Journal of Veterinary Internal Medicine

Journal of Veterinary Internal Medicine Open Access
ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE OPEN ACCESS
Small Animal Internal Medicine Nephrology/Urology

Effects of Paricalcitol on Renal Secondary Hyperparathyroidism and Proteinuria in Dogs With Chronic Kidney Disease

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Received: 29 October 2024 | **Revised:** 27 February 2025 | **Accepted:** 4 March 2025

Funding: This work was supported by the European College of Veterinary Internal Medicine - Companion Animals (ECVIM-CA) Clinical Studies Fund Purina Institute Resident Research Award.

Keywords: FGF-23 | mineral bone disorder | parathyroid hormone | vitamin D

ABSTRACT

Background: Renal secondary hyperparathyroidism (RHPT) is an inevitable consequence of chronic kidney disease (CKD). Paricalcitol might safely attenuate RHPT and proteinuria.

Hypothesis/Objective: Paricalcitol decreases parathyroid hormone (PTH) and proteinuria in dogs with CKD.

Animals: Thirteen dogs with naturally acquired CKD.

Methods: Placebo-controlled clinical trial. Dogs were randomly allocated to receive a placebo or paricalcitol (14 ng/kg/day) in a crossover design of 2, 12-week arms. Dogs were evaluated every 3 weeks. Associations between treatment, visit, and the outcome variables were assessed using generalized estimating equations.

Results: PTH decreased by 22% (95% CI, 7%–35%, $p = 0.006$) in the paricalcitol-treated dogs and increased by 18% (95% CI, 2%–37%, $p = 0.022$) in the placebo-treated dogs with each visit. FGF-23 at 12 weeks increased compared with baseline in the paricalcitol-treated (mean 6941 pg/mL, 95% CI, 1781–20 057 vs. 489 pg/mL, 95% CI, 188–1272, $p < 0.001$, respectively), but not in the placebo-treated dogs (696 pg/mL, 95% CI, 316–1531 vs. 955 pg/mL, 95% CI, 308–2963, $p = 0.529$). Urine protein-to-creatinine ratio at 12 weeks increased compared with baseline in the placebo-treated (0.8, 95% CI, 0.3–1.3 vs. 0.5, 95% CI, 0.2–0.9, $p = 0.04$, respectively), but not in the paricalcitol-treated dogs (0.6, 95% CI, 0.3–0.9 vs. 1.0, 95% CI, 0.1–1.8, $p = 0.35$). Ionized calcium was unchanged between baseline and 12 weeks in the paricalcitol- and placebo-treated groups (1.3 mmol/L, 95% CI, 1.29–1.35 and 1.34, 95% CI, 1.27–1.40 vs. 1.30, 95% CI, 1.25–1.35, $p = 0.12$ and 1.28, 95% CI, 1.24–1.32, $p = 0.034$, respectively). However, 7/13 dogs developed mild hypercalcemia. Adverse effects were not reported by the owners.

Conclusion and Clinical Importance: Paricalcitol attenuated RHPT and stabilized renal proteinuria in dogs with CKD.

1 | Introduction

Chronic kidney disease (CKD) is an irreversible, progressive disease that affects dogs, especially at older ages [1–3]. Renal secondary hyperparathyroidism (RHPT) is an inevitable consequence of CKD [4], which occurs early in the disease course, before abnormalities in plasma calcium and phosphorus are evident, or in some cases before azotemia ensues [5]. RHPT

Abbreviations: CaXP, calcium phosphorus product; CKD, chronic kidney disease; FGF-23, fibroblast growth factor-23; GEE, generalized estimating equations; i-Ca, ionized calcium; PTH, parathyroid hormone; RHPT, renal secondary hyperparathyroidism; UPC, urine protein-to-creatinine; VDAs, vitamin D analogs.

pathogenesis is complex; it begins with the decrease in the glomerular filtration rate, resulting in phosphate retention. Phosphate accumulation, combined with concurrent reduction in the kidney functional mass, leads to decreased activity of the renal enzyme 1α-hydroxylase, resulting in reduced production of calcitriol (1,25(OH)2-cholecalciferol), the active form of vitamin D. The combination of phosphate retention with the lower calcitriol concentration results in an increase in serum parathyroid hormone (PTH) [6]. As CKD progresses, PTH concentrations increase [5, 7, 8]. Fibroblast growth factor-23 (FGF-23) is a phosphaturic hormone that contributes indirectly to RHPT development in the advanced stages of CKD, as it inhibits renal 1α-hydroxylase activity, further decreasing calcitriol concentration and increasing PTH secretion [9, 10].

Clinical consequences of RHPT in people include mental dullness, weakness, anorexia, increased incidence of infections, carbohydrate intolerance, and fatty acid metabolism derangements, impaired cardiac and skeletal muscle function, inhibition of erythropoiesis, and altered red-cell osmotic resistance, as well as B-cell proliferation, T-cell and platelet dysfunction [11–13]. RHPT also promotes nephrocalcinosis and further progressive loss of renal function [5, 14]. Finally, mineral bone disease causing decreased bone density and quality is described in dogs with RHPT [15].

Vitamin D compounds might have renoprotective effects through multiple potential mechanisms, including reduction in PTH concentration that attenuates RHPT and reduction of proteinuria through activation of vitamin D receptors in podocytes, enhancing their health and downregulating angiotensinogen [1, 13, 16]. Indeed, calcitriol therapy delayed the development of uremic crises and was associated with prolonged survival in dogs with CKD [17]. Despite its ability to ameliorate the effects of RHPT, calcitriol treatment might lead to excessive calcium and phosphate absorption, resulting in hypercalcemia and hyperphosphatemia, which have substantial negative effects, most importantly worsening of CKD [17]. Therefore, prescribing either vitamin D or its metabolites to dogs with CKD as part of the medical management necessitates frequent monitoring, which requires high owner compliance and has financial implications. Due to its potentially harmful adverse effects and the need for close monitoring, this treatment has been largely neglected and is no longer recommended as part of the routine management of CKD in dogs [18].

Numerous synthetic vitamin D analogs (VDAs) were developed in order to advance the biological properties of the natural compound for different therapeutic applications [19]. Paricalcitol (19-nor-1-alpha-25-dihydroxyvitamin D2) is a second-generation VDA, indicated for the treatment of RHPT in human patients with CKD. It is considered to have a limited calcemic effect, decreased risk for hypercalcemia, and an antiproteinuric effect [20–22], although recent studies in human patients suggest that despite its superior effect in RHPT attenuation, its effects on calcium and phosphate concentrations might not be different from those of calcitriol [23, 24]. Paricalcitol treatment was evaluated in dogs with atopic dermatitis and was discontinued due to the development of hypercalcemia in half of the treated dogs [25]. Oxacalcitriol, another VDA, was investigated in dogs with RHPT and was suggested to have a larger therapeutic window compared with calcitriol [26].

We hypothesized that paricalcitol will attenuate RHPT in dogs with CKD, without resulting in hypercalcemia, and will also reduce proteinuria. The aims of this study were to determine the effects of a 12-week daily paricalcitol treatment on circulating PTH and FGF-23, as surrogates of RHPT, and to assess the frequency of treatment-related hypercalcemia in dogs with CKD. A secondary aim was to evaluate the short-term effect of paricalcitol on proteinuria.

2 | Materials and Methods

2.1 | Dogs and Study Design

This was a randomized, controlled study in a crossover design, conducted at the Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, teaching hospital, following approval of the Institutional Animal Care & Use Committee (approval number MD-21-16662-2). Adult dogs (≥12-month-old) diagnosed with stable CKD IRIS Stages 2–4 were considered for inclusion with their owners' signed consent. Diagnosis of CKD and stratification to IRIS stages were based on the IRIS guidelines [27]. Dogs were evaluated for concurrent medical conditions based on the results of complete blood count, biochemical profile, urinalysis, urine culture, and abdominal ultrasound. Dogs with concurrent major non-renal diseases, specifically diseases that can lead to hypo or hypercalcemia (e.g., primary hypo- or hyperparathyroidism, protein-losing enteropathy, hypo- or hyperadrenocorticism, or neoplasia), or other urinary tract diseases (e.g., acute kidney injury or acute exacerbation of CKD, urinary tract infection, urolithiasis) were excluded. Dogs with ionized hypercalcemia (i-Ca > 1.4 mmol/L), dogs in which phosphate serum concentration was not well managed (> 5 mg/dL), or dogs receiving any form of vitamin D compound or steroids were also excluded. Dogs that were not already fed a renal prescription diet were gradually transitioned to such a diet until they consumed the diet exclusively for at least 4 weeks before enrollment.

Dogs were randomized into two treatment groups. Each dog received either paricalcitol or placebo during the first 12-week arm, then transitioned to the other treatment for the second 12-week arm, with a 2-week washout period between arms. Group allocation was done by one of the researchers (Hilla Chen), using commercial block randomization software (Sealed Envelope), while the owners, attending clinicians, and other researchers were blinded to the treatment administered.

During the study, dogs continued to receive standard treatment for CKD management at the attending clinician's discretion. Importantly, no changes were made in the individualized standard treatment throughout the study period, nor in the 4 weeks preceding enrollment. If such a change was needed to provide the best standard of care, the dog was excluded from the study.

2.2 | Procedures

At the time of enrollment, history, physical examination findings, and blood pressure were documented. Blood samples were obtained for complete blood count (Advia 2120, Siemens, Erfurt, Germany), complete biochemical profile, and ionized calcium (i-Ca) measurement (Cobas 6000, Roche, Mannheim, Germany) which were performed within 60 min of collection. Serum aliquots were immediately frozen at −80°C pending PTH and FGF-23 analysis, and a urine sample was collected by cystocentesis for urinalysis, urine culture, and UPC. Paricalcitol (Zemplar, AbbVie LTD UK, Queenborough, Kent, England) was administered at 14 ng/kg orally once daily. This dose was chosen as it is equivalent to the dose used in human patients with CKD and is numerically four times higher compared to a dose of calcitriol administered at 3.5 ng/kg once daily on a ng/kg basis, similar to the relative doses of calcitriol and paricalcitol used in human patients with CKD (0.25 and 1 μg for a person daily, respectively). This dose was also lower than the doses previously used in dogs that resulted in frequent hypercalcemia (20–100 ng/kg) [25]. Depending on the dog's body weight, paricalcitol was either given as 1000 ng Zemplar capsule (EOD) or a 200 ng/mL suspension which was compounded at the hospital's pharmacy by a certified pharmacist. Placebo was given in a capsule or suspension form, respectively. During each arm, five additional evaluations were performed at 1, 3, 6, 9, and 12 weeks. The evaluation at Week 1 included i-Ca measurement to detect potential early development of hypercalcemia. At 3, 6, and 9 weeks, kidney function parameters (i.e., urea, creatinine), albumin, phosphate, and potassium, as well as i-Ca, were measured, and serum samples were immediately stored at −80°C pending PTH and FGF-23 analysis. At week 12, the last visit of the arm, the aforementioned parameters were evaluated, as well as UPC. If hypercalcemia (i-Ca > 1.40 mmol/L) was detected at any time during the study, treatment was paused for 1 week, and serum i-Ca concentration was reevaluated. If hypercalcemia persisted, the dog was withdrawn from the study. In cases where hypercalcemia resolved, treatment was reintroduced at a reduced dose of 25%. If hypercalcemia recurred following reintroduction of treatment, the dose was further reduced by another 25%. If hypercalcemia persisted or recurred despite these adjustments, the dog was withdrawn from the study.

Serum FGF-23 concentrations were measured using a previously validated ELISA kit (Kainos Laboratories, Tokyo, Japan) [28] and serum PTH concentration was measured with an intact PTH ELISA (Immutopics assay San Clemente, USA) previously used in dogs [29–31].

2.3 | Data Analyses

Summary data are presented as median (range). The distribution pattern of quantitative variables was assessed by the Shapiro–Wilk test. Natural log transformation was applied for variables that were not normally distributed in order to achieve normality. Linear correlations were assessed using Pearson's correlation coefficient test. Associations between treatment (paricalcitol or placebo) or time (baseline and 12 weeks) and the outcome variables (concentrations of PTH, FGF-23, and UPC) were evaluated using generalized estimating equations (GEE) with arm order (paricalcitol then placebo or placebo then paricalcitol) as an additional factor to control for potential confounding on the relationship of treatment or time and the outcome variables. Additional analysis was performed for PTH with the time variable including baseline, 3, 6, 9, and 12 weeks after treatment initiation. In all models, "dog" was set as the subject variable, and "time" was set as the within-subject variable. An exchangeable working correlation matrix was used to treat within-subject observations as equally correlated. Interaction effects between treatment, time, or arm order were evaluated. Separate analyses were performed, and results were reported separately for each variable level for which an interaction effect with a $p < 0.10$ was observed. Bonferroni correction was applied for multiple post hoc comparisons where applicable. Data were analyzed using SPSS 26.0 for Windows, and $p \leq 0.05$ was considered significant. Results of the GEE models are reported as adjusted means and 95% confidence interval (CI).

A priori sample size calculation (G*Power 3.1.9.7) was based on the reduction in PTH concentration as the main outcome. Assuming an effect size of 0.9, estimated based on a 50% reduction in PTH concentrations as a clinically significant difference, 12 dogs were required to detect a difference in PTH concentration with a power of 80% and a confidence level of 98.3% (α error = 0.0167). Alpha error was set as 0.05 divided by 3 (0.0167) to allow for α error of 0.05 (level of significance of 95%) after Bonferroni correction for three post hoc pairwise comparisons between time points.

3 | Results

Fifteen dogs were initially enrolled. Two dogs were withdrawn from the study: one dog on Week 9 of the first arm due to pyelonephritis and poor owner compliance, and another dog on Week 6 of the second arm due to acute decompensation of CKD. Thirteen dogs were included in the study, of which nine (69%) were females and four (31%) were males, with a median age of 3 years (range, 1–15) and a median body weight of 25.0 kg (range, 4.8–42.8). Breeds included: mixed (eight dogs), Labrador retriever, Golden retriever, Pekingese, Boxer, and Japanese Spits (one dog each).

Dogs were classified into CKD stages at the time of inclusion: 10, two, and one dog were classified into CKD Stages 2, 3, and 4, respectively. CKD etiologies included juvenile onset CKD (six dogs), idiopathic (three dogs), post-AKI (three dogs), and primary glomerular disease (one dog).

The median initial PTH concentration of all dogs was 135 pg/mL (range, 3–2400). Initial PTH serum concentration was below, within, and above the reference range (42–586 pg/mL) in four, five, and four dogs, respectively. The median initial FGF-23 concentration of all dogs was 569 pg/mL (range, 89–4216).

The median initial paricalcitol dose was 13.7 ng/kg (range, 10.3–15.1). Mild hypercalcemia (median 1.55 mmol/L, range, 1.41–1.67) developed in 7/13 (54%) dogs while treated with paricalcitol. In three dogs, the hypercalcemia was first documented on the last visit of the arm; therefore, dose reduction was not indicated. In the remaining four dogs, i-Ca normalized after paricalcitol dose reduction. The dose was reduced once in

TABLE 1 | Concentrations of ionized and total calcium, creatinine, and phosphorus, and the product of total calcium and phosphate in 13 paricalcitol- and placebo-treated dogs with chronic kidney disease at baseline and after 12 weeks of treatment.

	Paricalcitol			Placebo			*p*	*p*
	Mean,[a] 95% CI			Mean,[a] 95% CI			Time	Treatment
	Baseline	12 weeks	*p*	Baseline	12 weeks	*p*	All[b]	All[c]
Ionized calcium (mmol/L)[d]	1.30, 1.29–1.35	1.34, 1.27–1.40	0.12	1.30, 1.25–1.35	1.28, 1.24–1.32	0.34	—	—
Total calcium (mg/dL)	10.7,[e] 10.9–11.4	11.1,[e] 10.7–11.5	0.22	10.5,[e] 10.3–10.7	10.9,[e] 10.2–11.6	0.21	0.02	0.11
Creatinine (mg/dL)	2.8, 2.1–3.5	3.0, 2.3–3.63	0.75	2.5, 2.1–3.0	3.1, 2.2–4.1	0.08	0.01	0.60
Phosphate (mg/dL)	4.1, 3.8–4.5	4.7, 4.3–5.0	0.01	3.9, 3.5–4.3	4.5, 3.7–5.3	0.12	< 0.001	0.15
CaXP (mg^2/dL^2)	44, 37–49	51, 47–55	0.04	42, 37–47	49, 39–58	0.06	0.001	0.14

[a]Presented are means and 95% CI of separate GEE analyses for each treatment group (paricalcitol or placebo).
[b]*p* value for comparison between means at baseline and 12 weeks for all dogs analyzed in one model.
[c]*p* value for comparison between means of paricalcitol and placebo treatments for all dogs analyzed in one model.
[d]*p* values of interaction between time and group are < 0.1 for ionized calcium and > 0.1 for all other variables.
[e]Mean adjusted to arm order.

three dogs and twice in one dog, and no dog had to be removed from the study due to unresolved hypercalcemia. The final median paricalcitol dose of all dogs after dose adjustments was 12.3 ng/kg (range, 7.9–15). Treatment-related adverse reactions were not reported by any of the dogs' owners.

At baseline, PTH concentrations were positively correlated with urea concentrations ($r = 0.58$, $p = 0.04$), FGF-23 concentrations were positively correlated with creatinine concentrations ($r = 0.93$, $p = 0.02$) and total calcium concentrations ($r = 0.91$, $p = 0.03$). In addition, total calcium concentrations were positively correlated with creatinine ($r = 0.90$, $p < 0.001$).

3.1 | Concentrations of Calcium, Phosphate, and Creatinine

There was an interaction between treatment and time in the analysis of i-Ca ($p = 0.06$), but not in all other models, including creatinine ($p = 0.40$), total calcium ($p = 0.35$), phosphate ($p = 1.00$), and the product of total calcium and phosphate (CaXP; $p = 0.79$). Results of these variables are reported for each group in Table 1. Arm order had a significant effect in the analysis of total calcium ($p = 0.03$), but not in all other models, including i-Ca ($p = 0.15$), creatinine ($p = 0.63$), phosphate ($p = 0.90$), and the CaXP ($p = 0.14$). Concentrations of total calcium were significantly higher in dogs that were initially treated with paricalcitol and then placebo compared to dogs that were initially treated with placebo and then paricalcitol (mean, 11.3 mg/dL, 95% CI, 10.6–12.0 vs. mean 10.4 mg/dL, 95% CI, 10.2–10.7, $p = 0.03$).

3.2 | Concentrations of PTH and FGF-23, and UPC Ratio

There were interaction effects between treatment and visit in the analyses of PTH ($p = 0.02$), FGF-23 ($p < 0.001$), and UPC ($p = 0.09$). Therefore, results are reported separately for the paricalcitol- and placebo-treated dogs. Arm order did not have a significant effect in any of the models ($p = 0.84$, $p = 0.92$, and $p = 0.26$, respectively).

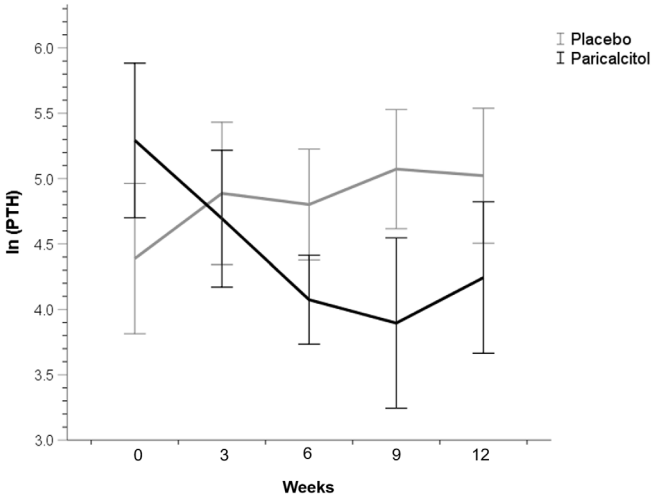

FIGURE 1 | Serum concentrations of PTH in 13 paricalcitol- and placebo-treated dogs with chronic kidney disease at baseline and after 3, 6, 9, and 12 weeks of treatment. Measured serum concentrations (mean and SD) are presented on a natural logarithmic scale.

Concentrations of PTH decreased by 22% (7%–35%, $p = 0.006$) with each visit in the paricalcitol-treated dogs and increased by 18% (2%–37%, $p = 0.02$) with each visit in the placebo-treated dogs (Figure 1). PTH concentrations were significantly lower at 12 weeks compared with baseline in the paricalcitol-treated dogs (mean 62 pg/mL, 95% CI, 22–173 vs. mean 166 pg/mL, 95% CI, 54–514, $p = 0.04$) but were not significantly different between 12 weeks and baseline in the placebo-treated dogs (mean, 157 pg/mL, 95% CI, 68–365 vs. mean 81 pg/mL, 95% CI, 27–237, $p = 0.11$, Figure 2A).

Concentrations of FGF-23 were significantly higher at 12 weeks compared with baseline in the paricalcitol-treated dogs (mean

6941 pg/mL, 95% CI, 1781–20 057 vs. mean 489 pg/mL, 95% CI, 188–1272, $p < 0.001$), but there was no difference in FGF-23 concentrations between 12 weeks and baseline in the placebo-treated dogs (mean 696 pg/mL, 95% CI, 316–1531 vs. mean 955 pg/mL, 95% CI, 308–2963, $p = 0.53$, Figure 2B).

The UPC ratio was significantly higher at 12 weeks compared with baseline in the placebo-treated dogs (mean, 0.8, 95% CI, 0.3–1.3 vs. mean, 0.5, 95% CI, 0.2–0.9, $p = 0.04$), but there was no difference in UPC ratio between 12 weeks and baseline in the paricalcitol-treated dogs (mean 0.6, 95% CI, 0.3–0.9 vs. mean 1.0, 95% CI, 0.1–1.8, $p = 0.35$, Figure 2C).

4 | Discussion

In our study, paricalcitol treatment decreased PTH concentrations in dogs with CKD, while there was no significant change in PTH concentrations in the placebo group. Although i-Ca concentrations did not significantly increase following 12 weeks of paricalcitol treatment, dose reduction was necessary in some of the dogs, as mild hypercalcemia was recognized during the treatment.

The results of this study suggest that a 12-week treatment with paricalcitol effectively decreases PTH concentrations, thus attenuating RHPT in dogs with CKD. Controlling RHPT is a major

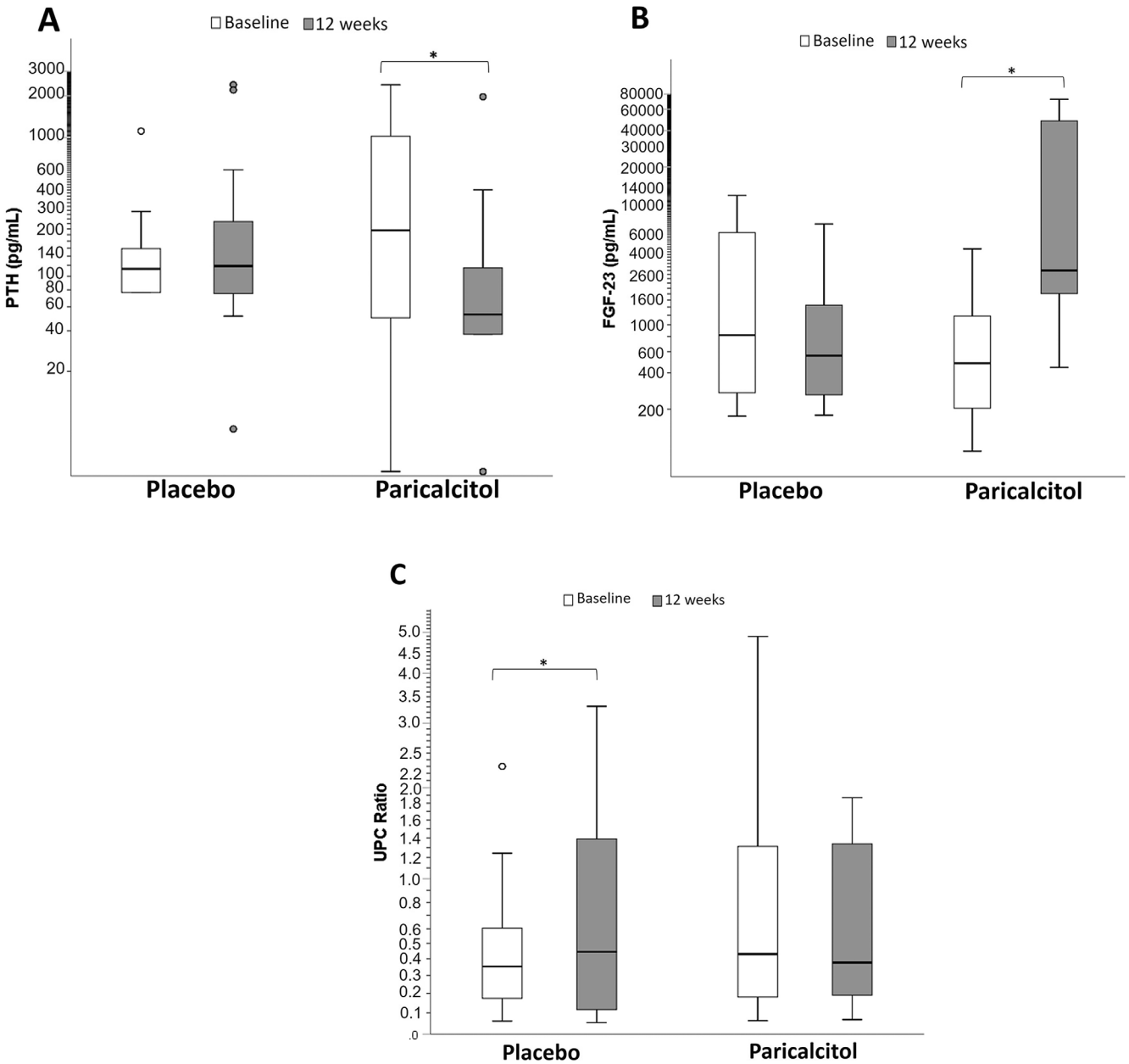

FIGURE 2 | Serum concentrations of PTH (A), FGF-23 (B), and UPC ratio (C), in 13 paricalcitol- and placebo-treated dogs with chronic kidney disease at baseline and after 12 weeks of treatment. Measured serum concentrations are presented on a natural logarithmic scale. The horizontal bar within the box plot indicates the median, the box contains the middle half of the results, and the whiskers indicate the range. *$p < 0.05$.

goal in the management of human patients with CKD [32–34]. Previous data suggest that treatment with vitamin D compounds is associated with prolonged survival in both dialysis-dependent and nondependent human patients, as well as dogs with CKD [17, 35]. The survival benefit likely results from better control of RHPT as well as from the renoprotective effects of these compounds against renal injury, including attenuation of processes implicated in CKD progression, such as inflammation, fibrosis, and proteinuria [36]. Reduction in RHPT was demonstrated in dogs with CKD treated with the VDA oxacalcitriol [26]. Yet, in a more recent study, treatment of dogs with CKD with the metabolite calcifediol did not result in PTH reduction [37]. Our study was designed to evaluate the short-term effect of a treatment with the VDA paricalcitol on RHPT rather than to evaluate its effect on survival. Based on the results, paricalcitol can be considered as part of the management of dogs with CKD to attenuate RHPT, and potentially delay its deleterious consequences. However, to further evaluate the effect of paricalcitol on slowing down CKD progression, longer-term investigation is warranted. Assessment of the change in UPC ratio as a marker of CKD progression might suggest a beneficial effect of paricalcitol treatment. Proteinuria worsened in the placebo group and remained unchanged in the paricalcitol-treated group. Although this change was modest and of questionable clinical significance, it might be attributed to the potential renoprotective effects of paricalcitol, which is known to reduce proteinuria in humans with CKD [20, 21, 38]. To thoroughly assess the renoprotective effects of paricalcitol, independent of PTH reduction, further evaluation, including long-term treatment and its influence on mineral bone disease, the hormonal axis, and CKD progression, is needed.

Vitamin D compounds are not routinely used in dogs due to the risk of hypercalcemia, necessitating close monitoring. The newer generation VDAs such as paricalcitol were initially considered to have all the benefits of activated vitamin D metabolites with fewer adverse effects, specifically hypercalcemia [39]. In our study, there were no significant changes in the i-Ca concentrations over the study period during the placebo and paricalcitol treatments. However, there was a significant difference in the direction of the change in i-Ca with the two treatments, an increase occurred in the paricalcitol group, while a decrease occurred in the placebo group. In addition, ionized hypercalcemia developed in about half of the dogs during paricalcitol treatment, necessitating dose reduction. Despite the fact that no dog had to be removed from the study, as the i-Ca concentrations were only mildly increased and hypercalcemia rapidly resolved with dose adjustments, the potential benefit of a less intensive monitoring protocol with paricalcitol treatment cannot be recommended based on these results. Our results are consistent with recent findings of a meta-analysis of human patients with CKD, in which treatment with paricalcitol was found to increase the risk for hypercalcemia compared with placebo [40]. The initial paricalcitol dose in our study was an extrapolation of the human dose, namely, numerically four times higher than calcitriol. Since dose reduction resulted in the resolution of hypercalcemia, possibly, an initial lower paricalcitol dose can be administered, reducing the risk of treatment-related hypercalcemia, as suggested in the human literature [40].

Phosphate concentrations increased during paricalcitol treatment. However, the increase in phosphate was mild and did not result in hyperphosphatemia in any of the dogs. Treatment-related increase in phosphate is a potential side effect in human patients with CKD [41]. Since hyperphosphatemia is a risk factor for CKD progression in dogs, it is crucial that phosphate is well controlled before initiating paricalcitol treatment.

Our results demonstrate a significant increase in FGF-23 during paricalcitol treatment. This is consistent with studies in both dogs and human patients treated with vitamin D compounds, including paricalcitol [37, 41, 42]. Activated vitamin D interacts with various factors in mineral metabolism, including calcium, phosphate, PTH, and the FGF23–klotho system. Increases in FGF-23 might result from either a direct stimulatory effect of the activated metabolites of vitamin D or through an indirect effect mediated through alterations in the phosphate or calcium concentrations. Since vitamin D increases circulating phosphate concentration resulting in FGF-23 increase [43], it is plausible that paricalcitol will have a similar effect. Indeed, despite the increase in circulating phosphate concentrations in all dogs, the finding was more evident during paricalcitol treatment, similar to what is seen in human patients treated with paricalcitol [41]. Concentration of i-Ca did not increase significantly during paricalcitol treatment, but mild hypercalcemia did develop in about half of the dogs. Calcium-induced increases in FGF-23 expression and circulating FGF-23 concentrations have been described in vitamin D receptor-deficient mice [44]. The strong positive correlation between concentrations of calcium and FGF-23 found at baseline in this study supports a similar effect of calcium on FGF-23 in dogs. Thus, the increases in i-Ca in some of the dogs could potentially also be contributed to the increase in FGF-23. Finally, although CaXP increased in all dogs, it was more evident in the paricalcitol-treated dogs. The CaXP strongly correlates with changes in FGF-23 during paricalcitol treatment in human patients with CKD [42], suggesting paricalcitol might be the cause of the FGF-23 increase. It is well established that FGF-23 is associated with all-cause mortality, cardiovascular dysfunction, and aortic calcification in dialysis-dependent human patients [41, 45, 46]. The implications of chronically elevated FGF-23 on the cardiovascular system and survival in general in dogs are currently unknown. It is plausible that due to their shorter lifespan compared with humans, those long-term influences of increased FGF-23 are less detrimental in dogs and cats with CKD. However, FGF-23 was also reported as a risk factor for mortality in dogs with CKD [47], therefore, decisions about whether a dog will benefit from paricalcitol treatment should be made on a case-by-case basis, including a careful assessment of the dog's hormonal axis to guide the decision.

This study has several limitations. The cohort size was relatively small, which might have led to a type 2 error in a few of the analyses. Most dogs included in this study were classified to IRIS Stage 2 CKD, during which PTH, FGF-23, as well as the phosphate, are usually within the normal range, and potentially, the results could have been more robust if more dogs with advanced disease were included. Despite that, an effect of paricalcitol treatment on PTH reduction was demonstrated. Moreover, the PTH concentrations of dogs were unknown before inclusion, and dogs were enrolled based on CKD diagnosis and not the presence of RHPT, which led to the inclusion of dogs with normal PTH concentrations. Although the paricalcitol effect was still evident even with those dogs included, further investigation

of the treatment effects in dogs with pre-diagnosed RHPT is warranted, as this should probably be the target population for this treatment. Another limitation is the lack of standardized CKD management of the dogs included in the study (e.g., use of phosphate binders and subcutaneous fluids, the type of renal diet fed to the dogs or the time of day in which the owners administered the paricalcitol). However, all dogs were managed by the same three internists and with a rather uniform protocol practiced in our institution, and each dog served as its own control.

In conclusion, paricalcitol effectively decreased PTH concentrations in dogs with CKD, and therefore is a potential treatment for RHPT. However, due to the possible adverse consequences of this treatment, including hypercalcemia, increased phosphate, CaXP, and FGF 23, paricalcitol initiation needs to be carefully considered on a case-by-case basis, including thorough assessment of the hormonal axis, calcium, and phosphate, to decide if the potential to benefit from the treatment is high enough in light of the potential unwanted consequences and need for intensive monitoring. If treatment is initiated, close monitoring of calcium and phosphate is recommended, and dose adjustment should be made accordingly.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Authors declare human ethics approval was not needed. The study was aproved by the Institutional Animal Care & Use Committee (approval number MD-21-16662-2). Dogs were included only after owner consent was obtained.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.