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Changes in Plasma Levels of Asymmetric Dimethylarginine in Chronic Kidney Disease Patients Treated for 8 Weeks with the Vitamin D Receptor Agonist Paricalcitol

Amy Barton Pai[1]*, Darren W Grabe[1,2], George Eisele[2], Syed Haqqie[2], Heena Patel[3], Alexander J Prokopienko[4], Thomas D Nolin[4] and Natsuki Kubotera[5]

[1]*Albany College of Pharmacy and Health Sciences, Albany, NewYork, USA*
[2]*Albany Medical College, Division of Nephrology and Hypertension, Albany, NewYork, USA*
[3]*Atlantic Health System, Morristown, New Jersey, USA*
[4]*University of Pittsburgh School of Pharmacy, Pittsburgh, Pennsylvania, USA*
[5]*Providence Health and Services, Portland, Oregon, USA*

Abstract

Objective: We investigated the effect of the vitamin D receptor agonist (VDRA), paricalcitol on biomarkers of endothelial dysfunction in patients with chronic kidney disease (CKD).

Methods: This was an 8-week, randomized, blinded, controlled trial of paricalcitol versus placebo in 40 CKD patients. Serum chemistry, asymmetric dimethyl arginine (ADMA), soluble intracellular adhesion molecule (ICAM), vascular adhesion molecule (VCAM), biomarkers of vascular reactivity (Regulated on activation, normal T cell expressed and secreted (RANTES), vascular endothelial growth factor (VEGF), monocyte chemoattractant protein (MCP-1)) and pro-inflammatory cytokines (interleukin-1(IL-1) interleukin (IL-6), tumor necrosis factor-α (TNFα)) at baseline, week 4 and week 8.

Results: Paricalcitol treated patients had significant reductions in asymmetric dimethylarginine (ADMA) serum concentrations after 4 weeks of treatment. There were no statistically significant changes in mean concentrations of cytokines or biomarkers of endothelial dysfunction among patients receiving paricalcitol or placebo. However, more than one-third of patients receiving paricalcitol had reductions in one or more of the analytes measured. Multiple logistic regression analyses showed that higher baseline serum concentrations of native vitamin D (1,25 OH vitamin D) were inversely associated with reduction of VCAM and all three cytokines evaluated.

Conclusion: Data from this pilot study show that VDRA paricalcitol is associated with short-term reductions in ADMA. Larger interventional studies are warranted.

Keywords: Paricalcitol; Chronic kidney disease; Asymmetric dimethylarginine vascular reactivity; Adhesion molecules; Cytokines

Abbreviations: VDRA: Vitamin D Receptor Agonist; ADMA: Asymmetric Dimethylarginine; ICAM: Soluble Intracellular Adhesion Molecule; VCAM: Vascular Adhesion Molecule; RANTES: Regulated on Activation Normal T cell Expressed and Secreted; VEGF: Vascular Endothelial Growth Factor; MCP-1: Monocyte Chemoattractant Protein; IL-1: Interleukin-1; IL-6: Interleukin-6; TNFα: Tumor Necrosis Factor-α; CKD: Chronic Kidney Disease; eGFR: Estimated Glomerular Filtration Rate; PTH: Parathyroid Hormone

Introduction

Patients with chronic kidney disease (CKD) have markedly increased cardiovascular disease morbidity and mortality compared to the general population. Cardiovascular disease in CKD is associated with a preponderance of non-traditional risk factors that promote accelerated atherosclerosis, inflammation and endothelial dysfunction. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase, a key regulator of vascular tone [1]. ADMA is a small water soluble compound that is persistently elevated in chronic kidney disease due to reduced urinary excretion. In a normal population cohort, low vitamin D concentrations were associated with elevated serum levels of ADMA [2]. In CKD, inflammation and oxidative stress also perpetuate a vicious cycle of vascular damage and endothelial dysfunction. Soluble cell adhesion molecules (ICAM, VCAM) promote monocytes adherence to activated endothelium are up-regulated in response to inflammatory stimuli, such as TNF-α [3]. The VDRA calcitriol has been shown to significantly reduce expression of cell adhesion molecules in human endothelial and proximal tubule cell culture models [4]. Calcitriol also reduced expression of inflammatory cytokines and ICAM-1 when incubated with immune-stimulated monocytes isolated from Type 2 diabetic patients [5,6]. Recently, both calcitriol and paricalcitol were found to downregulate expression of atherothrombotic mediators in human aortic smooth muscle cells [7]. Non-dialysis CKD patients with low native vitamin D levels, which infers low vitamin D receptor stimulation, were found to have higher concentrations of ICAM and reduced brachial artery flow-mediated dilation, indicative of endothelial dysfunction [8]. The purpose of this study was to evaluate the impact of paricalcitol treatment on serum

***Corresponding author:** Amy Barton Pai, Professor, Department of Pharmacy Practice, Albany College of Pharmacy and Health Sciences, 106 New Scotland Avenue, Albany, NY, USA, Tel: (518) 694-7203; E-mail: Amy.bartonpai@acphs.edu

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ADMA concentrations, biomarkers of vascular reactivity and cytokine activation in patients with chronic kidney disease [9].

Material and Methods

Patients

This was a randomized, blinded, parallel group study (NCT00915876) designed to evaluate and compare markers of oxidative stress, and vascular reactivity in CKD patients receiving oral paricalcitol or matching placebo. The study was approved by the Albany Medical Center Hospital Institutional Review Board. Inclusion criteria were: males or females ≥ 18 years of age at the start of screening, CKD with estimated glomerular filtration rate (eGFR) values between 15 to 60 mL/min/1.73 m^2 by the Modification of Diet in Renal Disease (MDRD) equation [10], not expected to start dialysis for 4 months, serum intact parathyroid hormone (PTH) 70-200 pg/mL during screening period. Patients taking angiotensin converting enzyme inhibitor or angiotensin receptor blocker regimen had to be on stable regimens for at least 30 days prior to screening. Patients were excluded from participation in the study if they had a history of any of the following diseases: congestive heart failure, myocardial infarction within the last 6 months, history of cerebrovascular accident, significant valvular disease, malignancy, were currently taking any vitamin D products had mean systolic blood pressure values > 190 or diastolic blood pressure values > 100 mm/Hg during the preceeding 30 day period prior to screening; were currently being titrated on therapy with an angiotensin converting enzyme inhibitor or angiotensin receptor blocker; had two consecutive serum calcium values greater than 10.2 mg/dL or Ca × P > 55 mg^2/dL^2; were currently receiving erythropoiesis stimulating agent or intravenous iron therapy or were pregnant or breastfeeding. All 40 patients completed the study and had evaluable data.

Study design

Forty VDRA naïve CKD patients were randomized by a computer generated schema to receive oral paricalcitol 1 mcg once daily or placebo. Study drugs were provided by Abbott Laboratories, Chicago, IL. Plasma samples were obtained at baseline, week 4 and week 8. A thirty-day supply of investigational drug product (paricalcitol 1 mcg or matching placebo) was dispensed at baseline and Day 28. Fixed dosing of paricalcitol was used to maintain a consistent exposure profile. Doses of angiotensin converting enzyme inhibitors or angiotensin receptor blockers were not permitted to be changed over the 8 week study period. Compliance was evaluated by pill count.

Bioanalytic methods

Whole blood samples were collected and processed at each study visit and serum and plasma were stored at -80°C for later analysis. Samples for routine laboratory chemistries were analyzed at Albany Medical Center Clinical Chemistry Laboratory (Albany, NY). These included; a basic metabolic panel (sodium, potassium, chloride, bicarbonate, creatinine, blood urea nitrogen), serum phosphorus, calcium and albumin 25 OH Vitamin D and parathyroid hormone. Serum concentrations of 25 OH Vitamin D_2 were measured by the LIAISON® 25 OH Vitamin D TOTAL Assay (DiaSorin, Inc, Stillwater, MN). Parathyroid hormone was measured by Beckman Coulter Access Intact PTH assay®. Asymmetric dimethylarginine (ADMA) concentrations were determined using the liquid chromatography/tandem mass spectrometric method of Kirchherr et al. with minor modification. Customized multiplex human cytokine panels were used (Bio-Plex Human Cytokine Singleplex sets, BioRad, Hercules, CA) for the simultaneous quantification of serum ICAM, VCAM, IL-1β, TNF-α, IL-6 and MCP-1, RANTES and VEGF [11]. Analysis was performed on the Luminex IS100™ analyzer (Luminex Inc., Austin, TX). The data were saved and evaluated as median fluorescence intensity using appropriate curve-fitting software (BioPlex Manager 4.1).

Statistical analysis

Data are reported as mean ± SD. Repeated measures ANOVA with grouping factors and time as a repeated measure, followed by Fisher's protected least significant difference test, was used to assess percent differences between groups at baseline (t = 0) and at 8 weeks after placebo or paricalcitol administration. If data were not normally distributed, a two-tailed Kruskal-Wallis test was used to assess differences between groups. Categorical data were analyzed with chi-square or Fisher's exact test. Forward selection and backward elimination multivariate logistic regression was used to determine predictive relationships for changes in biomarker concentration reduction >30%. (STATA/SE V.11, College Station, TX). Using this approach limits the effect of the order of the sequenced variables in the regression analysis. The variables included in the model were; duration of treatment (4 weeks vs. 8 weeks), 25 OH vitamin D (native vitamin D) concentration, race, screening hemoglobin concentration; diabetes and hypertension. A p value < 0.05 was considered significant. Assuming 25% variability in circulating ADMA concentrations [12] a sample size of 17 per group was determined to be adequate to detect a potential 15% reduction in ADMA from baseline with 80% power and an α = 0.05. To allow for potential dropouts 20 patients were recruited and randomized to each arm.

Results

All patients completed the week 4 and week 8 study visits. Compliance with study medication (pill count) in the paricalcitol and placebo groups was 95% and 96%, respectively. No statistically significant differences were observed between the paricalcitol and placebo groups in baseline characteristics (Table 1) or serum calcium and phosphorus (Table 2). PTH declined in patients on paricalcitol, however, the difference from baseline after 8 weeks of treatment was not statistically significant.

No significant differences in mean biomarker or cytokine concentrations were observed between paricalcitol and placebo groups at baseline (Table 3). Paricalcitol treated patients had significant reductions in ADMA serum concentrations at week 4 (Figure 1), compared to placebo treated patients and a trend toward maintenance

Parameter	Paricalcitol (n=20)	Placebo (n=20)
Age (years)	64 ± 14	67 ± 11
Sex (% female)	60	35
eGFR (mL/min/1.73m²)	30 ± 9	36 ± 11
PTH (pg/mL)	118 ± 35	108 ± 43
Corrected Calcium (mg/dL)	9.6 ± 0.3	9.5 ± 0.3
Phosphorus (mg/dL)	3.8 ± 0.7	3.7 ± 0.7
25-OH Vitamin D (ng/mL)	27 ± 12	30 ± 12
Hypertension (%)	80	90
Diabetes Mellitus (%)	30	40
Race (%)		
White	85	75
Black	15	20
Hispanic	0	5
Concomitant Medications (%)		
Statin	40	60
ACE inhibitor or ARB	70	55

Data reported as mean ± SD, ACE angiotensin converting enzyme, ARB angiotensin receptor blocker, p=NS for all baseline comparisons

Table 1: Baseline Characteristics of the Study Population.

Parameter	Paricalcitol			Placebo		
	Baseline	Day 28	Day 56	Baseline	Day 28	Day 56
Calcium (mg/dL)	9.56 ± 0.31	9.68 ± 0.37	9.62 ±0.4	9.53 ± 0.34	9.56 ± 0.34	9.56 ± 0.34
Phosphorus (mg/dL)	3.8 ± 0.69	3.99 ± 0.69	4.06 ± 0.89	3.67 ± 0.4	3.76 ± 0.57	3.71 ± 0.7
PTH (pg/mL)	123 ± 89	88 ± 38	95 ± 66	108 ± 43	102 ± 45	132 ± 72 (p=0.09)

Table 2: Mineral and bone disease-related biochemical changes during the study (mean ± SD).

Biomarker	Placebo	Paricalcitol	#Paricalcitol Responsive Number of patients (mean % decrease)
Vascular Reactivity Mean ± SD			
ICAM (ng/mL) Baseline Day 28 Day 56	 707 ± 504 679 ± 557 590 ± 458	 701 ± 603 627 ± 570 477 ±382	12 (40)
VCAM (ng/mL) Baseline Day 28 Day 56	 1147 ± 1000 1334 ± 1240 969 ±734	 1020 ± 835 1140 ±1138 689 ± 583	11 (32)
VCAM (ng/mL) Baseline Day 28 Day 56	 1147 ± 1000 1334 ± 1240 969 ±734	 1020 ± 835 1140 ±1138 689 ± 583	11 (32)
RANTES (pg/mL) Baseline Day 28 Day 56	 8.368 ± 4.406 7.789 ± 2.268 7.642 ± 2.741	 7.914 ± 2.427 8.383 ± 2.846 7.545 ± 3103	5 (48)
MCP-1 (pg/mL) Baseline Day 28 Day 56	 171 ± 428 162 ± 424 139 ± 422	 80 ± 102 75 ± 45 57 ± 29	0
VEGF (pg/mL) Baseline Day 28 Day 56	 207 ± 342 257 ± 382 206 ± 354	 420 ± 554 195 ± 245 114 ±151	11 (58)
Cytokines (pg/mL)			
IL-6 Baseline Day 28 Day 56	 8.1 ± 12.8 8.1 ± 12.6 8.2 ± 9.0	 12.4 ± 19.3 8.9 ± 14.8 7.5 ± 12.6	0
TNF-α Baseline Day 28 Day 56	 15.7 ± 26.4 15.7 ± 24.3 16.1 ± 25.1	 52.4 ± 180.7 63.7 ± 215.8 69.9 ± 213.9	9 (30)

p=NS for all aggregate comparisons for paricalcitol vs. placebo by ANOVA, #Response to paricalcitol defined as > 30% decrease in biomarker concentration from baseline to Week 8 of treatment.

Table 3: Biomarker concentrations during the study period and paricalcitol response rates.

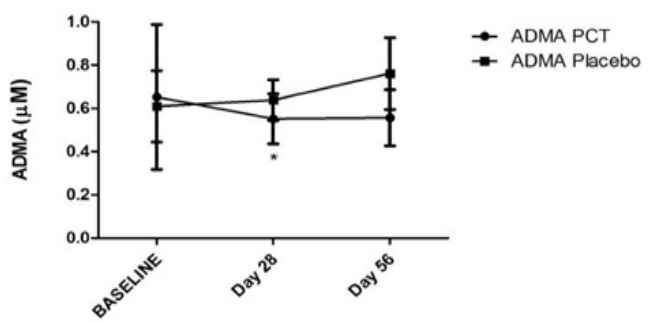

Figure 1: ADMA concentrations at baseline, week 4 (day 28) and week 8 (day 56) in patients receiving paricalcitol (PCT) or placebo.

Data reported as mean ± SD *p<0.05.

Vascular Reactivity Biomarkers	Coefficient	95% Confidence Interval	p value
ICAM			
Duration of treatment	0.44	0.18, 0.7	p=0.001
Screening hemoglobin value	-0.56	-1.002,-0.12	p=0.012
VCAM			
Duration of treatment	0.64	0.27,0.10	p=0.001
25, OH Vitamin D	-0.11	-0.18,-0.38	p=0.005
Black race	-2.49	-4.59,-0.41	p=0.039
Screening hemoglobin value	-1.26	-2.16,-0.36	p=0.009
Presence of diabetes	-1.91	-3.58,-0.257	p=0.043
Presence of hypertension	1.74	0.04, 3.43	p=0.044
VEGF			
Duration of treatment	0.043	0.016, 0.07	p=0.003
RANTES			
Duration of treatment	0.053	0.015,0.092	p=0.006
Screening hemoglobin value	-0.82	-1.48, -0.16	p=0.015
Cytokines			
IL-1			
Duration of treatment	0.62	0.03, 0.10	p=0.001
25, OH Vitamin D	-0.08	-0.134, -0.025	p=0.016
Presence of diabetes	-2.36	-2.73, 0.73	p=0.003
TNF-α			
Duration of treatment	0.035	0.009, 0.066	p=0.012
25, OH Vitamin D	-0.074	-0.13, -0.019	p=0.009
Screening hemoglobin value	-0.55	-1.07, -0.032	p=0.037

Table 4: Multivariate logistic regression analysis.

of this reduction was observed at 8 weeks. Large interpatient variability in vascular reactivity biomarkers and cytokine concentration profiles was observed. When analyzed in aggregate, mean (SD) biomarker concentrations did not change significantly at 4 or 8 weeks in either group (p = NS for all comparisons). However, up to 60% of paricalcitol treated patients exhibited >30% reduction from baseline of one or more biomarkers which was considered potentially clinically significant (Table 3). Reductions >30% were not observed in the placebo group. ICAM, VCAM, and IL-1 were the most frequently observed to have reductions with paricalcitol treatment. Multiple logistic regression was performed to elucidate what, if any, predictive clinical factors existed for response to each biomarker to guide future study design (Table 4). There were no predictive variables identified for MCP-1 response. Among other analytes, longer duration of treatment (week 8 vs. week 4) was significantly correlated with response to paricalcitol. Higher baseline concentrations of native vitamin D was found to be a negative predictor of response for VCAM and the pro-inflammatory cytokines IL-1, and TNF-α. Interestingly, black race was a very strong predictor of non-response to paricalcitol therapy for VCAM.

Study treatments were well tolerated during the 8 week study period. There were no serious adverse events reported. One patient receiving placebo was withdrawn after receiving one dose after reporting lower extremity weakness, the causality of which was determined to be a

supratherapeutic dose of gabapentin. The patient was replaced and their data is not included in the analyses.

Discussion

This is the first study to evaluate effect of oral paricalcitol therapy on reduction of ADMA concentrations in patients with advanced CKD. A significant reduction in ADMA concentration was observed in paricalcitol treated patients compared to placebo at 4 weeks. This may be explained by several biologically plausible mechanisms. Vitamin D has been associated with decreased lipid peroxidation and can act as an antioxidant [13]. This can modulate decreased production of ADMA via decreased Type 1 protein arginine methyltransferase (PRMT) activity and/or enhanced ADMA degradation via increased dimethyarginine dimethylaminohydrolase (DDAH) activity [14].

The effects of paricalcitol on other etiologies of endothelial dysfunction by measuring biomarkers of vascular reactivity and cytokine activation were also studied. In multivariate analysis of paricalcitol treated patients, there were several clinical factors that were significantly associated with reductions in principal biomarkers of endothelial dysfunction and inflammation. There was an association between higher native vitamin D concentrations and the lower likelihood of VCAM and pro-inflammatory cytokines reduction in patients receiving paricalcitol therapy. This could be explained by intact local tissue 1α-hydroxylase activity conversion of native vitamin D which is proposed to account for benefits of native vitamin D in patients with CKD despite their reduced renal conversion to active vitamin D. This would make supplementation with active vitamin D potentially less beneficial. Duration of treatment (response at week 4 vs. week 8) emerged as a significant positive predictor of response [15]. This may be related to individual differences in the time required for transcriptionally-mediated down regulation of biomarkers. Black individuals have been reported to have a high prevalence of low native vitamin D concentrations. In this analysis black race was found to be a significant negative predictor of response to paricalcitol therapy [16].

Limitations of this study include a relatively small sample size in the context of large interpatient variability in inflammation profiles. This likely limited our ability to fully model populations that may respond to paricalcitol treatment although the observed biomarker concentration variability is consistent with previous reports in advanced CKD patient cohorts. Although not statistically significant, a greater proportion of patients receiving paricalcitol were receive angiotensin converting enzyme inhibitor or angiotensin receptor blocker therapy which has been shown to improve endothelial dysfunction [17]. However, patients had to be on a stable regimen for inclusion in the study. Duration of treatment was noted as a significant predictor of biomarker reduction for responders, but it is unknown if additional patients may have responded with treatment for more than 8 weeks. Finally, it must be noted that there are many other factors that influence the inflammation observed in chronic kidney disease patients.

Our findings show that paricalcitol treatment was associated with short-term reduction of ADMA concentrations that are typically persistently elevated in CKD. Larger future studies should examine longer durations of treatment and explore pharmacodynamic endpoints for clinical changes in vascular reactivity potentially associated with paricalcitol therapy.

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