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Original Article

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Paricalcitol and Calcitriol Prevent Progression of Chronic Kidney Disease in Uremic Rats

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ABSTRACT

Objective: Chronic kidney disease is common, irreversible, and associated with high mortality and morbidity. In this study, we aimed to investigate the effects of calcitriol and paricalcitol on chronic kidney disease progression in an adenine-induced uremic rat model.

Methods: Male Wistar-albino rats were fed a diet containing 0.75% adenine + *1.2% phosphorus* for 3 weeks to induce chronic kidney disease. The rats were randomly divided into 6 groups: control group (n = 12), chronic kidney disease group (n = 12), chronic kidney disease+ calcitriol group (n = 12), chronic kidney disease + paricalcitol group (n = 12), calcitriol group (n = 6), paricalcitol group (*n* = 6). Animals were monitored for weight and systolic blood pressure. The kidney function parameters, parathyroid hormone, monocyte chemoattractant protein-1, and kidney histology were investigated. This research was supported by the Scientific Research Projects Unit (11-TIP-062).

Results: Kidney histology proved that the adenine-induced uremic rat model was successfully established. Serum urea, creatinine, and phosphorus were significantly higher in chronic kidney disease, chronic kidney disease + calcitriol, chronic kidney disease + paricalcitol groups compared with the control group ($P < .05$). Calcitriol and paricalcitol provide effective parathyroid hormone suppression and decrease serum monocyte chemoattractant protein-1 levels in uremic rats. All uremic rats, including the paricalcitol and calcitriol treatment groups, lost weight. Paricalcitol significantly lowered systolic blood pressure in uremic rats ($P = .006$). Serum calcium was higher in calcitriol group compared with chronic kidney disease group. Paricalcitol caused less hyperphosphatemia in non-uremic rats than calcitriol.

Conclusion: Paricalcitol and calcitriol therapy may prevent chronic kidney disease progression in uremic rats as they restore vitamin D metabolism during the disease.

Keywords: Adenine, calcitriol, uremic rat, paricalcitol

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INTRODUCTION

Chronic kidney disease (CKD) is a worldwide multisystem disease affecting most organs and systems that originate from kidneys.[1] Prevalence of CKD according to the 2017 Global Burden of Disease Study in the world increased by 28.2% from 2007 to 2017 among women and by 25.4% among men.[2] Mortality due to CKD is expected to increase from 1.2 million in 2016 to 3.1 million in 2040.[3] Cardiovascular disease is the leading cause of CKD-related mortality, in which hypervolemia and calcium–phosphorus imbalance are nontraditional risk factors of atherosclerosis.

Regulation of calcium and phosphorus homeostasis requires a complex interaction of feedback systems such as parathyroid hormone (PTH), calcitriol, Klotho, and fibroblast growth factor-23 (FGF-23). Parathyroid hormone (PTH), (FGF-23), and 1,25-(OH)2 vitamin D3 are the major “calciophosphoregulatory” hormones in mineral homeostasis.[4] Fibroblast growth factor-23,

a phosphaturic hormone as well as a counter-regulatory hormone for vitamin D, induces expression of the catabolic enzyme 24-hydroxylase to regulate the level of renal calcitriol.[5] CKD is known to strongly impair vitamin D metabolism.[6] In patients with CKD, CYP27B1-mediated production of 1,25-(OH)2 D3 is significantly reduced[7] and circulating concentrations of PTH and FGF-23 rise markedly.[6]

Calcitriol is the active form of vitamin D, and paricalcitol is a selective vitamin D receptor activator, which are both used in the treatment of secondary hyperparathyroidism. In patients with secondary hyperparathyroidism, paricalcitol lowers PTH levels with minimal serum calcium and phosphorus variations, unlike calcitriol.

Vitamin D receptors (VDRs) are widely distributed in tissues such as bone, kidney, parathyroid gland, intestine, immune system, myocardium, smooth muscle and are responsible for the pleiotropic effects of vitamin D. They may have anti-cancer, renin–angiotensin–aldosterone blocking, and anti-inflammatory effects.[8] Previous studies showed that the abnormalities in the vitamin D metabolism regardless of calcium homeostasis were associated with hypertension, disturbed muscle function, and susceptibility to infections, autoimmune diseases, and cancer.[9-11] The factors leading to the progression of CKD or to become end-stage kidney failure are poorly clarified. Animal models of CKD are often used to investigate the pathogenesis and mechanisms of disease progression and to test the potential interventions.[12] In this study, we aimed to investigate the effects of calcitriol and paricalcitol on CKD progression in the adenine-induced uremic rat model and whether these agents can prevent the progression of CKD.

METHODS

Ethics Committee Approval

Ethics committee approval was obtained from Ege University Animal Ethics Committee with the date of October 23, 2009 and approval number 2009-114.

Animals

All experimental procedures were carried out according to the ethical standards of Animal Research Committee, Türkiye, and all efforts were made to minimize the suffering of the animals. Wistar-albino rats weighing 180-220 g were purchased from Experimental Animal Breeding Farm, Türkiye. We preferred male rats because female rats are resistant to kidney damage[13] and less likely to bring about adenine-induced CKD.[14] Animals were housed in polycarbonate cages under controlled temperature (24°C ± 2°C) and humidity (55% ± 5%) conditions with a 12-hour light–dark cycle and fed with standard chow and water ad libitum. Adenine was purchased from Sigma-Aldrich, Inc. (Saint Louis, Mo, USA). Blood pressure (BP) was measured by a noninvasive tail-cuff system (IITC Life Science, Woodland Hills, Calif, USA) both at week 0 and week 3. Blood pressure results were presented as the mean of 5 measurements.

Experimental Protocols

Eight-week-old non-uremic male Wistar-albino rats were randomly divided into the following 6 groups: control group (n = 12), CKD group (n = 12), CKD + calcitriol group (n = 12), CKD + paricalcitol group (n = 12), calcitriol group (n = 6), and paricalcitol group (n = 6). Rats in the CKD and CKD + calcitriol and CKD+ paricalcitol group were fed a diet containing 0.75% adenine + 1.2% phosphorus for 0-3 weeks. The CKD + calcitriol group was treated with 0.04 µg/kg/day calcitriol (Calcijex® 5 µg/mL, 2 mL Abbott, Istanbul, Türkiye) and CKD + paricalcitol group was treated with 0.2 µg/kg/day paricalcitol (Zemplar® 2 µg/mL, 1, Abbott, Istanbul, Türkiye) during the study period for 0-3 weeks. The Control group, calcitriol, and paricalcitol group received normal adenine-free feed for 3 weeks. The calcitriol group was administered with 0.04 µg/kg/day calcitriol (Calcijex®), and the paricalcitol group was administered with 0.2 µg/kg/day paricalcitol (Zemplar®) for 0-3 weeks. At the end of the study, all rats were sacrificed after anesthesia with ketamine hydrochloride (Ketalar®, 50 mg/mL, 10 mL, Pfizer, Istanbul, Türkiye)) (40 mg/kg, intraperitoneal injection), and blood samples were obtained by cardiac puncture.

Biochemical analyses were performed on blood samples obtained after the animals were sacrificed and were performed once during the entire study. Biochemical measurements of rats in the control group and animals receiving calcitriol only and paricalcitol only were used to compare the groups receiving adenine only, adenine + calcitriol, and adenine + paricalcitol.

The levels of plasma urea, creatinine, phosphorus, albumin, total proteins, and calcium were measured using the corresponding commercial kits (Biolabo, Maizy, France). Plasma levels of PTH were assessed by enzyme-linked immunosorbent assays as per the manufacturer's instructions (Elabscience, Tex, USA).

Kidney Histology

Kidneys were fixed in 4% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm thickness, and stained with hematoxylin and eosin. Tubulointerstitial injury of kidney was evaluated semi-quantitatively under light microscope (BX, Olympus,

MAIN POINTS

- Secondary hyperparathyroidism is an important complication in patients with chronic kidney disease (CKD), and it is related to CKD progression to end-stage kidney disease.
- Impaired vitamin D metabolism regardless of calcium homeostasis was associated with hypertension, disturbed muscle function, and susceptibility to infections, autoimmune diseases, and cancer in general population.
- Paricalcitol and calcitriol may retard the progression of CKD in uremic rats by restoring vitamin D metabolism and suppressing inflammation.

Tokyo, Japan), as in previous studies, 0-4 grading was made according to the extent of the damaged tubulointerstitial area in the renal cortex.

Tubular degeneration (TD) was defined as stained bodies of varied sizes and vacuolization including acidophilus in the cytoplasm of the proximal tubule epithelial cells.

Grade 0: absence of TD; grade 1 (mild TD): small and a few focus TD immediately beneath the capsule (0%-10%); grade 2 (moderate TD): a few focal TD along the tubular segment (10%-25%); grade 3 (severe TD): diffuse and significant TD along the tubular segment (25%-50%); grade 4 (very severe TD): TD was greater than 50%.

Tubular necrosis (TN), loss of epithelial cells of the nucleus, dark acidophilic cytoplasm, shedding of tubular epithelial cells into the tubular lumen, and acellular parts of tubules are considered as TN.

Grade 1: small and a few focus TN immediately beneath the capsule (0%-10%); grade 2 (moderate TN): a few focal TN along the tubular segment (10%-25%); grade 3 (severe TD): diffuse and significant TN along the tubular segment (25%-50%); grade 4 (very severe TN): TN was greater than 50%.

Tubulointerstitial nephritis (TIN), was considered as infiltration of inflammatory cells in perivascular and interstitial areas.

Grade 0: absence of TIN; grade 1 (mild TIN): a few pieces of infiltration concentrated on perivascular area (0%-5%); grade 2 (moderate TIN): usually infiltrations involved in cortical interstitial and many focal areas (5%-10%); grade 3 (severe TIN): diffuse and significant infiltration areas (15%-25%); grade 4 (very severe TIN): TIN was greater than 50%.

Tubular regeneration (TR) and microcalcification (MC) were scored as follows: 0, normal; grade 1, <10%; grade 2, 10%-25%; grade 3, 25%-50%; grade 4, >50%.

Statistical Analysis

Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, Ill, USA) was used for statistical analysis. Data presented as a mean ± standard error. Kruskal–Wallis analysis and Mann–Whitney *U* test were used for comparisons of groups. A *P* value < .05 was considered significant.

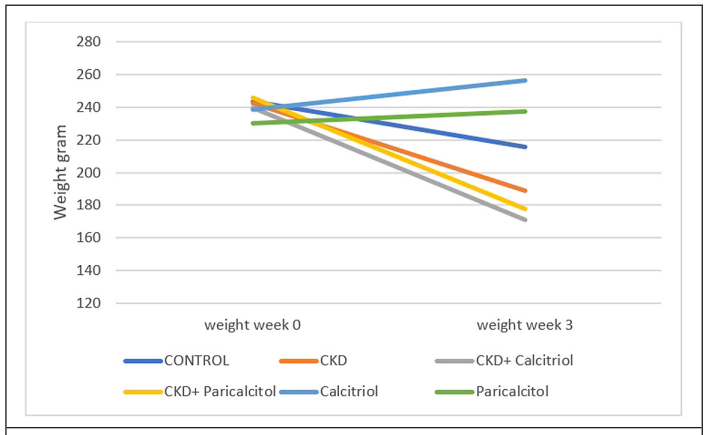

Figure 1. Change in body weight.

RESULTS

The mean weight of rats in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups at week 0 was 243.7 ± 3.46 g, 242.6 ± 4.00 g, 239.8 ± 3.46 g, 245.8 ± 3.46 g, 238.4 ± 2.82 g, 230.2 ± 2.44 g, respectively. The mean weight of rats at week 3 in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups was 215.8 ± 3.46 g, 188.9 ± 3.16 g, 170.9 ± 3.46 g, 177.9 ± 3.16 g, 256.3 ± 2.44 g, 237.4 ± 2.4 g, respectively. Initial weights of the rats were similar in all groups. Significant weight loss occurred in CKD, CKD + calcitriol, and CKD + paricalcitol groups compared to the control group ($P < .05$). Compared to the control group (215.8 + 3.46 g), there was a significant increase in weight in the calcitriol group ($P < .05$). The rats in the paricalcitol group gained weight, but it was not statistically significant. Compared to the CKD group, there is no weight difference between CKD + calcitriol and CKD + paricalcitol at the end of the third week (Table 1, Figure 1).

Mean systolic BP was 131.3 ± 2.44 mm Hg, 131.3 ± 3.74 mm Hg, 114.1 ± 2.44 mm Hg, 118.1 ± 3.46 mm Hg, 126.3 ± 2.23 mm Hg, 115.4 ± 2.6 mm Hg in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups at week 0, respectively. At the third week, mean BP was 121.3 ± 2.44 mm Hg,

Table 1. Weight and Blood Pressure Changes of Rats

	Control (N = 12)	CKD (N = 10)	CKD + Calcitriol (N = 12)	CKD + Paricalcitol (N = 9)	Calcitriol (N = 6)	Paricalcitol (N = 5)
Weight, (g), Week 0	243.7 ± 3.46	242.6 ± 4.00	239.8 ± 3.46	245.8 ± 3.46	238.4 ± 2.82	230.2 ± 2.44
Weight, (g), Week 3	215.8 ± 3.46	188.9 ± 3.16	170.9 ± 3.46[a]	177.9 ± 3.16[a]	256.3 ± 2.44[a,b,c,d]	237.4 ± 2.4[b,c,d]
BP, mm Hg, Week 0	131.3 ± 2.44	131.3 ± 3.74	114.1 ± 2.44	118.1 ± 3.46	126.3 ± 2.23	115.4 ± 2.6
BP, mm Hg, Week 3	121.3 ± 2.44	143.5 ± 2.23	135.0 ± 3.16	118.1 ± 3.31[b,c]	111.2 ± 1.7[b,c]	115.0 ± 2.00

BP, systolic blood pressure; CKD, chronic kidney disease.
[a]Group vs. control; [b]group vs. CKD, [c]group vs. CKD + calcitriol; [d]group vs. CKD + paricalcitol.

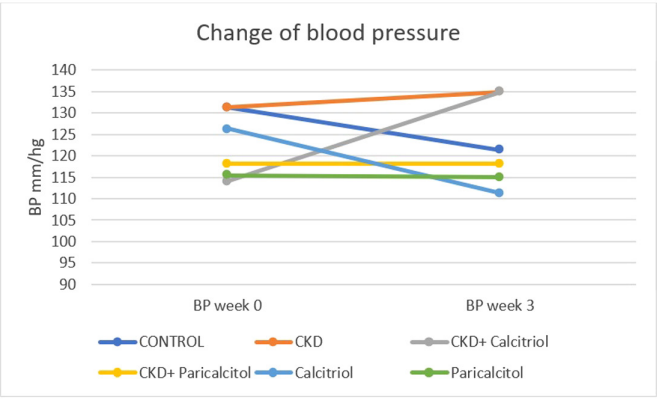

Figure 2. Change in blood pressure.

143.5 ± 2.23 mm Hg, 135.0 ± 3.16 mm Hg, 118.1 ± 3.31 mm Hg, 111.2 ± 1.7 mm Hg, 115.0 ± 2.00 mm Hg in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively (Table 1). In the third week, no significant difference was observed in terms of mean systolic BP in any group, compared with the control group. The highest systolic BP was measured in the CKD group in the third week. Systolic BP changes of the groups were given in Figure 2.

Mean creatinine value was 0.7 ± 0.11 mg/dL (normal range: 0.2-0.8 mg/dL), 2.1 ± 0.77 mg/dL, 2.2 ± 0.52 mg/dL, 2.7 ± 0.35 mg/dL, 0.6 ± 0.26 mg/dL, 0.6 ± 0.14 mg/dL in control, CKD, CKD+ calcitriol, CKD+ paricalcitol, calcitriol and paricalcitol groups, respectively. Mean urea was 43.8 ± 11.17 mg/dL (normal range: 30-44.1 mg/dL), 225.1 ± 71.1 mg/dL, 242.8 ± 78.1 mg/dL, 235.2 ± 37.9 mg/dL, 48.2 ± 21.6 mg/dL, and 41.2 ± 14.11 mg/dL in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol, respectively. Mean creatinine and urea values were significantly higher in CKD, CKD + calcitriol, CKD + paricalcitol groups compared with control group ($P < .05$). As expected, there was no difference in terms of creatinine and urea between control, calcitriol, and paricalcitol groups. Creatinine and urea were significantly higher in uremic groups including CKD, CKD + calcitriol and CKD + paricalcitol compared with calcitriol and paricalcitol groups (Table 2).

Mean serum calcium level was 8.24 ± 0.73 mg/dL (normal range: 3.2-8.5 mg/dL), 7.65 ± 0.65 mg/dL, 8.11 ± 0.66 mg/dL, 8.04 ± 0.34 mg/dL, 8.37 ± 0.29 mg/dL, 8.26 ± 0.6 mg/dL in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. There was no significant difference between groups compared with control group in terms of serum calcium. Serum calcium level was significantly higher in calcitriol group compared with CKD group ($P < .05$). Serum calcium values were similar in groups except calcitriol compared with CKD group (Table 2).

Mean phosphorus level was 5.53 ± 1.11 mg/dL (normal range:3-8.3 mg/dL), 11.15 ± 3.41 mg/dL, 11 ± 2.97 mg/dL, 11.11 ± 1.46 mg/dL, 5.9 ± 0.72 mg/dL, 4.6 ± 0.38 mg/dL in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. Serum phosphorus level was significantly higher in CKD, CKD + calcitriol, and CKD + paricalcitol groups compared with control group ($P < .005$). The rats in calcitriol and paricalcitol groups had similar serum phosphorus levels with control group. Serum phosphorus level was similar in CKD, CKD + calcitriol, and CKD + paricalcitol groups, and these were significantly higher than serum phosphorus level in calcitriol and paricalcitol groups. Significantly higher phosphorus level was observed in the calcitriol group compared with the paricalcitol group ($P < .05$) (Table 2).

Serum PTH level was 90.4 ± 3.31 pg/mL, 245.2 ± 2.82 pg/mL, 67.5 ± 2.44 pg/mL, 124.1 ± 3.00 pg/mL, 136.6 ± 2.44 pg/mL, 214.2 ± 2.23 pg/mL in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. The serum PTH level of the CKD and paricalcitol groups was significantly higher than the control group ($P < .05$). Significantly lower PTH levels were obtained in CKD + calcitriol and CKD + paricalcitol groups compared with the CKD group ($P < .05$) (Table 2). Serum PTH was significantly lower in CKD + calcitriol group compared with calcitriol and paricalcitol groups ($P < .05$).

Table 2. Serum Chemistries in Normal and Uremic Rats

	Control (N = 12)	CKD (N = 10)	CKD + Calcitriol (N = 12)	CKD + Paricalcitol (N = 9)	Calcitriol (N = 6)	Paricalcitol (N = 5)
Creatinine (mg/dL)	0.7 ± 0.11	2.1 ± 0.77[a]	2.2 ± 0.52[a]	2.7 ± 0.35[ac]	0.6 ± 0.26[b,c,d]	0.6 ± 0.14[b,c,d]
Urea (mg/dL)	43.8 ± 11.7	225.1 ± 71.1[a]	242.8 ± 78.1[a]	235.2 ± 37.9[a]	48.2 ± 21.6[b,c,d]	41.2 ± 14.11[b,c,d]
Calcium (mg/dL)	8.24 ± 0.73	7.65 ± 0.65	8.11 ± 0.66	8.04 ± 0.34	8.37 ± 0.29[b]	8.26 ± 0.6
Phosphorus (mg/dL)	5.53 ± 1.11	11.15 ± 3.41[a]	11 ± 2.97[a]	11.11 ± 1.46[a]	5.9 ± 0.72[b,c,d]	4.6 ± 0.38[b,c,d,e]
PTH (pg/mL)	90.4 ± 3.31	245.2 ± 2.82[a]	67.5 ± 2.44[b]	124.1 ± 3.00[b]	136.6 ± 2.44[c]	214.2 ± 2.23[a,c,d]
Total protein (g/dL)	6.14 ± 0.29	6.07 ± 0.23	6.14 ± 0.25	6.08 ± 0.31	5.8 ± 0.2	5.9 ± 0.19
MCP-1 (ng/mL)	1.5 ± 3.46	11.7 ± 3.00[a]	5.05 ± 3.46[a,b]	5.4 ± 2.82[a,b]	1.5 ± 2.44[b,c,d]	1.8 ± 2.23[b,c,d]

[a]Group vs. control; [b]group vs. CKD; [c]group vs. CKD + calcitriol; [d]group vs. CKD + paricalcitol; CKD, chronic kidney disease; PTH, parathyroid hormone.

Table 3. Histopathological Findings in Normal and Uremic Rats

	Control (N = 12)	CKD (N = 10)	CKD + Calcitriol (N = 12)	CKD + Paricalcitol (N = 9)	Calcitriol (N = 6)	Paricalcitol (N = 5)
Tubular degeneration	0 ± 2.44	1.2 ± 3.00	1.00 ± 3.46	1.0 ± 3.00	0 ± 2.44	0 ± 2.23
Tubular necrosis	0 ± 2.44	1.0 ± 3.00	0.6 ± 3.46	1.0 ± 3.00	0.17 ± 2.44	0 ± 2.23
Tubular regeneration	0.2 ± 2.44	3.1 ± 3.00	2.2 ± 3.46	2.0 ± 3.00	0.50 ± 2.44	0 ± 2.23
Tubulointerstitial nephritis	0.2 ± 2.44	1.8 ± 3,00	2.6 ± 3.46	2.9 ± 3.00	0.17 ± 2.44	0 ± 2.23
Microcalcifications	0 ± 2.44	2.6 ± 3.00	3.6 ± 3.46	3.5 ± 3.00	0 ± 2.44	0 ± 2.23

CKD, chronic kidney disease.

Lower serum PTH level was detected in the calcitriol group than the paricalcitol group, and there was no statistically significant difference (Table 2).

Serum total protein was 6.14 ± 0.29 g/dL (normal range: 5.6-7.6 g/dL), 6.07 ± 0.23 g/dL, 6.14 ± 0.25 g/dL, 6.08 ± 0.31 g/dL, 5.8 ± 0.2 g/dL, 5.9 ± 0.19 g/dL in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. There was no statistically significant difference in terms of serum albumin level between the groups (Table 2).

Serum monocyte chemoattractant protein-1 (MCP-1) was 1.5 ± 3.46ng/mL,11.7±3.00ng/mL,5.05±3.46ng/mL,5.4±2.82ng/mL, 1.5 ± 2.44 ng/mL, 1.8 ± 2.23 ng/mL in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. Serum MCP-1 was significantly lower in the control group compared with uremic rats including CKD, CKD + calcitriol, and CKD + paricalcitol groups ($P < .05$). The level of MCP-1 was similar in the control, calcitriol, and paricalcitol groups. Monocyte chemoattractant protein-1 was significantly higher in CKD group than in CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups ($P < .05$). While there was no difference in terms of MCP-1 levels between CKD + calcitriol and CKD + paricalcitol groups, the MCP-1 levels of these 2 groups were significantly higher than calcitriol and paricalcitol groups ($P < .05$) (Table 2).

Histopathological findings were scored as TD, TN, TR, TIN, and MC. Tubular degeneration was 0 ± 2.44, 1.2 ± 3.00, 1.00 ± 3.46, 1.0 ± 3.00, 0 ± 2.44, and 0 ± 2.23 in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. Tubular necrosis was 0 ± 2.44, 1.0 ± 3.00, 0.6 ± 3.46, 1.0 ± 3.00, 0.17 ± 2.44, and 0 ± 2.23 in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. Tubular regeneration was 0.2 ± 2.44, 3.1 ± 3.00, 2.2 ± 3.46, 2.0 ± 3.00, 0.50 ± 2.44, and 0 ± 2.23 in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. Tubulointerstitial nephritis was 0.2 ± 2.44, 1.8 ± 3,00, 2.6 ± 3.46, 2.9 ± 3.00, 0.17 ± 2.44, and 0 ± 2.23 in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively. Microcalcification was 0 ± 2.44, 2.6 ± 3.00, 3.6 ± 3.46, 3.5 ± 3.00, 0 ± 2.44, and 0 ± 2.23 in control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups, respectively (Table 3). As expected, TD, TN, TR, TIN, and MC scores were higher in uremic rats in CKD, CKD + calcitriol, CKD + paricalcitol groups (Figure 3). Histopathological data of groups was presented in Figure 4.

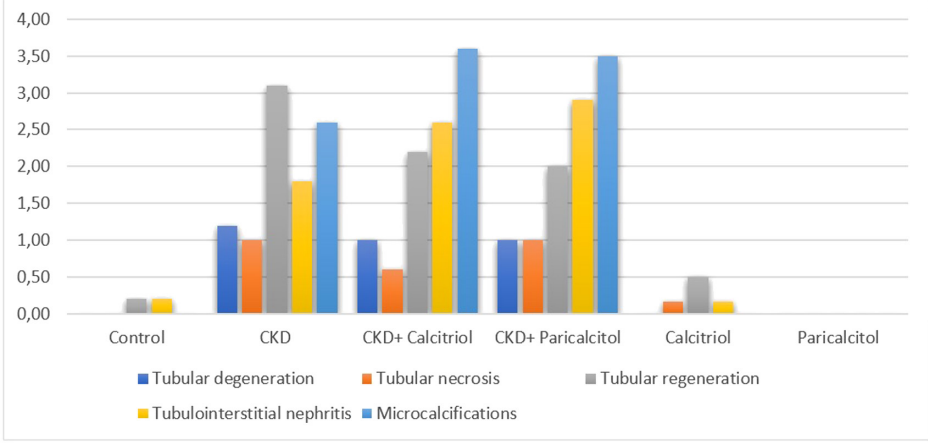

Figure 3. Comparison of histopathological data between groups.

DISCUSSION

This is the first experimental study that highlights the effects of calcitriol and paricalcitol on CKD progression in an adenine-induced rat model.

The 0.75% adenine diet model is a well-established animal model that produces rapid-onset kidney disease with extensive tubulointerstitial fibrosis, tubular atrophy, crystal formation, and marked vessel calcification. Kidney structural damage that occurs after the fourth week of oral adenine is irreversible, as in chronic kidney failure.[15,16] Since irreversible damage was not desired in our study, a diet with adenine was given for 3 weeks to create a CKD model.

Here, we investigated the effect of calcitriol and a vitamin D analog paricalcitol on the progression of CKD in an adenine-induced uremic rat model. In our study, histopathological assessment of kidney tissues proved that the oral 0.75% adenine-induced CKD model was successfully established (Table 3, Figure 4). In addition, significantly higher serum urea, creatinine, phosphorus, and PTH values were obtained in adenine groups at the end of the third week compared with the control group.

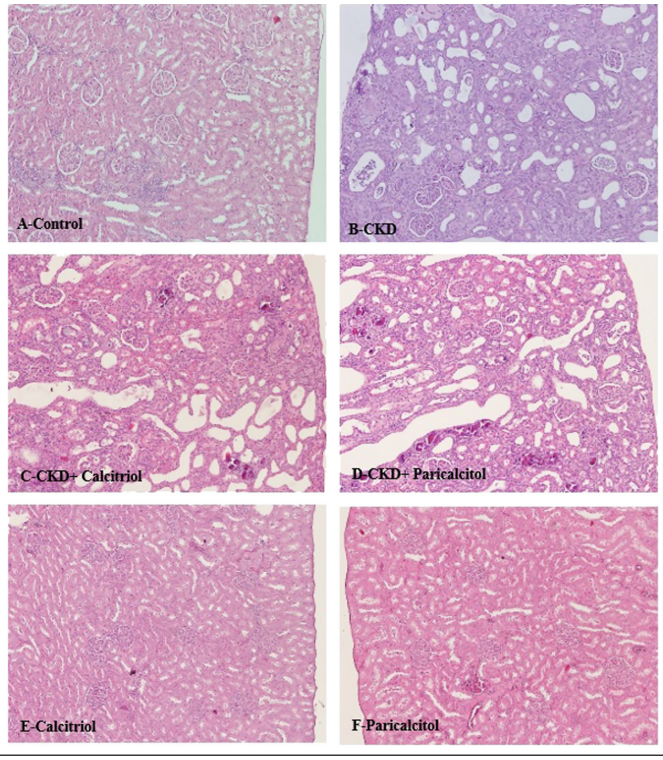

Figure 4. Histopathological evaluation of control, CKD, CKD + calcitriol, CKD + paricalcitol, calcitriol, and paricalcitol groups (hematoxylin and eosin, ×4). (A) Structures of glomerulus and tubules are normal in the control group. (B) In the CKD group, there are vacuolization, dilatation, necrosis in the tubules, and inflammatory cell infiltration in the perivascular and interstitial area. (C-D). In CKD + calcitriol and CKD + paricalcitol groups, there are vacuolization, dilatation, necrosis, inflammatory cell infiltration in the perivascular, interstitial area, and medulla papillary microcalcification in tubular structures. (E-F). Structures of glomerulus and tubules are normal in the calcitriol and paricalcitol groups. CKD, chronic kidney disease.

Cardús et al[17] tested the effects of calcitriol and its analog paricalcitol on vascular calcification in uremic rat model induced with 5/6 nephrectomy. Calcitriol significantly increased the vascular calcification. Paricalcitol had no enhancing effect on vascular calcification despite having similar levels of serum calcium and phosphorus as rats treated with calcitriol. As expected, calcium levels in all CKD groups decreased and non-CKD groups given paricalcitol and calcitriol increased but not reached statistical significance in our study.

An increase in systolic BP was observed in rats treated with hypercalcemic doses of calcitriol and paricalcitol. However, diastolic BP only raised in rats treated with paricalcitol. A similar decrease in PTH level was obtained with paricalcitol and calcitriol.[17] Wu-Wong et al[18] also reported that calcitriol increased aortic calcification more than paricalcitol with the similar serum calcium-phosphorus levels. Mizobuchi et al[19] claimed that different vitamin D receptor activators including calcitriol, paricalcitol, and doxercalciferol have different effects on vascular calcification in uremic rats. While higher dose of calcitriol and doxercalciferol significantly increased the calcium–phosphate product and the aortic calcium content, the same dose of paricalcitol had no effect.

In our study, serum calcium, phosphorus, and PTH levels were significantly higher in uremic rats including CKD, CKD + calcitriol, and CKD + paricalcitol groups compared with control group. Serum calcium–phosphorus levels and the effect of PTH suppression were similar in animals treated with calcitriol and paricalcitol. However, significantly higher phosphorus level was observed in calcitriol group compared with the paricalcitol group, both were non-uremic animals. This may be related to the fact that paricalcitol directly affects the VDRs and parathormone levels rather than the intestines to absorb calcium and phosphorus, unlike calcitriol.

A growing data claim that vitamin D deficiency is related to increased cardiovascular disease risk in hypertension; moreover, short-term vitamin D deficiency may increase BP directly.[20] In our study, while paricalcitol improved significantly systolic BP in uremic rats, positive effects of calcitriol were observed. We suggested that the improvement in systolic BP with calcitriol was associated with the suppressive effect of vitamin D on the renin–angiotensin–aldosterone system.[21-24]

In our study, rats in the control and paricalcitol groups gained weight. Significant weight loss occurred in uremic rats compared to the control group. In CKD, we expect no weight gain on either treatment with calcitriol or paricalcitol. Non-CKD animals given paricalcitol gained weight may be attributable to increased appetite or any other mechanisms of paricalcitol.

Vitamin D has major immunomodulatory actions like the enhancement of the innate immune system and inhibition of the adaptative immune responses.[23] Previous studies showed that paricalcitol reduces cyclosporine-induced kidney injury in rats by anti-inflammatory and antifibrotic effects.[25,26] In addition, paricalcitol effectively decreased the renal interstitial fibrosis induced with 7/8 nephrectomy model of experimental CKD through a combination of inhibitory actions on the RAS system, inflammation, and epithelial/mesenchymal transition.[27] Monocyte chemoattractant protein is a chemokine that regulates monocyte chemotaxis and T-lymphocyte differentiation by binding to CC chemokine receptor 2 and has an important role in regulating chemotaxis both in health and in the pathogenesis of inflammatory diseases, atherosclerosis, and cancer.[28]

In addition, it is proved that MCP-1 is one of the first chemokines described and plays a critical role in kidney disease caused by inflammation.[29] We studied serum MCP-1 levels to investigate the anti-inflammatory effect of calcitriol and paricalcitol in CKD progression. The levels of MCP-1 were significantly higher in uremic rats compared with the control group. There was no difference between calcitriol and paricalcitol in terms of suppressing MCP-1 levels. As expected, the MCP-1 levels were in the normal range in non-uremic rats that received only calcitriol and paricalcitol.

Although, our study has some limitations such as the lack of data on serum FGF-23 levels and vascular calcification, we suggest that our study may contribute to the literature due to its unique experimental design. This is the first experimental study that highlights the effects of calcitriol and paricalcitol on CKD progression in adenine induced rat model.

CONCLUSION

We investigated the effect of calcitriol and a vitamin D analog paricalcitol on the progression of CKD in an adenine-induced uremic rat model. Calcitriol and paricalcitol provide effective PTH suppression in uremic rats, suppress inflammation due to decreasing serum MCP-1 levels, and there is no difference between both molecules in terms of PTH and MCP suppression. Paricalcitol significantly improved systolic BP in uremic rats, and calcitriol had a positive effect on this issue, but that was not reached statistical significance. Additionally, paricalcitol caused less hyperphosphatemia in non-uremic rats than calcitriol. Our results suggest that paricalcitol and calcitriol may ameliorate the progression of CKD due to positive effects both on calcium–phosphorus homeostasis and on pleiotropic effects of vitamin D such as suppression of inflammation.

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