Chapter 3

Sodium Restriction Potentiates the Renoprotective Effects of Combined Vitamin D Receptor Activation and ACE Inhibition in Established Proteinuric Nephropathy

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ABSTRACT

**Background.** Renin-angiotensin-aldosterone system (RAAS) blockade provides renoprotective effects in chronic kidney disease (CKD); yet progressive renal function loss remains common. Dietary sodium restriction potentiates the renoprotective effects of RAAS blockade. Vitamin D receptor activator (VDRA) treatment reduces proteinuria, inflammation and fibrosis, but whether these effects depend on sodium intake has not been studied. We hypothesized that the renoprotective effects of VDRA treatment, with or without RAAS blockade, are modulated by sodium intake.

**Methods.** Six weeks after the induction of adriamycin nephrosis in Wistar rats, i.e. with established proteinuria, animals were treated with the VDRA paricalcitol, lisinopril, the combination, or vehicle; each treatment was given during either a high- (2% NaCl) or a low-sodium (0.05% NaCl) diet for 6 weeks. We assessed proteinuria, blood pressure, renal macrophage accumulation and renal expression of the pre-fibrotic marker alpha-smooth muscle actin (α-SMA) at the end of the treatment.

**Results.** Both paricalcitol and lisinopril individually, as well as in combination, reduced proteinuria and glomerular and interstitial inflammation during a low-sodium diet, but not during a high-sodium diet. All interventions also reduced focal glomerulosclerosis and interstitial expression of α-SMA during the low-sodium diet, while similar trends were observed during the high-sodium diet. The renoprotective effects of paricalcitol were not accompanied by blood pressure reduction. As proteinuria was already abolished by lisinopril during the low-sodium diet, the addition of paricalcitol had no further effect on proteinuria or downstream inflammatory or pre-fibrotic changes.

**Conclusion.** The renoprotective effects of the VDRA paricalcitol are blood pressure independent but do depend on dietary sodium status. The combination of RAAS blockade, dietary sodium restriction and VDRA may be a promising intervention to further retard renal function loss in CKD.
3.1 Introduction

Blockade of the renin–angiotensin–aldosterone system (RAAS) with an angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) is the mainstay of chronic kidney disease (CKD) therapy. ACEIs and ARBs retard renal function loss by reducing blood pressure and proteinuria \(^1\), yet renoprotection is incomplete in many patients. Several strategies have been employed to potentiate the efficacy of single-agent RAAS blockade. Reduction in volume overload by dietary sodium restriction and/or diuretic therapy enhances the efficacy of ACEIs to reduce proteinuria and blood pressure \(^2,3\). In addition, lower sodium intake has been associated with improved cardiorenal protection by RAAS blockade \(^4,5\). Dual RAAS blockade combining an ACEI with an ARB, a renin inhibitor or an aldosterone blocker, on the other hand, did not translate into better renal outcomes but rather increased side effects including hyperkalaemia, hypotension and acute kidney injury \(^6\). Additional renoprotective therapy adjunct to single-agent RAAS blockade, but with a more favourable safety profile, is warranted.

Vitamin D receptor activator (VDRA) therapy also reduces proteinuria, glomerulosclerosis and interstitial fibrosis, as shown in experimental studies \(^7\). Recent small- to medium-sized clinical trials confirmed the antiproteinuric capacity of vitamin D analogues during RAAS blockade in patients \(^8\). However, presently it is not known whether the renoprotective effects of VDRAs depend on sodium intake. Interestingly, a post hoc analysis of the VITAL study in diabetic nephropathy suggested that the VDRA paricalcitol, given during background RAAS blockade, reduced albuminuria more prominently in patients with higher baseline dietary sodium intake \(^9\).

This led us to prospectively investigate whether the renoprotective effects of paricalcitol, either as monotherapy or in combination with RAAS blockade, depend on dietary sodium intake. We addressed this study question in a rat model of established proteinuric nephropathy [adriamycin nephrosis (AN)].

3.2 Materials and Methods

3.2.1 Animals

Adult male Wistar rats (Crl:WI, Charles River) weighing 160–180 g at the start of the study were used. Animals were housed in a temperature-controlled room with a 12-h light–dark cycle and had free access to food and water during the entire study period. All surgical procedures took place under isoflurane anaesthesia. Experimental protocols were approved by the Animal Experiments Committee of the University of Groningen, the Netherlands.

3.2.2 Study design

After acclimatization for 1 week, all animals were trained daily for tail-cuff blood pressure measurement for 3 weeks. AN was induced by injection of 1.5 mg/kg of adriamycin (Doxorubicin®) into the tail vein \((n = 67)\). In our model of AN, proteinuria develops gradually after a single injection of adriamycin and interventions start after 6 weeks, when proteinuria is established. After 6 weeks, animals were put in metabolic cages for 24-h
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urine collection and determination of proteinuria and stratified according to proteinuria into eight experimental groups. To compare the extent of pre-fibrotic damage at the start of treatment, on the following day, a renal biopsy was taken via abdominal incision. Immediately after surgical removal of the small part of the lower pole from the left kidney, gelfoam (Spongostan®, Ferrosan, Copenhagen, Denmark) was applied to reach haemostasis. Careful precaution was taken to obtain biopsies of similar size. Biopsy procedures do not affect the level of proteinuria and renal function. Treatments started upon recovery from surgery and lasted for another 6 weeks until Week 12. Animals were treated with the ACEi lisinopril in a dose aiming for an optimal antiproteinuric effect (75 mg/L in drinking water; L)\textsuperscript{11}, paricalcitol (160 ng/kg three times per week orally; P), both (P/L) or vehicle (V); all the treatments were given during either a high-sodium (2% NaCl; HS) or a low-sodium (0.05% NaCl; LS) diet. The animals received NaCl-adjusted rat chow (Harlan, Horst, the Netherlands); apart from the sodium content, both diets were identical and had a standard composition. All groups contained six to nine animals. At the end of the study, blood was collected by cannulation of the abdominal aorta, kidneys were perfused in situ with saline and removed, fixed in 4% formaldehyde and embedded in paraffin.

3.2.3 Measurements

Body weight was measured weekly during the study period. Twenty-four hour urine was collected 2-weekly in metabolic cages from Week 6 until Week 12 with measurement of water intake. Systolic blood pressure (SBP) was measured 2-weekly in trained conscious rats using tail-cuff method (CODA 6, Kent Scientific). Urinary protein excretion was measured by the pyrogallol red molybdate method. Serum and urine creatinine, urea, sodium and potassium were all analysed on a multi-test analyser system (Roche Modular, Hoffmann-La Roche, Basel, Switzerland).

3.2.4 Real-time PCR

Quantitative real-time polymerase chain reaction (q-PCR) was performed for monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-alpha (TNF-alpha). RNA was extracted from snap-frozen kidney tissue using the Trizol method (Invitrogen, Carlsbad, CA, USA). The RNA concentrations were measured using a nanodrop UV spectrometer (Nanodrop Technologies, Wilminton, DE, USA). Subsequently, cDNA was synthesized using Superscript II RT with random hexamer primers (Invitrogen). We used a primer on demand to detect TNF-alpha gene expression (Qiagen, Venlo, the Netherlands, Catalogue No. PPR06411F-200) and custom primers for MCP-1 (forward: CCGACTCATTGGGATCATCTT, reverse: TGTCTCAGCCAGATGCGATTAAT) and GAPDH (forward: CATCAAGAAGGTGAAGC, reverse: ACCACCCTGTTGCTGTAG). Gene expression levels were measured by real-time PCR based on the SYBR Green methodology. Gene expression was normalized for the expression of the housekeeping gene GAPDH and calculated relative to the vehicle group for each sodium diet.

3.2.5 Immunohistochemical procedures

Paraffin sections (4 µm) were deparaffinized and stained with periodic acid-Schiff (PAS) to evaluate focal glomerulosclerosis (FGS). Macrophages/monocytes (ED1) and the pre-
fibrotic marker for myofibroblast transformation alpha-smooth muscle actin (α-SMA) were detected using mouse monoclonal antibodies (ED1: Serotec Ltd, Oxford, UK and α-SMA: clone 1A4, Sigma, St Luis, MO, USA). Sections were subjected to heat-induced antigen retrieval by overnight incubation in 0.1 M Tris/HCl buffer (pH 9) at 80°C. Endogenous peroxidase was blocked with 0.075% H$_2$O$_2$ in phosphate-buffered saline (pH 7.4) for 30 min. Primary antibodies were incubated for 60 min. Binding was detected using sequential incubations with peroxidase-labelled rabbit anti-mouse and peroxidase-labelled goat anti-rabbit antibodies (Dakopatts, Glostrup, Denmark) for 30 min. Peroxidase activity was developed by using 3,3’-diaminobenzidine tetrachloride containing 0.03% H$_2$O$_2$ for 10 min. Counterstaining was performed using Mayer’s haematoxylin.

3.2.6 Quantification of histopathological changes

Sections were examined in a blinded fashion. The severity of FGS was assessed on PAS sections by semi-quantitative scoring of 50 glomeruli per slide on a scale of 0–4. FGS was scored positive if collapse of capillary lumina, mesangial matrix expansion, hyalinosis and adhesion of the glomerular tuft to Bowman’s capsule were simultaneously present. If 25% of the glomerulus was affected, a score of 1 was adjudged, 50% was scored as 2, 75% as 3 and 100% as 4. The ultimate score per animal was obtained by multiplying the degree of change by the percentage of glomeruli with the same degree of injury and addition of these scores.

The extent of interstitial α-SMA protein expression was measured using computer-assisted morphometry. Thirty cortical interstitial fields (200× magnification) were measured with exclusion of arteries and glomeruli, and percentage of the stained area was determined. The ultimate score was calculated by the average of all fields per section. Glomerular and interstitial macrophages were counted manually in, respectively, 50 glomeruli and 30 interstitial fields per section, and the mean number per section/animal was calculated.

3.2.7 Statistical analysis

Statistical analyses were performed using PASW Statistics 18.0.3 (SPSS, Armonk, NY, USA) and GraphPad Prism (version 3.02, GraphPad Software, San Diego, CA, USA). Data were tested for normality, and when non-parametric distribution was present, differences between groups were analysed by Kruskal–Wallis and Mann–Whitney U tests; otherwise, the ANOVA with Bonferroni post hoc test was used. We display histopathological scores relative to the mean of each vehicle control group, i.e. either HS + vehicle or LS + vehicle, respectively, since our primary goal was to evaluate the efficacy of the pharmacological interventions compared with vehicle during either diet. The appropriate vehicle-treated group was used as control group. Data are presented as mean ± standard error of the mean unless otherwise stated. For all analyses, P<0.05 was considered statistically significant.
### 3.3 Results

#### 3.3.1 Animal characteristics

Six weeks after adriamycin injection, i.e. before the treatment was started, α-SMA immunostaining in renal biopsies was similar across all groups (Supplementary material, Figure 3.7, P > 0.05 for all comparisons). Mean 24-h urinary sodium excretion at the end of the experiment was in accordance with the high-sodium diet versus low-sodium diet (Table 3.1). One animal in the LS/paricalcitol group with a discrepantly high 24-h sodium excretion (1420 µmol/24 h, i.e. above the 99th percentile), indicating inadequate dietary sodium restriction, was excluded from further analysis. Body weight was similar across all groups at the start of the treatment, but after 6 weeks of the treatment, weight was lower in animals on LS/lisinopril and LS/paricalcitol/lisinopril compared with LS/vehicle. Both groups also displayed reduced creatinine clearance compared with LS/vehicle. In all other groups, creatinine clearance was comparable. Water intake was similar for all groups. During the treatment, one animal in the HS/L group died.

#### 3.3.2 Proteinuria and blood pressure

The course of proteinuria during the 6-week treatment period is shown in Figure 3.1. Proteinuria (>30 mg/24 h) was present in all animals at the onset of the treatment. During HS diet (Figure 3.1A), none of the treatments reduced proteinuria compared with vehicle. In contrast, during LS diet (Figure 3.1B), proteinuria was strongly reduced by paricalcitol monotherapy (P < 0.01 versus LS/vehicle at 12 weeks) and almost completely abolished by lisinopril monotherapy (P < 0.01) or combination therapy (P < 0.01).

SBP at the start of the treatment was comparable between groups (Figure 3.2). During high-sodium diet (Figure 3.2A), lisinopril, both as monotherapy and in combination with paricalcitol, moderately reduced blood pressure (P < 0.01 versus HS/vehicle at 12 weeks). During low-sodium diet, the antihypertensive effect was more pronounced in both groups (Figure 3.2B). Paricalcitol monotherapy did not influence SBP, neither during HS nor during LS diet. Blood pressure or proteinuria was not modulated by the sodium diet alone (P > 0.05 LS/vehicle versus HS/vehicle).

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**Table 3.1:** Group characteristics. Data are expressed as median (interquartile range). Data were obtained after 6 weeks of treatment, unless otherwise indicated. *P < 0.05 versus low sodium/vehicle. **P < 0.05 versus corresponding high-sodium group.

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<td>Body weight (g)</td>
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<tr>
<td>At treatment start</td>
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<td>515 (489−545)</td>
<td>511 (466−525)</td>
<td>463 (435−506)</td>
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<td>At treatment end</td>
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<td>511 (558−622)</td>
<td>595 (516−619)</td>
<td>533 (523−584)</td>
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<td>856 (565−1095)</td>
<td>3111 (1162−5502)</td>
<td>814 (552−2517)</td>
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<td>Creatinine clearance (mL/min)</td>
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<td>3.6 (3.1−4.3)</td>
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Sodium Restriction Potentiates

Figure 3.1: Proteinuria. Proteinuria course during 6 weeks of treatment with high-sodium (A) or low-sodium (B) diet combined with paricalcitol, lisinopril or both. Lowering of proteinuria was significant for all the treatments during low, but not high, sodium diet. **P < 0.01 versus LS/paricalcitol, LS/lisinopril and LS/paricalcitol/lisinopril groups.

Figure 3.2: Systolic blood pressure (SBP). SBP course during 6 weeks of treatment with high-sodium (A) or low-sodium (B) diet combined with paricalcitol, lisinopril or both. Treatment with lisinopril monotherapy or lisinopril + paricalcitol reduced SBP during both sodium diets (**P < 0.01 versus vehicle), although the reduction was most prominent during the low-sodium diet. Paricalcitol did not significantly reduce blood pressure.

3.3.3 Glomerular inflammation and FGS

The extent of glomerular inflammation was assessed by quantification of glomerular macrophage accumulation. In all adriamycin rats, macrophages were detected in the glomeruli, around renal arteries and injured tubuli. Both paricalcitol monotherapy and paricalcitol/lisinopril reduced glomerular inflammation during LS (Figure 3.3B) but not during HS diet (Figure 3.3A); animals on lisinopril monotherapy showed a similar trend (P = 0.06). FGS was significantly reduced by paricalcitol, lisinopril and their combination during LS diet (Figure 3.3D). During the HS diet, similar trends were observed, although none of the differences during the HS diet reached statistical significance (Figure 3.3C).
Figure 3.3: Glomerular inflammation and glomerulosclerosis. Glomerular macrophage accumulation after 6 weeks of treatment during high- (A) or low-sodium diet (B). Glomerular inflammation was reduced by paricalcitol monotherapy or paricalcitol + lisinopril (lisinopril monotherapy borderline significant) during low, but not high, sodium diet. FGS after 6 weeks of treatment during high- (C) and low-sodium diets (D). FGS was reduced by all the treatments during the low-sodium diet. During the high-sodium diet, similar yet non-significant trends were observed. ∗P < 0.05 versus LS/vehicle.

3.3.4 Interstitial inflammation and pre-fibrotic changes

Both paricalcitol and lisinopril monotherapy as well as their combination reduced interstitial macrophage influx compared with vehicle during LS (Figure 3.4B) but not HS diet (Figure 3.4A). Representative images are presented in Figure 3.4C. Real-time PCR demonstrated that renal expression of the pro-inflammatory genes MCP-1 (Figure 3.5A and B) and TNF-alpha (Figure 3.5C and D) was (borderline) reduced by all the treatments during the low-sodium diet, but not during the high-sodium diet.

Interstitial expression of α-SMA was used to determine the extent of pre-fibrotic tubulointerstitial changes. Representative images according to treatment groups are presented in Figure 3.6C. Paricalcitol, both alone and in combination with lisinopril, significantly reduced α-SMA expression during the LS diet, while treatment with lisinopril during the LS diet resulted in a similar trend (Figure 3.6B). During the HS diet, only non-significant trends were observed for all interventions compared with vehicle (Figure 3.6A). When we assessed changes in α-SMA expression from the start of the treatment (assessed in pre-treatment biopsies) to the end of the treatment, we found that progression of α-SMA expression was (borderline) significantly reduced by all interventions, irrespective of the diet (Figure 3.6C and D). Progression of α-SMA was (numerically) least in animals treated with LS/lisinopril/paricalcitol (Figure 3.6D).
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Figure 3.4: Interstitial inflammation. Average macrophage accumulation per tubulointerstitial field after 6 weeks of treatment during high- (A) and low-sodium diets (B). All interventions significantly reduced interstitial inflammation during the low, but not high, sodium diet. (C) Representative photomicrographs of all groups. *P < 0.05 versus LS/vehicle.

Figure 3.5: Renal gene expression of MCP-1 and TNF-alpha. Real-time PCR for MCP-1 (A and B) and TNF-alpha (C and D) revealed that after 6 weeks of treatment during high-sodium diet, none of the interventions affected these pro-inflammatory genes (A and C), whereas during the low-sodium diet all the treatments showed (borderline) reduction in pro-inflammatory gene expression (B and D). *P < 0.05 versus LS/vehicle.
Figure 3.6: Interstitial α-SMA expression. Tubulointerstitial pre-fibrotic changes were determined by quantification of interstitial α-SMA expression per tubulointerstitial field at baseline and after 6 weeks of treatment. Scores were evaluated at the end of the study during high- (A) and low-sodium diets (B). α-SMA expression was reduced by paricalcitol monotherapy or paricalcitol + lisinopril (lisinopril monotherapy borderline significant) during low, but not high, sodium diet. During high-sodium diets, non-significant trends were observed. Progression of α-SMA was assessed by subtracting baseline scores from scores at the end of the treatment, during high- (C) or low-sodium diet (D). Both during high- and low-sodium diets, a (trend to) reduction in α-smooth muscle progression was observed; this was most pronounced during paricalcitol/lisinopril/low-sodium diet. (E) Representative photomicrographs at the end of the treatment for all groups. *P < 0.05 versus LS/vehicle.

3.4 Discussion

The main finding of this study is that in a rat model of established proteinuric nephropathy, treatment with the VDRA paricalcitol is effective in reducing proteinuria and glomerular and interstitial inflammation when combined with a low-sodium diet, but not during a high-sodium diet. These renoprotective effects of paricalcitol were independent of blood pressure and were observed either when given as monotherapy or in combination with the ACEI lisinopril. To our knowledge, this is the first study to demonstrate that renoprotective effects of VDRA therapy depend on sodium intake.

Current renoprotective strategies in proteinuric CKD are based on single-agent RAAS blockade, aiming for optimal reduction in blood pressure and proteinuria. Our data are in
Sodium Restriction Potentiates line with previous preclinical\textsuperscript{13,14} and clinical\textsuperscript{2,3} studies documenting that sodium intake potentiates the antiproteinuric efficacy of ACEIs \textsuperscript{13,14}. The strong antiproteinuric effect of lisinopril during the low-sodium diet was accompanied by a drop in renal function, in line with earlier reports. Such effect probably reflects a reversible haemodynamic effect, which has been associated with improved long-term prognosis in patients\textsuperscript{15,16}.

During the high-sodium diet, the antiproteinuric response to RAAS blockade was blunted, in accordance with previous clinical studies\textsuperscript{17}. Surprisingly, a post hoc analysis of the VITAL clinical trial in diabetic nephropathy suggested that paricalcitol was more effective to reduce albuminuria in patients with higher dietary sodium intake\textsuperscript{9}. However, in the VITAL study, dietary sodium intake was estimated from a single 24-h urine collection at baseline. In contrast, in our prospective study, specifically designed to further address this subject, we found that paricalcitol, particularly when combined with lisinopril, reduced proteinuria during a low-sodium diet, but not a high-sodium diet. This was accompanied by significant anti-inflammatory effects in response to paricalcitol (with or without lisinopril), further supporting the notion that vitamin D analogues, similar to RAAS blockade, require dietary sodium restriction to provide renoprotective effects.

Despite the fact that the treatment with lisinopril during LS diet reduced proteinuria practically to control levels, this did not translate into optimal reduction of tubulointerstitial α-SMA (Figure 3.5B). Previous results from our group showed that the dose of lisinopril used in this study exerts a powerful antiproteinuric effect\textsuperscript{11} that can be further potentiated by adding a low-sodium diet\textsuperscript{18}. However, similar to our current findings, in these studies, the reduction in proteinuria and glomerular damage dissociated from the tubulointerstitial effects, that ranged from no protection to outright worsening. Our current study extends these findings by showing that the VDRA paricalcitol under low-sodium conditions can further potentiate renoprotective effects by ameliorating interstitial pre-fibrotic changes, and by optimally preventing progression of interstitial pre-fibrotic lesions (Figure 3.5C and D). As interstitial fibrosis strongly predicts renal outcome\textsuperscript{19}, this additional tubulo-interstitial protection could have therapeutic relevance to long-term outcome. Yet the effects of VDRA (with or without ACEI) treatment on glomerular (FGS) and interstitial (α-SMA) (pre-)fibrotic markers were only marginally different when comparing low- and high-sodium diets, as opposed to the clearly differential effects on proteinuria and renal inflammation.

Vitamin D analogues have demonstrated renoprotective effects, either when given as a monotherapy or in combination with RAAS blockade\textsuperscript{7,20}. Beneficial effects of vitamin D analogues have been attributed to reduction in proteinuria by a direct protective effect on podocytes, subsequently resulting in reduced inflammation and (pre-)fibrotic processes\textsuperscript{20}. Several studies have investigated the combination of vitamin D and RAAS blockade, mainly in experimental models of diabetic nephropathy\textsuperscript{21−23}. In all of these studies, monotherapy showed relatively modest effects on the development of albuminuria and glomerulosclerosis, while combined treatment provided a strong effect. In our study, under low-sodium diet, paricalcitol monotherapy significantly reduced proteinuria as well as interstitial inflammation and pre-fibrotic changes compared with vehicle. We did not, however, observe an additive effect on proteinuria when paricalcitol was combined with lisinopril, most probably because the antiproteinuric effect of lisinopril was already strongly potentiated by the low-sodium diet, limiting the potential additional effect of paricalcitol on proteinuria as well as downstream inflammatory and fibrotic changes.
Since paricalcitol did not affect blood pressure in our study, it is highly unlikely that the renoprotective effects of paricalcitol are mediated by an effect on blood pressure.

Our finding that dietary sodium restriction potentiates the renoprotective efficacy of VDRA treatment, combined with ACE inhibition, is in line with previous studies using ACEIs, ARBs, their combination, renin inhibitors and neprilysin inhibitors. Although the underlying mechanism is not elucidated by our study, we hypothesize that a protective effect of paricalcitol on glomerular haemodynamics, contributing to the observed reduction in proteinuria, may be offset by the low-sodium diet. An ongoing clinical trial further investigates the effects of paricalcitol during a high- or a low-sodium diet on glomerular haemodynamics.

Limitations of our study include the fact that the extent of proteinuria and subsequent renal damage was relatively mild, which could have resulted in an underestimation of the renoprotective effects of paricalcitol, particularly in the paricalcitol + lisinopril group. Since proteinuria and inflammation precede the development of (pre-)fibrotic changes, this might also explain the similar effects of paricalcitol (and/or lisinopril) during a low-sodium diet versus a high-sodium diet on (early) fibrosis markers, which was discrepant with the effects on proteinuria and inflammation. Blood pressure was measured by the tail-cuff method, which is less precise than telemetric measurement. Furthermore, the animals treated with lisinopril (either with or without paricalcitol) during the low-sodium diet did not gain weight during the treatment period and displayed a reduced creatinine clearance, which may indicate drug toxicity. The effect of combined ACE inhibition and volume depletion on renal function was stronger than generally observed in the clinical setting, probably since sodium restriction was relatively strict in our study (0.05%). We cannot exclude that some of our findings were influenced by the reduced creatinine clearance; paricalcitol monotherapy during low sodium, however, did not affect renal function. Furthermore, the dose of paricalcitol used in our study was relatively low compared with other experimental studies. On the other hand, the dose of paricalcitol (160 ng/kg three times per week p.o.) was chosen on purpose to better approach the dose used in the clinical setting when compared with most published studies using 2- to 3-fold higher doses.

Another strong point of our study is its design where treatment is started during established proteinuria, better reflecting the clinical setting compared with studies initiating treatment before onset of renal injury.

In conclusion, the VDRA paricalcitol alone and in combination with the ACEI lisinopril reduced proteinuria and reduced glomerular and tubulointerstitial inflammation during a low-sodium diet, but not during a high-sodium diet, in a rat model of proteinuria-induced renal damage. Our findings provide the basis for future studies in models characterized by more severe renal damage and with a longer follow-up. Whether the combination of a VDRA with dietary sodium restriction may enhance the renoprotective effects of RAAS blockade in CKD patients is currently being addressed in ongoing clinical trials, such as the ViRTUE trial in non-diabetic CKD and the PROCEED trial (ClinicalTrials.gov identifier: NCT01393808) in diabetic nephropathy; results of both trials are eagerly awaited.
References


18. Kramer AB, Bos H, van Goor H et al. Sodium intake modifies the negative prognostic value of renal damage prior to treatment with ACE inhibitors on proteinuria induced by adriamycin. Nephron Physiol 2006; 103: 43–52


**Supplementary material**

**Figures**

**Figure 3.7:** Results of a pilot study to identify the optimal recombinant FGF23 dose for the induction of hypophosphatemia. Mice (n = 5 per group) were treated with either a 40 µg/kg or a 160µg/kg dose of FGF23, administered twice with a 12-hour interval, using peritoneal injections. Blood samples were taken 12 hours after the second injection. We observed a significant decrease in serum phosphate (p<0.05) in mice treated with the high dose, but not in mice treated with the low dose, as compared to vehicle control animals.