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Paricalcitol Versus Calcifediol for Treating Hyperparathyroidism in Kidney Transplant Recipients

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Introduction: Secondary hyperparathyroidism (SHPT) and vitamin D deficiency are common at kidney transplantation and are associated with some early and late complications. This study was designed to evaluate whether paricalcitol was more effective than nutritional vitamin D for controlling SHPT in *de novo* kidney allograft recipients.

Methods: This was a 6-month, investigator-initiated, multicenter, open-label, randomized clinical trial. Patients with pretransplantation iPTH between 250 and 600 pg/ml and calcium <10 mg/dl were randomized to paricalcitol (PAR) or calcifediol (CAL). The intention-to-treat population (PAR: n = 46; CAL: n = 47) was used for the analysis. The primary endpoint was the percentage of patients with serum iPTH >110 pg/ml at 6 months. Secondary endpoints were bone mineral metabolism, renal function, and allograft protocol biopsies.

Results: The primary outcome occurred in 19.6% of patients in the PAR group and 36.2% of patients in the CAL group ($P = 0.07$). However, there was a higher percentage of patients with iPTH <70 pg/ml in the PAR group than in the CAL group (63.4% vs. 37.2%; $P = 0.03$). No differences were observed in bone turnover biomarkers and bone mineral density. The estimated glomerular filtration rate was significantly higher in the CAL group than in the PAR group without differences in albuminuria. In protocol biopsies, interstitial fibrosis and tubular atrophy tended to be higher in the PAR group than in the CAL group (48% vs. 23.8%; $P = 0.09$). Both medications were well tolerated.

Conclusion: Both PAR and CAL reduced iPTH, but PAR was associated with a higher proportion of patients with iPTH <70 pg/ml. These results do not support the use of PAR to treat posttransplantation hyperparathyroidism.

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Kidney allograft recipients are considered patients with chronic kidney disease—mineral and bone disorder (CKD-MBD), including hyperparathyroidism and post-transplantation bone disease.[1] Immunosuppression, severity of hyperparathyroidism, disturbed vitamin D levels, and the degree of renal function achieved play a key role in CKD-MBD disturbances after transplantation. Therefore, various guidelines recommend exhaustive monitoring of mineral metabolism in patients with transplants.[2,3]

Secondary hyperparathyroidism (SHPT) is a common complication in CKD. Although kidney transplantation rapidly restores the glomerular filtration rate (GFR) and

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the renal capacity to respond to parathyroid hormone (PTH),[4,5] in 20% to 30% of cases, parathyroid gland resistance to inhibitory feedback persists several years after transplantation.[5] Inappropriately high PTH levels are associated with hypercalcemia, hypophosphatemia, renal allograft calcifications and dysfunction, loss of bone mineral density (BMD), an increased risk of fracture, vascular calcification, and an increased risk of cardiovascular events.[6–9] These conditions are named tertiary hyperparathyroidism or persistent hyperparathyroidism after kidney transplantation.[10]

Reduced vitamin D levels are frequent and persist in being low after kidney transplantation.[1] Vitamin D metabolism can be influenced by allograft dysfunction, persistent hyperparathyroidism, and elevated fibroblast growth factor-23 (FGF23).[1] Although FGF23 reduces conversion of 25 to 1,25-(OH)2-D, PTH and hypophosphatemia[11] have the opposite effect and can increase 1,25(OH)2-D synthesis, which can reduce the 25(OH) levels by increasing its conversion.[1] Some observational studies have investigated the correlation between vitamin D deficiency and graft outcomes.[1,12] It has been reported that low 25(OH)D levels predict GFR decline and mortality.[13,14] Although international guidelines recommend replenishment of vitamin D, there are few studies on vitamin D therapy in renal transplantation.[1] Therefore, there is a lack of recommendations regarding the use of nutritional vitamin D, active vitamin D, or vitamin D receptor activator (VDRA).

In CKD patients, a large number of studies comparing paricalcitol (PAR), a VDRA, versus active vitamin D (calcitriol) have shown the efficacy of PAR on reducing intact parathyroid hormone (iPTH) with a significantly lower risk of hypercalcemia. PAR and calcitriol, in the CKD population, have been associated with reduced cardiovascular disease and mortality,[15] although patients who received PAR had a survival advantage over those who received calcitriol.[15,16] PAR also showed antiproteinuric and nephroprotective effects, based primarily on the partial inhibition of the renin-angiotensin system, as well as endothelial protection, and anti-inflammatory and immunomodulatory properties.[17–19]

Currently, there are few studies on PAR in transplantation patients. Amer *et al.*[20] demonstrated that PAR was superior to placebo in reducing 1-year post-transplantation SHPT, although there were no differences in BMD, proteinuria, and renal function. In contrast, in prevalent kidney allograft recipients with SHPT, Trillini *et al.*[21] showed that PAR reduced iPTH, attenuated bone remodeling and mineral loss, and reduced estimated GFR (eGFR) and proteinuria. However, there is a lack of studies that have compared PAR with nutritional vitamin D. Thus, this is the first clinical trial aimed to evaluate whether PAR is more effective than calcifediol (CAL) for controlling post-transplantation hyperparathyroidism in *de novo* kidney transplantation patients with significant SHPT before transplantation.

METHODS

Study Population

This was an investigator-initiated, multicenter, parallel-group, open-label, randomized prospective clinical trial. The study was promoted by the Spanish Society of Nephrology and approved by The Spanish Drug Agency (EudraCT 2013-001326-25) and registered as Clinical Trial NCT01939977. Study participants were identified among recipients of kidney transplantations ages 18 years or older with SHPT who at the baseline evaluation fulfilled the following selection criteria: written informed consent; serum iPTH levels between 250 and 600 pg/ml in the 24 hours before the transplantation; panel of preformed antibodies <20% in the 24 hours before the transplantation or considered by the investigator to be a low immunological risk; serum calcium (corrected for albumin) <10 mg/dl in the 24 hours before the transplantation; and receiving standard immunosuppressive treatment with basiliximab, tacrolimus, mycophenolate mofetil, or mycophenolic acid and steroids. No change in the immunosuppressive regimen was allowed throughout the whole study period. Main exclusion criteria were >1 previous kidney transplantation, positive crossmatch, ABO incompatible transplantation, patients who received cinacalcet 48 hours before transplantation, and patients with any active viral infection. Use of cinacalcet, biphosphonates, and/or any type of vitamin D apart from the study medication was not allowed during the study. Women of child-bearing potential were tested for pregnancy at screening visit and were informed to avoid pregnancy during the study. Patients who did not meet inclusion and/or exclusion criteria after the screening period were considered to be a screening failure and were not included in the statistical analysis.

Study Groups

An algorithm was used to generate random numbers by computer for a simple randomization; patients who satisfied the inclusion and/or exclusion criteria were assigned sequentially at a 1:1 ratio to the PAR or the CAL group. The PAR group was treated with oral PAR (Zemplar, Abbvie Inc., North Chicago, Illinois). The starting dose of PAR was 1 μg/d, but this dose could be adjusted based on the serum iPTH and serum calcium levels (Supplementary Figure S1). Briefly, the PAR dose was increased to 2 μg/d if iPTH was >110 pg/ml and

calcium was ≤10 mg/dl. PAR dose was reduced to 1 μg every other day if iPTH was ≤110 pg/ml and calcium was >10.3 mg/dl. PAR was discontinued if calcium was >10.3 mg/dl and iPTH was >110 pg/ml. The CAL group was treated with oral CAL (Hidroferol, FAES Farma, Madrid, Spain). The starting dose was 5 drops/d (20 μg or 1200 IU), and the maintenance dose was adjusted based on the 25(OH)D levels (Supplementary Figure S2). Study medication was started in the first 7 days post-transplantation as soon as the patient satisfied the eligibility criteria and was continued up to 6 months post-transplantation. Dose adjustments of the study medications were performed at months 1 and 3. During the randomization process, patients were stratified based on if the patient was a first-time recipient of a kidney or if the patient had already had a previous kidney transplantation.

Primary and Secondary Efficacy Endpoints

The primary outcome of this study was to demonstrate the superiority of PAR treatment at early renal post-transplantation in the control of iPTH (percentage of patients with serum iPTH >110 pg/ml at 6 months) compared with the use of CAL. Secondary objectives were changes on bone turnover biomarkers and BMD, the effect on recipient alloimmune response (clinical and subclinical acute rejection, *de novo* donor-specific antibodies, parameters of renal function, blood pressure and pulse-wave velocity, and acute (subclinical rejection and borderline changes) and chronic (interstitial fibrosis and tubular atrophy [IFTA]) allograft damage and calcification (in hematoxylin and eosin stained samples) in the 6-month protocol biopsy. The assessment of serum iPTH, CAL, calcium, phosphorus, FGF23, and bone turnover biomarkers was performed in a central laboratory. A pathologist who was unaware of study groups diagnosed protocol biopsies. From a safety point of view, the frequency of adverse events related to treatment with PAR and CAL, and the incidence of adverse effects that required treatment discontinuation between the 2 arms were evaluated.

Statistical Analyses

Continuous variables with normal or symmetrical distribution were reported as mean ± SDs. The categorical variables were described with frequencies and percentages. The differences between the 2 groups for continuous variables were analyzed using Student's *t* test or the Wilcoxon rank-sum test, as appropriate. The differences between categorical variables were analyzed using the χ^2 distribution and Fisher exact test, as appropriate. All statistical tests were considered significant if the *P* value was <0.05 for 2-tailed tests. The primary efficacy variable was analyzed using the intention-to-treat (ITT) and per-protocol approach. Statistical analysis was performed with the SAS program (SAS Institute, Cary, North Carolina). All safety analyses were conducted on the ITT data sheet using the MedRA code and classifying them as an adverse event or serious adverse event.

The study sample size was calculated based on previous publications on the effect of PAR on bone mineral metabolism in stage 3 and 4 CKD patients. It was assumed that the difference between the arm treated with PAR versus the arm treated with CAL in the proportion of patients with iPTH >110 pg/ml would be 20%. It was expected that the percentage of patients treated with PAR compared with those who received CAL with iPTH >110 pg/ml would be 20% and 40%, respectively. Based on this assumption and using the Fisher exact test with a 2-tailed significance level of 0.05 and a power of 80%, the sample size would be 90 patients per treatment group, for a total of 180 patients. The planned recruitment period was 12 months (between January 2014 and December 2014) and then extended until February 2015 due to the low recruitment rate. Recruitment was stopped on February 2015, after including 148 screened patients. The reason was budget limitations related to the nature of the funding source.

RESULTS

Study Population

Of 148 screened participants, 48 patients were excluded because they did not fulfill the inclusion criteria, 2 patients decided not to participate in the study, and 4 patients were excluded for other reasons. Thus, 94 participants were randomized: 46 patients to PAR and 48 patients to CAL (Figure 1). One patient was not included in the ITT analysis because this patient did not receive study medication due to protocol deviation related to the inclusion criteria. Baseline characteristics of the 2 groups were similar (Table 1). The initial PAR dose was 1 μg/d. At 3 months, PAR doses were as follows: 1 μg/d (50%); 1 μg/48 hours (8.7%); 2 μg/d (39.1%); and discontinued in 2.2%. At 6 months, PAR doses were as follows: 1 μg/d (60.9%); 1 μg/48 h (8.7%); 2 μg/d (21.7%); and discontinued in 8.7%. The CAL starting dose was 5 drops/d. At 3 months, CAL doses were changed as follows: 5 drops/d (36.2%); 7 drops/d (61.7%); and discontinued in 2.1%. At 6 months, doses were 5 drops/d (80.9%), 7 drops/d (14.9%), and 4.2% discontinued (Table 2).

Primary Efficacy Analysis

If not specified, all the comparisons were performed on the ITT population. The number of patients with serum iPTH levels >110 pg/ml at 6 months of follow-up was

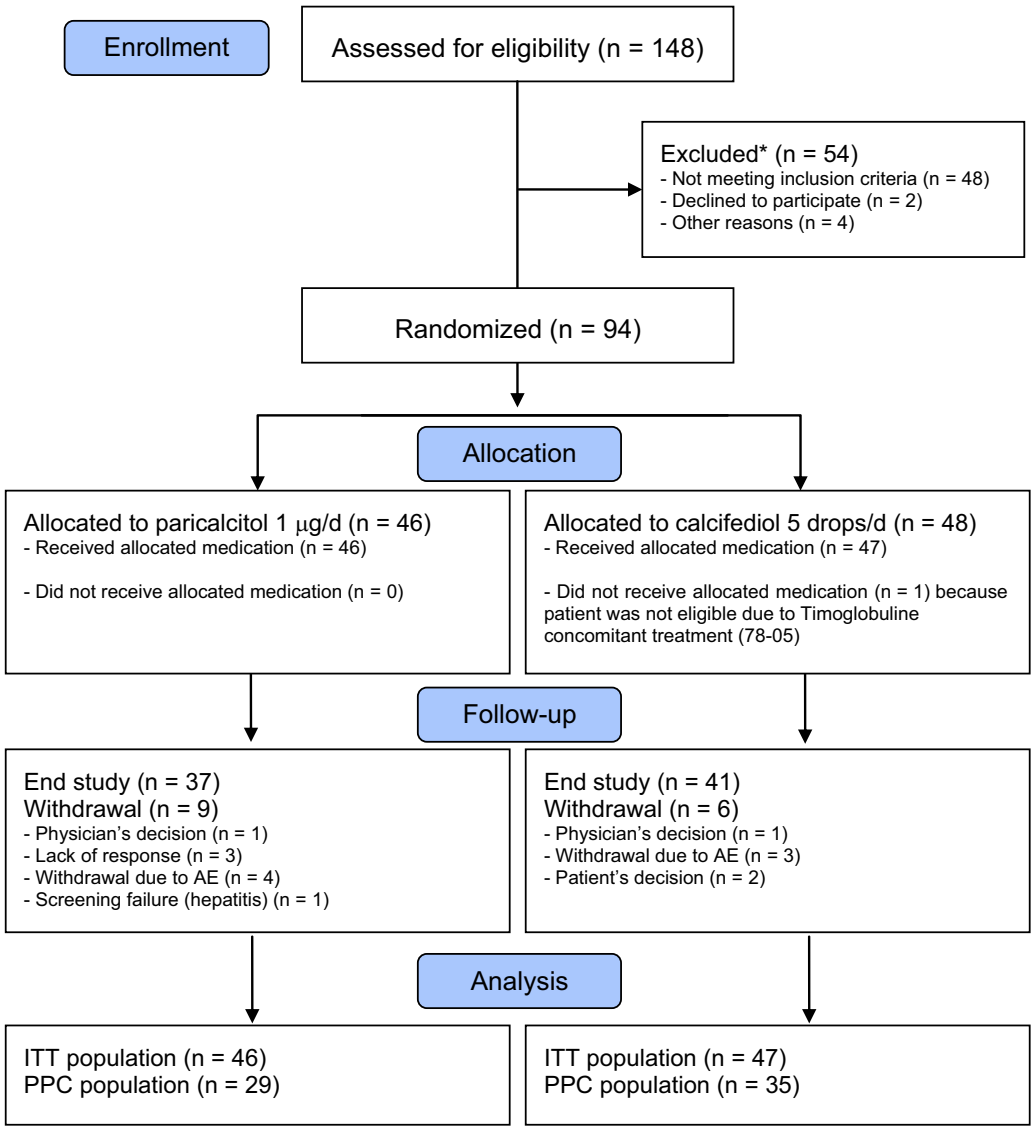

Figure 1. CONSORT flow diagram of the study population. The intention-to-treat population (ITT) was defined as randomized patients who took at least 1 dose of study medication. The per-protocol (PPC) population was defined as the ITT patients who fulfilled the medication algorithm without major protocol deviations. There were 112 protocol deviations (1 related to selection criteria, 50 related to procedures, and 4 related to forbidden medication). AE, adverse event.

lower in the group treated with PAR, but this was not statistically significant (9 patients vs. 17 patients; 19.6% vs. 36.2%; $P = 0.07$). However, when analyzing the per-protocol population, a statistically significant result was observed (2 patients vs.12 patients; 6.9% vs. 34.3%; $P = 0.008$). We also observed that serum iPTH levels were significantly lower in patients who received PAR (76 ± 55 pg/ml vs.101 ± 55 pg/ml; $P = 0.0036$) at 6 months of follow-up (Figure 2a and Table 2). No significant difference was observed between the groups in the proportion of patients with a reduction in serum iPTH levels of ≥30% (95.1% vs. 88.4% in PAR and CAL groups, respectively; $P = 0.26$). The proportion of patients with iPTH between 70 and 110 pg/ml was 14.6% in the PAR group and 25.6% in the CAL group ($P = 0.056$). As shown in Figure 2b, there was a significantly higher proportion of patients in the PAR group with iPTH <70 pg/ml (63.4% vs. 37.2%; $P = 0.028$).

Effect on Mineral and Bone Disorder Outcomes

All bone turnover biomarkers (osteocalcin, bone alkaline phosphatase, and C-terminal telopeptide) significantly decreased at month 6 in comparison to baseline (Table 2), without significant differences between groups. FGF23 also decreased in both treatment groups, although, at 6 months of follow-up, FGF23 values were lower in patients treated with CAL. Femoral neck and lumbar spine BMD, as assessed by dual-energy x-ray absorption, showed no significant differences between treatments. Similar BMD results were observed when the per-protocol population was analyzed (data not shown).

Table 1. Patient baseline characteristics

Variable	All patients ($N = 93$)	Paricalcitol ($n = 46$)	Calcifediol ($n = 47$)	P value
Age (yr)	57.5 ± 13.8	58.9 ± 13.0	56.2 ± 14.5	0.38
Sex (M/F)	63/30	32/14	31/16	0.71
Caucasian, n (%)	87 (93.5)	44 (95.7)	43 (91.5)	0.36
BMI (kg/m^2)	26.6 ± 4.6	26.9 ± 4.8	26.3 ± 4.4	0.56
Cause of ESRD				
Diabetes	12	6	6	
Hypertension	5	1	4	
Glomerulonephritis	21	13	8	
APKD	9	2	7	
Unknown	26	15	11	
Other	20	9	11	0.34
Previous transplant (yes/no)	6/87	3/43	3/44	1.00
Pretransplant PRA (%)	1.0 ± 4.2	1.5 ± 5.7	0.4 ± 1.4	0.97
Donor type (live/deceased)	20/73	9/37	11/36	0.65
Donor age (yr)	59.1 ± 15.9	61.3 ± 15.2	56.9 ± 16.4	0.25
Donor sex (M/F)	44/49	21/25	23/24	0.60
HLA DR match (0/1/2)	14/40/39	7/22/17	7/18/22	0.60

APKD, adult polycystic kidney disease; BMI, body mass index; ESRD, end-stage renal disease; HLA, human leukocyte antigen; HLA-DR, human leukocyte antigen–antigen-D related; PRA, panel reactive antibodies.

Other Secondary and Exploratory Outcomes

Overall, acute rejection was diagnosed in 7 patients (7.5%). Incidence of acute rejection was 10.9% and 4.3% in the PAR and CAL groups, respectively ($P = 0.23$). Incidence of delayed graft function was 11.8% (17.4% in the PAR group and 6.4% in the CAL group; $P = 0.12$).

GFR was estimated by Chronic Kidney Disease Epidemiology Collaboration. Both study groups showed a similar eGFR at 1 month. However, eGFR at 3 and 6 months was significantly higher in the CAL group than in the PAR group (Figure 3a). Furthermore, at 6 months, the percentage of patients with eGFR <30 ml/min per 1.73 m^2 was 26.9% and 13.9% in the PAR and CAL groups, respectively ($P = 0.18$). Likewise, eGFR ≥60 ml/min per 1.73 m^2 was 14.6% in the PAR group and 27.9% in the CAL group ($P = 0.18$) (Figure 3b). In contrast, no significant differences between treatments were observed for albuminuria (Table 2) or proteinuria (data not shown).

Six-month protocol biopsies were performed in 36 patients (17 treated with PAR and 19 treated with CAL). Protocol biopsies were not performed if the patients had allograft failure (n = 2), medical and technical contraindications (n = 10), medical decisions (n = 15), and decisions by the patients (n = 30). There were no differences with regard to subclinical acute rejection and borderline changes. However, the presence of IFTA tended to be higher in the PAR group than in the CAL group (Figure 4). Tubulointerstitial calcifications in protocol biopsies were seldom found (only 1 patient in the PAR group). Of 93 analyzed

Table 2. Evolution of serum calcium, phosphate, iPTH, biomarkers of bone turnover and renal function

	Paricalcitol			Calcifediol		
Measurement	Baseline	Month 3	Month 6	Baseline	Month 3	Month 6
iPTH (pg/ml)	338 ± 135	83 ± 50[a,b]	76 ± 55[a,b]	315 ± 118	99 ± 39[a,b]	101 ± 55[a,b]
FGF23 (pg/ml)	633 (102; 19,468)	—	157 (37; 46,314)[a]	607 (37; 23,241)	—	101 (37; 6208)[a,c]
Calcium (mg/dl)	8.89 ± 0.62	9.58 ± 0.6[a]	9.69 ± 0.5[a]	8.93 ± 0.64	9.52 ± 0.5[a]	9.57 ± 0.5[a]
Phosphorus (mg/dl)	4.27 ± 1.36	3.25 ± 0.7[a]	3.42 ± 1.0[a]	4.15 ± 1.12	3.04 ± 0.5[a,c]	3.10 ± 0.6[a]
25(OH)D3 (ng/ml)	18.7 ± 11.2	—	—	17.8 ± 8.2	47.6 ± 18.1[a]	49.5 ± 25[a]
Dosing (%)		2 μg/d (39.1) 1 μg/d (50) 1 μg/48 h (8.7) Stop (2.2)	2 μg/d (21.7) 1 μg/d (60.9) 1 μg/48 h (8.7) Stop (8.7)		1800 UI/d (61.7) 1200 UI/d (36.2) Stop (2.1)	1800 UI/d (14.9) 1200 UI/d (80.9) Stop (4.2)
Bone resorption biomarker						
C-terminal telopeptide (ng/ml)	1.33 ± 0.75	—	0.44 ± 0.30[a]	1.29 ± 0.60	—	0.56 ± 0.30[a]
Bone formation biomarkers						
Alkaline phosphatase (UI/l)	13.8 ± 6.9	—	11.7 ± 9.1[a]	14.5 ± 7.4	—	11.8 ± 5.1[a]
Osteocalcin (UI/l)	11.7 ± 9.2	—	4.0 ± 3.6[a]	12.6 ± 8.5	—	5.2 ± 4.1[a]
Renal function						
eGFR (ml/min)	—	40 ± 19	43 ± 19	—	49 ± 18[c]	52 ± 21[c]
Albuminuria (mg/g)	—	103 ± 164	59 ± 111	—	51 ± 52	48 ± 61
Bone mineral density						
Femoral neck (T-score)	−1.05 (1.5)	—	−1.13 (1.7)	−1.28 (1.3)	—	−1.25 (1.3)
Lumbar spine (T-score)	−0.64 (1.8)	—	−0.47 (1.8)	−0.45 (1.9)	—	−0.77 (1.8)
Pulse-wave velocity						
PWV (m/s)	8.8 ± 3.2		9.4 ± 2.8	8.7 ± 2.5		9.3 ± 3.9
PWV <10 m/s	75%		64.7%	68.2%		70.6%

eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor-23; iPTH, intact parathyroid hormone; PWV, pulse-wave velocity.
The FGF23 results are reported and analyzed as median (minimum, maximum).
[a] $P < 0.05$ value versus baseline value.
[b] $P < 0.05$ calcifediol versus paricalcitol.
[c] $P < 0.10$ calcifediol versus paricalcitol.

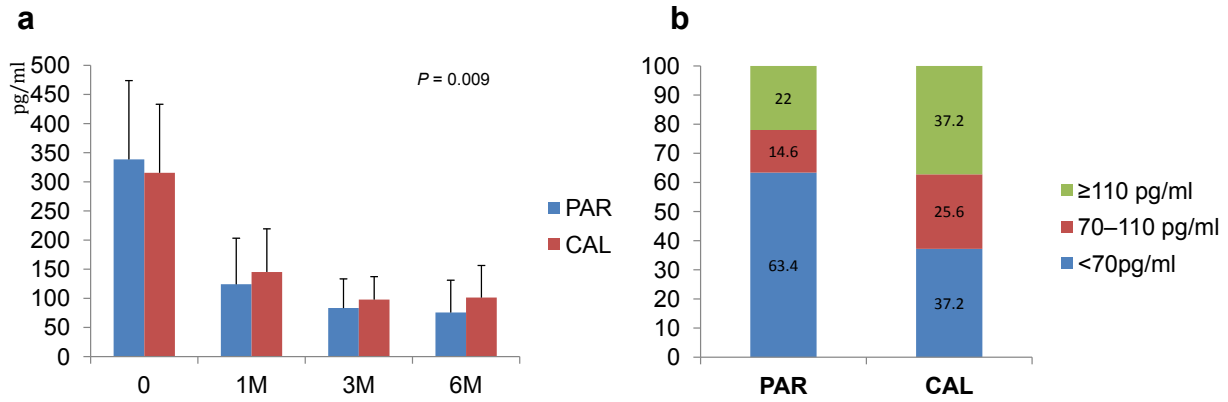

Figure 2. Serum intact parathyroid hormone (iPTH) levels and iPTH distribution at 6 months. (a) Both paricalcitol (PAR) and calcifediol (CAL) treatments were associated with significant reduction of serum iPTH mean values at 1, 3, and 6 months after transplantation. Serum iPTH mean levels were lower in the PAR group than in the CAL group ($P = 0.009$). (b) iPTH mean values distribution at 6 months after transplantation was different between the PAR and CAL groups. The percentage of serum iPTH <70 pg/ml was higher in the PAR group than in the CAL group ($P = 0.028$). The percentage of patients with serum iPTH on target (70–110 pg/ml) was 14.6% in the PAR group and 25.6% in the CAL group ($P = 0.056$).

patients, 82 were tested to detect the presence of anti-human leukocyte antigen antibodies at the moment of transplantation and at month 6. Ten patients showed a positive result at transplantation, 5 in each study group, but only 1 in the CAL group had a donor-specific antibody. At 6 months of follow-up, 2 patients (1 in each study arm) developed *de novo* donor-specific antibodies.

Hypertension was evaluated by 24-hour ambulatory blood pressure at baseline and at 6 months. Baseline results were similar in both study groups. Daytime systolic blood pressure at 6 months was 132 ± 15 and 136 ± 16 mm Hg in the PAR and CAL groups, respectively ($P = 0.29$). Daytime diastolic blood pressure at 6 months was 79 ± 11 and 78 ± 10 mm Hg in the PAR and CAL groups, respectively ($P = 0.62$). At nighttime, similar results were observed between treatments (systolic blood pressure was 117 ± 41 and 129 ± 27 mm Hg; $P = 0.20$, and diastolic blood pressure was 67 ± 25 and 72 ± 15 mm Hg; $P = 0.89$; in the PAR and CAL groups, respectively). Similarly, no significant differences in the pulse-wave velocity were observed between treatments (Table 2). Use of renin-angiotensin system blockade was 13% and 14.9% in the PAR and CAL groups, respectively.

Safety

All patients were alive at the end of the study. Two patients lost their grafts, both in the CAL group (primary nonfunction and surgical-related complication). Of 93 analyzed patients, 79 (84.9%) had at least 1 adverse event, 41 in the PAR group and 38 in the CAL group (89.1% vs. 80.9%; $P = 0.26$). Most side effects were nonserious (261 of 328) in both arms, and only 3 events were considered possibly or probably related to the study drug (2 in the PAR group and 1 in the CAL group). Sixty-seven serious adverse events were reported in 44 patients (Table 3), 37 in 24 participants who received PAR and 30 in 20 patients who received CAL (52.2% vs. 42.6%; $P = 0.35$). No serious adverse event was related to the study drug. Urinary

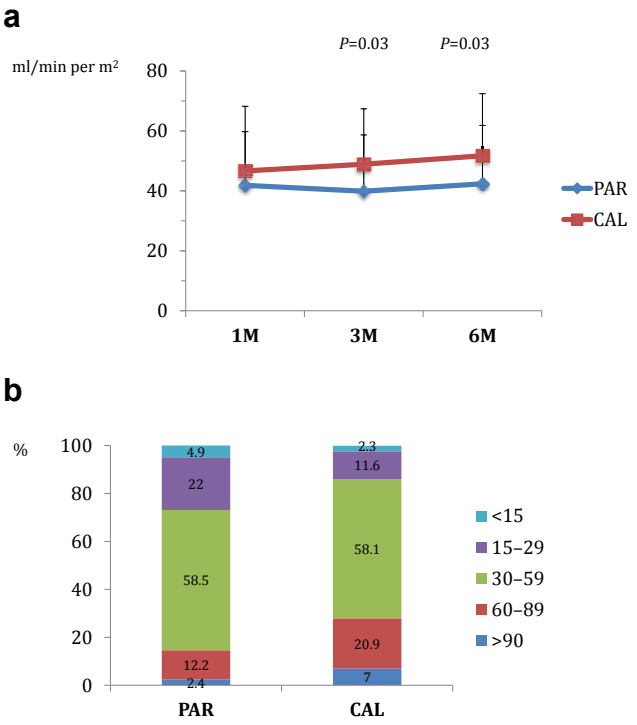

Figure 3. Estimated glomerular filtration rate (eGFR) evolution and stage of chronic disease distribution at 6 months after transplantation. (a) Estimated Chronic Kidney Disease Epidemiology Collaboration eGFR at 3 and 6 months after transplantation was significantly lower in the paricalcitol (PAR) group than in the calcifediol (CAL) group. (b) eGFR distribution according CKD stages at 6 months after transplantation. The percentage of patients with eGFR ≥60 ml/min per 1.73 m^2 was nearly double that in the CAL group than in the PAR group.

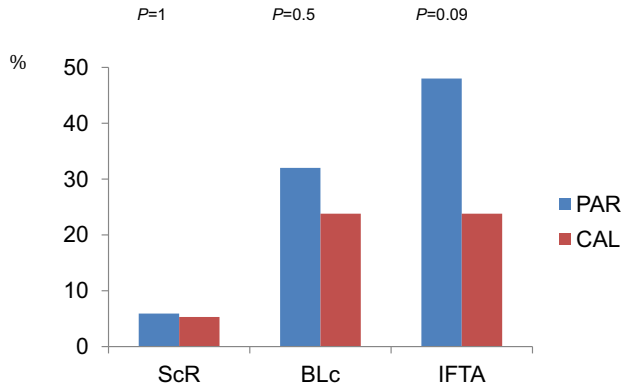

Figure 4. Histological findings in 6-month protocol biopsies. No differences were observed regarding inflammation categories (subclinical rejection [ScR] [5.6%] and borderline changes [BLc] [28.3%]). Overall, interstitial fibrosis and tubular atrophy (IFTA) was diagnosed in 37% of protocol biopsies. However, this diagnosis was nearly double in the paricalcitol (PAR) group than in the calcifediol (CAL) group (48% vs. 23.8%; $P = 0.09$).

tract infection, renal impairment, and diarrhea were the most commonly reported adverse events in 23.7%, 15.1%, and 11.8% of patients, respectively. Cytomegalovirus infection was 4.3% in each study group. Overall, hypercalcemia, defined as serum calcium >10.3 mg/dl, was reported in 7.1% and 4.7% in the PAR and CAL groups, respectively ($P = 0.67$). Hypercalcemia that caused dose adjustment of study medications was numerically higher in the PAR group. At month 1, hypercalcemia was observed in 7% and 2.2% in the PAR and CAL groups, respectively ($P = 0.35$). At month 3, hypercalcemia was observed in 12.2% and 4.4% in the PAR and CAL groups, respectively ($P = 0.19$).

DISCUSSION

Among the CKD population, CKD-MBD is common and becomes even worse after kidney transplantation. There is a significant bone loss in the first year after transplantation, followed by mild improvement thereafter.[22,23] In addition to pretransplantation pathogenic mechanisms, immunosuppression and vitamin D deficiency may account for impaired bone formation and mineralization in this setting. The Kidney Disease Improving Global Outcomes guidelines recommend vitamin D replenishment as first-line therapy in SHPT when calcidiol is <30 ng/ml. Surprisingly, most studies are observational, and interventional studies are scarce. Moreover, the natural history of PTH after transplantation is that PTH will decrease in many patients during the first 6 to 12 months,[11] which is associated with recovery of renal function and could be a confounding factor in clinical trials. To minimize this effect and in contrast with the study by Amer *et al.*,[20] we decided to include only patients with clinically

Table 3. Summary of patients with adverse events

Adverse event	Paricalcitol $n = 46$	Calcifediol $n = 47$	Total $N = 93$
Patients who reported at least 1 adverse event $P = 0.26$ (χ^2)	41 (89.1)	38 (80.9)	79 (84.9)
Patients who reported an adverse event possibly or probably related to the study drug $P = 0.62$ (χ^2)	2 (4.3)	1 (2.1)	3 (3.2)
Patients who had serious adverse events $P = 0.35$ (χ^2)	24 (52.2)	20 (42.6)	44 (47.3)
Patients who reported a serious adverse event possibly or probably related to the study drug	0 (0.0)	0 (0.0)	0 (0.0)
Patients who were withdrawn due to an adverse event $P = 0.68$ (Fisher)	2 (4.3)	4 (8.5)	6 (6.5)
Patients who reported moderate or severe adverse events $P = 0.14$ (χ^2)	34 (73.9)	28 (59.6)	62 (66.7)

Values are n (%).

significant SHPT before transplantation (iPTH 250–600 pg/ml). Wolf *et al.*[24] demonstrated that patients with pretransplantation iPTH >300 pg/ml had iPTH levels at 6 months that were approximately 200 pg/ml, and persistent SHPT (defined as PTH >65 pg/ml) was observed in >90% of these patients. Thus, the achievement of iPTH normalization at 6 months in the patients enrolled in the paridoinal study was unlikely done without treatment.

Our study was the first one to compare the use of PAR with nutritional vitamin D replenishment in *de novo* renal allograft recipients with SHPT before transplantation. We found that, although PAR seemed more effective than CAL to reduce iPTH, it was associated with a higher risk of iPTH <70 pg/ml. Although the ideal iPTH level after kidney transplantation is unknown and probably will depend on the GFR achieved, our results suggested that PAR, rather than CAL, might exacerbate adynamic bone disease. Finding that controlled PTH production was associated with reduced serum levels of biomarkers of bone formation (e.g., osteocalcin and bone alkaline phosphatase) and at the same time, reduced biomarker of osteoclastic-mediated bone reabsorption (e.g., C-terminal telopeptide)[25] provided consistent evidence that both therapies might effectively suppress the high-turnover bone disease that characterizes SHPT. This also suggested that, in the long term, vitamin D replenishment and PAR could help to prevent progressive bone mass loss and excess risk of pathological bone fractures that are invariably associated with SHPT, particularly in recipients of kidney transplantations.[26,27] However, because osteocalcin and C-terminal telopeptide are renally cleared, the

reduction in their levels, rather than changes on bone mineral turnover, might merely be related to the recovery of renal function achieved after kidney transplantation.[28] Despite the observation on bone biomarkers and in agreement with previous studies, we observed bone loss during the first 6 months after transplantation in both study groups. This finding could be mainly related to immunosuppression, which was homogenous per protocol and consistent with the current standard of care. Amer *et al.*[20] compared PAR versus placebo in incident renal allograft recipients regardless of the pre-transplantation iPTH level. They found PAR was able to reduce iPTH but also without benefits in BMD. In contrast, Trillini *et al.*[21], in prevalent kidney transplantation with SHPT, showed that PAR versus non-PAR therapy was associated with iPTH reduction and lumbar BMD improvement. There was a previous study that demonstrated that calcium plus calcitriol attenuated BMD changes at 1 year.[29] The ongoing VITALE (Vitamin D Supplementation in Renal Transplant Recipients) study[30] is comparing a high dose of cholecalciferol versus a low dose of cholecalciferol in prevalent kidney allograft recipients with vitamin D deficiency. Although the primary endpoint explored vitamin D pleiotropic effects, there was a secondary endpoint to assess changes in BMD and bone fractures at 2 years. Further studies with more prolonged follow-up are required to assess the effects of both PAR and vitamin D replenishment on BMD and bone fractures.

FGF23 and PTH regulate each other in a negative feedback loop, in which PTH stimulates FGF23 synthesis and FGF23 suppresses iPTH production action by the Klotho-FGFR1 complex in the parathyroid gland.[1,31] However, in CKD, reduced Klotho accounts for high iPTH despite elevated FGF23. After transplantation, FGF23 levels can remain elevated for months despite restored renal function, and, in combination with high iPTH, these levels might contribute to inappropriately low levels of calcidiol.[1] High levels of FGF23 have been associated with endothelial dysfunction, volume overload, and an increased risk of cardiovascular mortality.[1,11,31] In our study, the reduction of FGF23 seemed higher in the CAL group than in the PAR group. Experimental data suggest that VRDAs could increase FGF23. Recently, Donate-Correa *et al.*[31] found that PAR treatment in prevalent kidney transplants with SHPT was associated with an increased Klotho, reduced iPTH, and increased FGF23. However, more studies are needed to investigate whether PAR and vitamin D replenishment induce different effects on the imbalanced FGF23-PTH-vitamin D axis.

We also investigated some of the pleiotropic effects attributed to PAR.[32] In particular, we monitored immune response in a homogenous cohort that received basiliximab induction therapy, tacrolimus, mycophenolate, and prednisone immunosuppression. The incidence of both clinical and subclinical acute rejection was low, in line with the results achieved with current immunosuppression in the low immunological risk population.[33] There were no differences between PAR and CAL regarding rejection and *de novo* donor-specific antibody development. These findings suggested that PAR, in comparison with nutritional vitamin D replenishment, was not associated with a higher prevention of immunologically mediated allograft damage.

We found that PAR treatment was associated with lower GFR than CAL, whereas proteinuria levels were similar. Although unexpected, these results were consistent with a previous study carried out in prevalent renal transplant recipients with SHPT. Trillini *et al.*[21] suggested that decreased creatinine tubular secretion, increased creatinine generation, or both, could explain this finding because VDRAs did not affect inulin clearance, which is the gold standard for measuring GFR. However, in agreement with a lower eGFR, we also found a higher proportion of chronic renal allograft lesions (IFTA) in 6-month protocol biopsies in patients treated with PAR. This finding was an apparent discrepancy with a previous study that compared PAR versus placebo, in which PAR was associated with reduced moderate IFTA in protocol biopsies.[20] Because our study was the first to compare PAR with vitamin D replenishment and took previous studies into account, our results suggested that the observed differences in kidney function and renal damage might rely on the beneficial effect of vitamin D replenishment rather that on the nephrotoxicity of PAR. It was reported that vitamin D downregulated renin expression, transforming growth factor β1 and tubulointerestitial fibrosis in animal models of kidney damage.[34] This effect could be particularly relevant in our cohort of patients with a high proportion of transplanted kidneys from older donors. The ongoing VITA-D (the cholecalciferol substitution in vitamin D deficient kidney transplant recipients) study will provide further information regarding the immunomodulatory and renoprotective effects of cholecalciferol in renal allograft recipients with vitamin D deficiency.[35] In contrast, tubulointerstitial calcifications in protocol biopsies were rare and only observed in 1 patient treated with PAR. Thus, renal calcification did not seem to be associated with the eGFR differences between groups.

The cause of posttransplantation proteinuria could be multifactorial, including immunological and

nonimmunological factors. Many studies suggested that PAR reduces albuminuria in kidney transplantations, although the mechanism accounting for this effect is not clear.[21] Nevertheless, Amer *et al.*[20] reported similar proteinuria in a clinical trial that compared PAR versus placebo in incident renal allograft recipients. Our results were concordant with this study and showed that PAR and vitamin D replenishment had a similar effect on the level of proteinuria and albuminuria observed after transplantation.

In CKD patients, vitamin D is considered protective against cardiovascular disease (CVD).[1,32] Vitamin D is an endocrine suppressor of the renin-angiotensin-aldosterone system.[36] Although CVD is the main cause of mortality in renal transplantation, few studies have reported an association between vitamin D deficiency and mortality in this population.[1] In this regard, the short-term follow-up in our study was a limitation to assess CVD. To estimate cardiovascular risk, we measured changes in arterial stiffness and blood pressure. Experimental studies suggested that PAR is superior to doxercalciferol to prevent vascular calcification and arterial stiffness in uremic rats.[37] Lundwall *et al.*[38] found that PAR was able to reduce pulse-wave velocity and to ameliorate endothelial dysfunction in CKD patients. These results were not confirmed in our study because we found similar blood pressure and arterial stiffness in patients treated with PAR and CAL.

Overall, treatment was well tolerated with few episodes of hypercalcemia. Despite a slight trend to more serious and nonserious adverse events with PAR therapy, all treatment-related adverse events were nonserious and generally mild; patients fully recovered after treatment withdrawal. Our study had some limitations and strengths. Limitations were sample size because the enrollment goal was not achieved, short-term follow-up, absence of 25(OH)D3 assessment in the PAR group, and lack of preimplantation renal biopsies. Some of these limitations were caused by budget constraint related to the academic nature of the study. Regarding 25(OH)D3, it seems likely that, without supplementation, the calcidiol levels remained low, as demonstrated by Perrin *et al.*[39] Nevertheless, this was the first clinical trial that compared PAR with CAL in *de novo* kidney allograft recipients with a clinically relevant degree of SHPT before transplantation. Major strengths were study design, a homogeneous and representative kidney transplant population with pretransplantation SHPT, homogeneous immunosuppression, protocol biopsies, and assessment of donor-specific antibodies.

In conclusion, in *de novo* kidney transplantation recipients with SHPT, both PAR and vitamin D replenishment reduced iPTH at 6 months after transplantation. However, PAR treatment was associated with a higher proportion of patients with iPTH <70 pg/ml, higher FGF23, lower eGFR, and more IFTA in 6-month protocol biopsies. Although these results should be interpreted with caution due to statistical power limitation, they did not support the use of PAR to treat posttransplantation hyperparathyroidism. Long-term controlled clinical trials are needed to corroborate our short-term findings.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. Paricalcitol dose-adjustment algorithm. The starting dose of Paricalcitol was 1 μg/d. Paricalcitol dose was increased to 2 μg/d if iPTH $>$ 110 pg/ml and calcium $\leq$ 10 mg/dl. Paricalcitol dose was reduced to 1 μg every other day if iPTH $\leq$ 110 pg/ml and calcium $>$ 10.3 mg/dl. Paricalcitol was discontinued if calcium $>$ 10.3 mg/dl and iPTH $>$ 110 pg/ml.

Figure S2. Calcifediol dose-adjustment algorithm. The starting dose was 5 drops (20 μg or 1200 IU)/d and the maintenance dose was adjusted based on the 25(OH)D levels.

Consortium Checklist. CONSORT 2010 checklist of information to include when reporting a randomized trial.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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