximum concentration (C_{max}), was far higher after IV than MR administration: mean baseline corrected C_{max} was 110.3 ng/mL for the IV group and 6.9 and 14.2 ng/mL for the oral MR groups. Exposure was approximately dose-proportional with the oral MR 450 μg and 900 μg doses.

2.4.2. Serum 1,25-dihydroxy vitamin D

Mean baseline concentrations of serum 1,25-dihydroxyvitamin D were 19.3, 21.2 and 26.5 pg/mL for the IV (448 μg) and MR (450 μg and 900 μg) treatment groups, respectively. Mean baseline-adjusted concentrations over the 96-hour post-dose

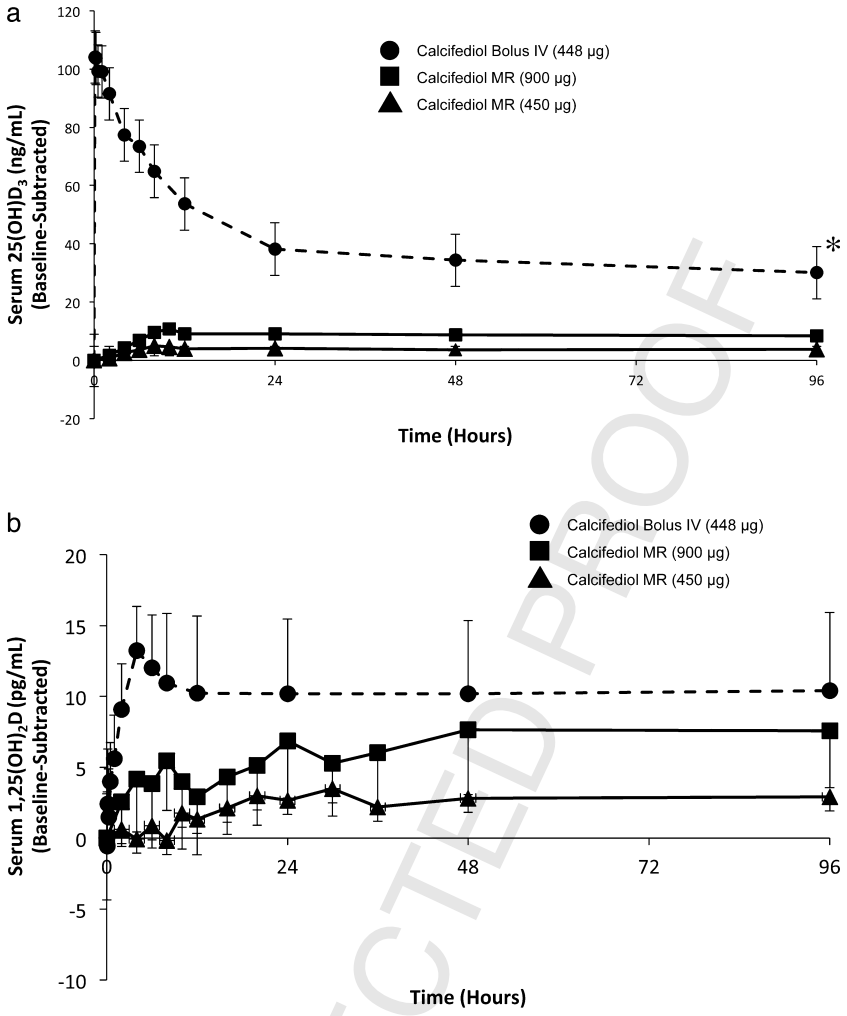

Fig. 4. Effect of bolus IV or oral MR calcifediol administration on serum levels of calcifediol and 1,25-dihydroxyvitamin D in patients. Patients with stage 3 or 4CKD, SHPT and vitamin D insufficiency were treated with a single bolus IV injection of 448 µg calcifediol (solid circles) or single doses of oral MR calcifediol (450 µg–solid triangles; 900 µg–solid squares). Serum samples obtained at the indicated time points were analyzed for (A) calcifediol ($25(OH)D_3$) and (B) 1,25-dihydroxyvitamin D. Data are corrected for baseline values. Asterisk denotes significant differences at all time points post-treatment between IV and MR treatment groups ($P < 0.05$).

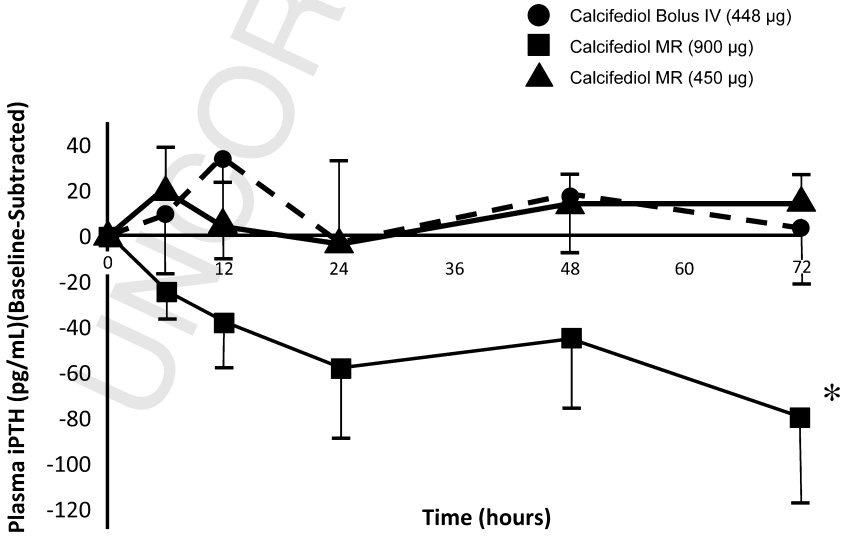

Fig. 5. Effect of bolus IV or oral MR calcifediol administration on plasma iPTH levels in patients. Patients with stage 3 or 4CKD, SHPT and vitamin D insufficiency were treated with a single bolus IV injection of 448 µg calcifediol (solid circles) or single doses of oral MR calcifediol (450 µg–solid triangles; 900 µg–solid squares). Plasma samples obtained at the indicated time points were analyzed for iPTH. Data are corrected for baseline values. Plasma iPTH was not determined at 96-hours post-dose. Asterisk denotes significant difference between IV and MR treatment groups at 72 h. ($P < 0.05$).

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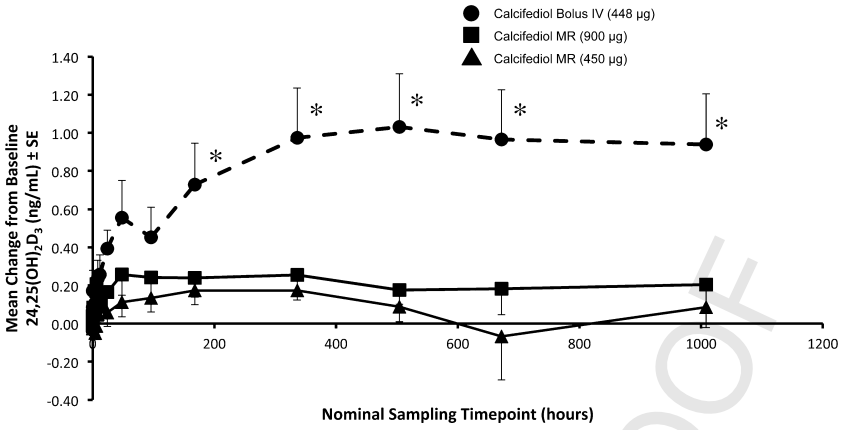

Fig. 6. Effect of bolus IV or oral MR calcifediol administration on plasma 24,25-dihydroxyvitamin D levels in patients. Patients with stage 3 or 4CKD, SHPT and vitamin D insufficiency were treated with a single bolus IV injection of 448 μg calcifediol (solid circles) or single doses of oral MR calcifediol (450 μg–solid triangles; 900 μg–solid squares). Plasma samples obtained at the indicated time points were analyzed for 24,25-dihydroxyvitamin D_3 levels. Data are expressed as percent of baseline values. Asterisks denote significant differences between IV and MR treatment groups ($P < 0.05$).

period are shown for the three treatment groups in Fig. 4B. Following bolus IV calcifediol, mean concentration of serum 1,25-dihydroxyvitamin D rapidly increased by up to 13 pg/mL at 6 h post-dose. In contrast, mean concentrations in the oral MR groups gradually increased and peaked at approximately 3 and 7 pg/mL over baseline, respectively, by 48 h post-dose. The mean AUC was 7449 and 2530 pg.h/mL for the IV (448 μg) and MR (900 μg) treatment groups, respectively, and these values did not differ significantly. AUC in the 450 μg MR group was negligible.

2.4.3. Plasma iPTH

Baseline levels of plasma iPTH were 184 pg/mL for the IV group, and 168 and 238 pg/mL, respectively, for the MR 450 and 900 μg groups. Mean percent changes in iPTH from baseline were minimal over the post-dose period for the bolus IV and lower oral MR dose groups. However, mean percent reduction in plasma iPTH was significant and sustained for the higher oral MR dose, reaching approximately 20% between 24 and 72 h post-dose (Fig. 5). No significant increases in serum calcium were observed in any treatment group during the post-dose period (data not shown).

2.4.4. Serum 24,25-dihydroxyvitamin D_3

Baseline levels of 24,25-dihydroxyvitamin D_3 were 1.13 ng/mL for the IV group, and 0.86 and 0.87 ng/mL, respectively, for the MR 450 and 900 μg groups. Mean values fluctuated around baseline for the MR 450 μg group and increased approximately 0.2 ng/mL for the MR 900 μg group. Mean values increased more dramatically over the course of the study for the IV group and reached levels approximately 1.0 ng/mL over baseline by two weeks post-dose, remaining at this level to the end of the study (Fig. 6).

3. Conclusions

Numerous non-clinical and clinical studies have investigated the therapeutic potential of vitamin D supplementation to control SHPT and manage metabolic bone disease in CKD patients [19]. Although there is general consensus that vitamin D repletion has an important role in treating these patients, the body of published literature shows that supplementation with cholecalciferol or ergocalciferol is generally unreliable in correcting vitamin D insufficiency and ineffective in controlling SHPT [10,13,20]. Further, there is no consistent view regarding how vitamin D supplements should best be administered. Published studies have used daily doses of from 700 to 4000 IU/day, weekly doses of 5000 to 50,000 IU, and monthly doses of 50,000 to 300,000 IU.

The impact of rate of administration on effectiveness of vitamin D therapies has been poorly investigated. In this paper, we present results from parallel studies in which calcifediol was delivered either rapidly as an IV bolus, or gradually *via* an oral MR formulation, to vitamin D deficient rats or patients with stage 3 or 4CKD, SHPT and vitamin D insufficiency. Our findings suggest that rate of delivery is an important determinant of vitamin D hormone production and therefore of therapeutic efficacy and that gradual delivery allows more effective treatment of both vitamin D insufficiency and SHPT in CKD patients.

In the presented studies, bolus IV calcifediol produced rapidly rising and higher drug exposures than oral MR calcifediol, due to a substantially faster calcifediol release rate and higher bioavailability. IV dosing also caused abrupt, large increases in serum 1,25-dihydroxyvitamin D_3. In vitamin D deficient rats, IV dosing triggered high expression of CYP24A1 and, subsequently, FGF23, then near-complete suppression of CYP27B1 and significant iPTH lowering. MR calcifediol yielded equivalent iPTH suppression by gradually elevating drug exposure and had no dramatic impact on serum 1,25-dihydroxyvitamin D, serum FGF23, CYP24A1 and CYP27B1. The gradual increase of CYP24A1 expression in the MR treated animals is likely due to the gradual restoration of vitamin D status in these animals. In CKD patients, IV administration yielded higher serum 24,25-dihydroxyvitamin D_3 levels, consistent with greater CYP24A1 activity, but negligible PTH suppression. Conversely, MR administration gradually raised serum calcifediol and 1,25-dihydroxyvitamin D without significantly elevating serum 24,25-dihydroxyvitamin D, and produced meaningful, sustained iPTH suppression.

Data from these studies indicate that renal production of calcitriol is driven by the supply of calcifediol until CYP27B1 is suppressed. The faster calcifediol is supplied, the more calcitriol is produced initially. The abrupt increase in serum calcifediol after bolus IV dosing produced a corresponding surge in serum calcitriol, which in turn triggered upregulation of CYP24A1 in both kidney and parathyroid gland. Increased expression of CYP24A1 appears to have attenuated the further rise of serum calcitriol (serum 1,24,25-trihydroxyvitamin D_3 was not measured) and, after suppression of renal CYP27B1, drove serum calcitriol in the rats back to baseline levels at 24 h post-dose. In contrast, MR dosing gradually increased both serum calcifediol and calcitriol, yielding calcitriol exposures that were greater in the rats and nearly equivalent in patients.

In rats, the strong upregulation of CYP24A1 by bolus IV dosing appeared to have been triggered both by the rapid

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increase in calcitriol levels and the significant elevation of FGF23 expression. These same factors may have also caused the almost complete and sustained suppression of renal CYP27B1. Although, at the end of the treatment period, serum calcitriol returned to baseline levels, FGF23 remained elevated. We do not presently know the mechanism sustaining FGF23 levels; however, this would likely continue to suppress CYP27B1 expression and maintain CYP24A1 elevation. This FGF23 "memory" effect would be expected to have an impact on the efficacy of subsequent dosing, further supporting gradual repletion over bolus treatments.

Previous studies have demonstrated that increased expression of CYP24A1 in kidney and extra-renal target tissues is differentially regulated following increased calcitriol production [21–23]. This differential regulation may depend on whether the target tissue in question can respond to FGF23 and whether FGF23 levels have been increased by vitamin D treatment.

The observed PTH lowering in rats was equivalent at 24 h post-dose after both IV and MR dosing. However, we postulate that PTH suppression would not have been sustained for much longer after IV dosing because CYP24A1 was increased in both kidney and parathyroid gland, serum FGF23 was elevated and CYP27B1 was suppressed. This is supported by the greater and more sustained PTH suppression observed in CKD patients between 24 and 72 h after the 900 μg MR dose.

Bolus IV administration of calcifediol induced a 40-fold surge in kidney CYP24A1 expression by 8 h post-dose. This rapid induction of CYP24A1 was similar to that observed previously in rats (46-fold increase in kidney and 25-fold increase in intestine) following 2.5 weeks of high-dose vitamin D (three treatments per week of 25,000 IU each) [23]. This previous study demonstrated that consecutive rapid administrations of vitamin D progressively raise CYP24A1 levels, attenuating the intended impact of treatment. Recent clinical studies have shown that treatment of CKD patients with bolus cholecalciferol results in a shift of vitamin D balance to net degradation with increased production of 24,25-dihydroxyvitamin D_3, reduced production of 1,25-dihydroxyvitamin D and increased FGF23 expression [24]. Consistent with our findings, bolus cholecalciferol was not effective at suppressing iPTH. In our study, patients receiving bolus calcifediol exhibited elevated and sustained production of 24,25-dihydroxyvitamin D_3. This likely reflects elevated CYP24A1 expression both in the kidney as well as in other vitamin D target tissues, but the mechanism underlying continued production of 24,25-dihydroxyvitamin D_3 over 42 days is unknown.

It is notable that both rat and patient responses to different rates of calcifediol administration were similar. This supports the use of the model to further investigate mechanisms affecting the efficacies of different vitamin D repletion regimens including comparisons between oral IR and MR formulations both in single-dose and repeat dose studies.

Taken together, the studies presented herein indicate that the rate at which vitamin D therapy is administered can have a significant impact on treatment outcomes. Further, they support continued investigation of MR calcifediol as a treatment of SHPT in patients with CKD and vitamin D insufficiency.

Acknowledgements

Support for these studies was provided by OPKO Health, Renal Division. We thank Drs. Christian Helvig and Dominic Cuerrier for technical suggestions. M.P. is supported by funding from the Canadian Institutes of Health Research.

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