Antifibrotic, nephroprotective effects of paricalcitol versus calcitriol on top of ACE-inhibitor therapy in the COL4A3 knockout mouse model for progressive renal fibrosis

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ABSTRACT

Background. The COL4A3−/− mouse serves as animal model for progressive renal fibrosis. Using this animal model, the present study investigates the nephroprotective effects of Paricalcitol versus Calcitriol alone and on top of ACE-inhibitor therapy.

Methods. Eighty six mice were divided into six groups: (PC) paricalcitol (dose equipotent), (PLAC) vehicle 0.1 mL i.p.

Methods. Eighty six mice were divided into six groups: (PC) paricalcitol (dose equipotent), (PLAC) vehicle 0.1 mL i.p. five times per

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week, (ACE + PC) Paricalcitol plus Ramipril, (ACE + CA) Calcitriol plus Ramipril and (ACE + PLAC) vehicle plus Ramipril. ACE therapy started pre-emptively in Week 4, PC/CA therapy was initiated in 6-week-old animals with ongoing renal fibrosis and lasted for 8 weeks. Four to six animals were sacrificed after 9.5 weeks and kidneys were further investigated using histological, immunohistological and Western-blot techniques. Survival until end-stage renal failure was determined in the remaining animals.

**Results.** PC, but not CA, prolonged lifespan until renal failure by 13% compared with untreated controls (P = 0.069). ACE-inhibition prolonged lifespan by >50%. Added on top of ACE inhibition, ACE + PC (but not ACE + CA) even further prolonged lifespan by additional 18.0% (P < 0.01 versus ACE + PLAC) and improved renal function (blood urea nitrogen; P < 0.05 versus ACE + CA). Accumulation of extracellular matrix and renal scarring was decreased in PC and ACE + PC-treated mice.

**Conclusions.** The present study demonstrated a substantial nephroprotective and antifibrotic effect of the vitamin D-receptor activator Paricalcitol on top of early ACE inhibition in the COL4A3−/− model of progressive kidney fibrosis. The synergistic effect of Paricalcitol on top of RAAS-blockade might as well be valuable in other chronic kidney diseases.

**Keywords:** Alport syndrome, collagen, fibrosis, nephroprotection, renal insufficiency

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**INTRODUCTION**

Kidney fibrosis, associated with renal failure, is known to be the most common final stage of progressive renal disease. Alport syndrome (AS) is a hereditary disorder associated with progressive renal failure and kidney fibrosis. The disease is characterized by mutations in genes encoding for either the α3, 4 or 5 chain of type IV collagen, leading to defect glomerular basement membrane (GBM). In the course of the disease, thickening and splitting of the GBM are accompanied by haematuria, proteinuria and progressive renal fibrosis leading to end-stage renal failure.

Several animal models of AS have been established, one of which is a COL4A3 knockout mouse model by Cosgrove et al. [1]. This model allows examination of renal fibrosis without disturbing factors like hypertension or surgical manipulations like partial nephrectomy. By the help of this model, several treatments including renin–angiotensin–aldosterone system (RAAS) blockade, stem-cells and antifibrotic therapy have been evaluated [2–6].

Vitamin D deficiency has been recognized as a very relevant problem in chronic kidney disease (CKD), which can be observed even in early stages of the disease. Several mechanisms can lead to vitamin D deficiency: (1) the final hydroxylation of 1,25-(OH)²D³ in the kidney is suppressed early in the course of CKD; (2) the megalin-mediated re-uptake of filtrated 25-(OH)²D³ is impaired in patients with proteinuria due to the loss of vitamin D-binding protein; (3) further, high levels of phosphate, which occur in CKD, lead to suppression of 1α-hydroxylase activity [7]. Vitamin D deficiency can lead to further damage to the renal and cardiovascular system. Low plasma levels of vitamin D have been shown to act as independent inverse predictors of death and disease progression in patients with CKD [8]. Additionally, vitamin D in its activated form 1,25-dihydroxyvitamin D3 acts as a hormone in bone metabolism but also as a down-regulator of the RAAS [9]. The RAAS plays a key role in the development of renal damage, partly independent from high blood pressure. The main mechanism for this renal damage seems to be the profibrotic potential of angiotensin II. High intrarenal levels of angiotensin II, for example in diabetic kidneys, induce proinflammatory and profibrotic mechanisms like cytokine production, podocyte damage and up-regulation of extracellular matrix production [10–12].

Paricalcitol is a recently developed analogue of activated vitamin D. It reduces renin-expression at doses above those necessary to suppress Parathormone without inducing hypercalcemia [13]. This seems to be one of the advantages of Paricalcitol in comparison with Calcitriol, in which induction of hypercalcemia is an important side effect. Furthermore, Paricalcitol displays a more obvious dose response. In previous studies, beneficial effects of a combination therapy of Paricalcitol and RAAS-blockers in mice with diabetic nephropathy have been described [9]. There is growing evidence that there are differences in the outcome in a therapy with either Calcitriol or a selective activator of the vitamin D receptor like Paricalcitol [14]. A recent epidemiological study in patients on haemodialysis showed a beneficial effect on survival in patients receiving Paricalcitol compared with those receiving Calcitriol [15].

The aim of the present study is to investigate possible favourable effects of Paricalcitol versus Calcitriol on the progress of kidney fibrosis in a non-hypertensive, non-diabetic animal model. Additionally, the study explores monotherapy versus the combination with the ACE-inhibitor Ramipril to analyse possible synergistic effects. Ramipril is known to prolong lifespan in Alport mice [16] and humans [17]. Paricalcitol and Calcitriol monotherapy were initiated in 6-week-old Alport mice, corresponding to Stage II disease (overt proteinuria) in human Alport patients [17]. This timepoint of overt proteinuria is the most relevant for initiation of nephroprotective therapy in all other CKDs leading to progressive fibrosis. ACE-inhibitor therapy was started at 4 weeks of age before onset of proteinuria in Alport mice to maximize the therapeutic effect of RAAS blockade [16]. The early onset of RAAS blockade increased the timespan as ‘therapeutic window’ for treatment with Paricalcitol and Calcitriol in the Alport mice: therefore, the present study was able to investigate the nephroprotective and antifibrotic effect of Paricalcitol on top of early on ACE inhibition in the COL4A3−/− model of progressive kidney fibrosis.

Beyond the established indication for Paricalcitol, hyperparathyroidism, our study investigates a possible role of Paricalcitol therapy because of its antifibrotic nephroprotective effects in patients with progressive renal fibrosis.
MATERIALS AND METHODS

Animals and medication

Genotyping of COL4A3+/− mice (Jackson Lab, ME, USA) was carried out by PCR as described before [16]. Treatment protocols for the mice were previously approved by local German authorities and supervised by veterinarians. Animal diet R/MH (V153x; Sniff, Soest, Germany) contained 19% protein, 3.3% fat and 4.9% fibre. PC and CA were provided by Abbott (Abbott Pharma AG, Ludwigshafen, Germany), dosages were applied according to recommendations by the manufacturer. Dosage of Paricalcitol was applied intraperitoneal five times a week at 0.1 mcg/kg as this is dose equipotent to Calcitriol 0.03 mcg/kg. Mice were bred on a clean 129/SvJ genetic background for >20 generations in a pathogen-free environment.

Homozygous COL4A3+/− mice (n = 86) were divided into six groups:

(i) (PC): with Paricalcitol 0.1 mcg/kg,
(ii) (CA): Calcitriol 0.03 mcg/kg (dose equipotent),
(iii) (PLAC): vehicle 0.1 mL i.p. five times per week,
(iv) (ACE + PC): Paricalcitol plus Ramipril,
(v) (ACE + CA): Calcitriol plus Ramipril and
(vi) (ACE + PLAC): vehicle plus Ramipril.

ACE therapy started pre-emptively in Week 4 at the maximal tolerated dosage10 mg/kg/day p.o. in drinking water, PC/CA therapy was initiated in 6-week-old animals with ongoing renal fibrosis and lasted for 6 weeks. Duration of PC/CA therapy had to be limited to 6 weeks in order to avoid suffering of animals due to recurrent intraperitoneal injections. Four (PC, CA, PLAC, ACE + PC, ACE + CA) to six (ACE + PLAC) animals were sacrificed after 9.5 weeks and kidneys were further investigated using histological, immunohistological and Western-blot techniques. Survival until end-stage renal failure was determined in the remaining 8 (PC, CA, PLAC, ACE + PC, ACE + CA) to 10 (ACE + PLAC) animals.

Renal function tests

Proteinuria was measured using a 4–12% Novex Tris Glycine polyacrylamide gradient gels (Life Technologies, Carlsbad, USA) stained with Coomassie Blue and analysed by densitometry, a semiquantitative method described previously [16]. For measurements of urea, serum samples were obtained when mice were sacrificed. Serum urea was measured on a Cobas8000 Modular Analyzer Series (Roche Diagnostics, Mannheim, Germany).

Histology and immunostaining

Kidneys for histological examination and immunochemistry analysis were obtained when mice were sacrificed and immersion fixed as described previously [16]. Three micrometers thick sections were prepared on a Reichert-Jung 2040 Autocut Microtome (Leica, Wetzlar, Germany) and fixated on glass slides. Sections were dewaxed in xylene and rehydrated in decreasing alcohol concentrations. Sections were demasked using proteinase K for laminin or citrate buffer (pH 7.6) for fibronectin and incubated over night at 4°C with either anti-laminin 111 (ab11575, Abcam, Cambridge, UK) or anti-fibronectin (BM422, Acris Antibodies, San Diego, USA). Secondary antibodies were used as negative controls. After washing in TBST sections were incubated with a cy3-stained secondary anti-rabbit antibody (611-104-122, Rockland Immunchemicals, Gilbertsville, USA) or a TRITC conjugated secondary anti-mouse antibody (T5393, Sigma-Aldrich, St. Louis, USA), washed again in TBST and mounted with mounting-medium. Photographs were taken from six different positions of the section and evaluated for increased deposition of extracellular matrix on a Zeiss Axiosvert S100 TV microscope (Göttingen, Germany). Staining for fibronectin and laminin was used to evaluate the grade of fibrosis. Photographs of the immunostained sections were scored by three independent, blinded individuals [16]. This score ranged from 0 = no or normal deposition of fibronectin or laminin to 3 = heavily increased deposition.

Immunoblotting and ELISA

For this immunoblotting of TGFβ levels, three to four kidneys from different animals were pooled to minimize individual differences. Protein concentration was determined by BCA protein assay (Pierce, Rockford, USA). Thirty micrograms of protein aliquots of whole kidney extracts of 9.5-week-old untreated and treated animals were dissolved in SDS-sample buffer, separated by electrophoresis in a 15% SDS–polyacrylamide gel, transferred to nitrocellulose membrane, and blocked. Mouse anti-TGFβ1 (R&D Systems, Minneapolis, USA) was incubated for 60 min. The membrane was then incubated with secondary antibody conjugated with HRP (Agilent, Santa Clara, USA), and the blot was developed using chemoluminescence. Protein-expression was analysed densitometrically using the Kd1 analyzer software (Kodak, Rochester, USA). Each immunoblot was repeated three times.

ELISA was performed according to the instructions of the manufacturer (eBioscience, San Diego, USA).

Statistics

Data are presented as mean ± SEM. One-tailed Student t-test was used for renal function tests (Albuminuria, high-molecular-weight proteinuria, BUN) to analyse the hypothesis if dual therapy has stronger nephroprotective effects than monotherapies (for example, PLAC versus CA or PC, ACE + PLAC versus ACE + PC or ACE + CA). For all other statistical analysis mentioned throughout the text and the figures, we used two-way analysis of variance (ANOVA) and log-rank statistic (survival analysis).

RESULTS

Effect of therapy on lifespan of Alport mice until death from renal failure

Placebo (vehicle)-treated mice will be named ’untreated’ throughout the text, because the term ’Placebo’ should be
reserved to human beings. Untreated Alport mice died at a mean age of 66.3 days (range of 59–78, standard deviation (SD) 6.7) (Figure 1a). The Kaplan–Meier survival of CA-treated Alport mice was almost identical to untreated mice (66.6 days; range of 61–77, SD 5.3; not significant versus PLAC). PC therapy improved lifespan until death from renal failure by 13.3% to 75.1 days (range of 70–84, SD 5.0; P = 0.069 versus PLAC) (Figure 1b). ACE-treated Alport mice lived significantly longer than PLAC-, CA- or PC-treated mice (101.7 days, range 77–142, SD 19.1; P < 0.001 versus PLAC, CA and PC) (Figure 1a and b). Lifespan of ACE + CA-treated mice increased to 112.9 days (range 91–138 days, SD 14.3; data not significant versus ACE + PLAC). ACE + PC significantly prolonged lifespan by 18.0% to 120.0 days (range of 111–133, SD 8.4; P < 0.01 versus ACE + PLAC and not significant versus ACE + CA).

**Effect of therapy on renal function tests**

Albuminuria (Figure 2a) and high-molecular-weight proteinuria (Figure 2b) was quantified densitometrically and the values of albumin excretion measured in 6.0-, 7.5- and 9.5-week-old mice. In Week 7.5, albuminuria and high-molecular-weight proteinuria (mainly immunoglobulins) were >10-fold higher in untreated Alport mice compared with wild-type mice (Figure 2a and b). Compared with untreated Alport mice, CA did not improve proteinuria at all. In contrast, PC lowered albuminuria by ~40% and high-molecular-weight proteinuria by ~60%. ACE + PLAC and ACE + PC lowered albuminuria and high-molecular-weight proteinuria by ~80%. ACE + CA lowered albuminuria by ~80% and high-molecular-weight proteinuria by ~50% (all data compared with untreated Alport mice, all mice in Week 7.5).

Due to the advanced kidney fibrosis and ongoing oliguric renal failure, comparison of proteinuria in 9.5-week-old Alport mice was less meaningful. However, all treatment combinations with Ramipril (ACE + PLAC, ACE + PC, ACE + CA) still had a substantial effect on albuminuria and high-molecular-weight proteinuria (Figure 2a and b).

Loss of renal function was determined by values of blood urea nitrogen (BUN) in 9.5-week-old mice with different treatment modalities (Figure 3). Wild-type mice showed a BUN of 27 ± 2.9 mg/dL. BUN of untreated Alport mice did not differ significantly from CA- or PC-treated mice. BUN of all ACE-treated mice (ACE + PLAC, ACE + CA, ACE + PC) significantly improved compared with untreated Alport mice. In Alport mice treated with ACE + PLAC, the BUN was diminished to 53 ± 9.6 mg/dL. In ACE + CA-treated Alport mice, BUN was lowered to 65 ± 5.6 mg/dL, which was significantly worse than in ACE + PC-treated Alport mice with a BUN of 39 ± 0.8 mg/
dL (P < 0.05). The BUN value of ACE + PC-treated mice was significantly better than of the ACE + PLAC group.

**Effect of therapy on extracellular matrix accumulation**

Kidney sections from 9.5-week-old mice were scored by three to four blinded observers: in wild-type mice, extracellular laminin deposition (Figure 4d) was very low with a low glomerular score of 0.38 ± 0.1 (Figure 4g) and a low tubulo-interstitial score of 0.45 ± 0.11 (Figure 4h). In contrast, untreated Alport mice (Figure 4a) showed a very high deposition of laminin (glomerular score 2.0 ± 0.14; P < 0.001 versus wild type; tubulo-interstitial score 2.03 ± 0.11; P < 0.001 versus Wild type) (Figure 4g and h). PC therapy (Figure 4f) reduced the glomerular matrix accumulation to a value of 1.64 ± 0.12 (n.s. versus untreated mice) as well as did CA therapy (score 1.36 ± 0.24; P < 0.01 versus untreated mice) (Figure 4g). In parallel, therapy reduced tubulo-interstitial matrix deposition to a value of 1.83 ± 0.19 (PC) and 1.92 ± 0.38 (CA) (data not

**FIGURE 3:** Renal function. Blood urea nitrogen in untreated mice versus different treatment modalities. wt, wild type. All mice are 9.5 weeks of age (n = 3). *P < 0.05; ***P < 0.001.

**FIGURE 4:** Glomerular and tubulo-interstitial accumulation of extracellular matrix. Immune-fluorescence staining of laminin 111, ×200-fold. (a) Untreated Alport mice severe glomerular and tubular matrix deposition; (b) ACE + CA-treated Alport mice; (c) CA-treated Alport mice; (d) wild-type mice without matrix accumulation; (e) ACE + PC-treated Alport mice with less severe glomerular and minor tubular matrix deposition; (f) PC-treated Alport mice with extensive glomerular and significant tubular matrix accumulation; (g) score of glomerular extracellular matrix accumulation and (h) score of tubulo-interstitial extracellular matrix accumulation. wt, wild type. All mice 9.5 weeks of age (n = 3). ***P < 0.001; **P < 0.01; *P < 0.05.
significantly; Figure 4h). ACE + PC (Figure 4e) markedly reduced the glomerular matrix accumulation to a score of 1.13 ± 0.09 (P < 0.001 versus untreated mice) as well as ACE + PLAC alone (score 0.70 ± 0.02; P < 0.001 versus untreated mice) or the ACE + CA combination (score 1.09 ± 0.16; P < 0.001 versus untreated mice). In parallel, therapy reduced tubulo-interstitial matrix deposition by 43% to a value of 1.15 ± 0.11 (ACE + PC; P < 0.05), minus 3% to a value of 1.31 ± 0.25 (ACE + CA; P < 0.05) and to 0.80 ± 0.02 (ACE + PLAC; P < 0.01 versus untreated Alport mice) (Figure 4h).

Effect of therapy on scar-tissue formation in the kidney

Wild-type mice (Figure 5d) showed a very low glomerulosclerosis-score of 0.24 ± 0.04 (Figure 5g) and no relevant tubulo-interstitial fibrosis (score 0.2 ± 0.05; Figure 5h). In contrast, untreated Alport mice (Figure 5a) showed a prominent scarring (glomerular score 1.95 ± 0.06; P < 0.001 versus wild type; and tubulo-interstitial score 2.23 ± 0.08; P < 0.001 versus wild type) (Figure 5g and h). PC therapy (Figure 5f) significantly reduced the glomerular scar-tissue formation to a value of 1.74 ± 0.03 (n.s. versus untreated mice) as well as did CA therapy (score 1.65 ± 0.39; n.s. versus untreated mice) (Figure 5g). In parallel, therapy reduced tubulo-interstitial fibrosis to a value of 2.01 ± 0.07 (PC) and 1.82 ± 0.29 (CA) (data not significant; Figure 5h). ACE + PC (Figure 5e) markedly reduced the glomerular scarring to a score of 1.43 ± 0.13 (n.s. versus untreated mice; n.s. ACE + PLAC versus ACE + PC or versus ACE + CA) as well as ACE + PLAC alone (score 1.1 ± 0.3; P < 0.05 versus untreated mice) or the combination of ACE + CA (score 1.5 ± 0.11; n.s. versus untreated mice). In parallel, therapy reduced tubulo-interstitial fibrosis by 58% to a value of 0.91 ± 0.18 (ACE + PC; P < 0.001), minus 44% to a value of 1.22 ± 0.09 (ACE + CA; P < 0.001) and to 1.33 ± 0.26 (ACE + PLAC, ACE + PC, ACE + CA; all are P < 0.001 versus untreated Alport mice; n.s. ACE + PLAC versus ACE + PC or versus ACE + CA) (Figure 5h).

Effect of therapy on expression of profibrotic and proinflammatory cytokines

Kidneys of Alport mice showed a >10-fold up-regulation of TGFβ compared with wild-type mice in Western-blot analysis (Figure 6a). Treatment with ACE + PLAC reduced TGFβ by
85% (see [16]), while the combination ACE + CA led to a decrease of TGFβ synthesis by 94%. CA and ACE + CA reduced TGFβ expression below wild-type level. PC reduced TGFβ expression by 63 ± 4.6%; ACE + PC further reduced TGFβ expression by 74 ± 4.4%.

In order to further analyse the impact of therapy, the expression of the cytokine interleukin 6 (IL6) was measured in homogenates of kidneys of Alport mice treated with various combinations using an ELISA (Figure 6b). In untreated Alport mice, IL6 expression was decreased by 65% compared with kidneys of wild-type mice. CA increased IL6 expression to the value found in wild-type mice, but the combination of ACE + CA increased IL6 expression only to 79% of the wild-type level. Treatment of Alport mice with PC alone even lowered the IL6 value from 29 to 18%. Treating Alport mice with a combination of ACE + PC increased IL6 expression almost to the level measured in wild-type mice (96% of wild-type value).

**FIGURE 6:** Expression of profibrotic TGFβ and proinflammatory IL6. (a) Western blot of profibrotic TGFβ in 9.5-week-old Alport mice treated with either CA, ACE + CA, PC, ACE + PC or ACE + PLAC. Expression was quantified by densitometry (n = 3). (b) ELISA of proinflammatory IL6 using 100 μg protein of the supernatants of homogenized lysates of kidneys of 9.5-week-old Alport mice treated with either CA, ACE + CA, PC, ACE + PC or ACE + PLAC (n = 3).

**DISCUSSION**

In the present study, COL4A3 knockout mice as an animal model for human AS with progressive renal fibrosis were treated with Paricalcitol/Calcitriol alone or in combination with Ramipril. ACE inhibition started at 4 weeks of age, corresponding to early onset of ACE-inhibitor therapy in children with AS. However, Ramipril was started 1 week later than in previous experiments [16], because the present study focussed on the additive effect of Paricalcitol/Calcitriol on top of ACE-inhibitor therapy and not on the maximum effects of Ramipril monotherapy. Data from Alport animal models [16] and humans with AS [17] demonstrated a beneficial effect on delaying renal failure and thereby prolonging life-expectancy by pre-emptive ACE inhibition. Paricalcitol/Calcitriol therapy was started in Week 6, similar to a clinical setting in humans with AS with progressive proteinuria and ongoing renal fibrosis.

According to our hypothesis, the combination of Paricalcitol on top of ACE inhibition might delay progressive renal fibrosis and renal failure due to a synergistic effect. This hypothesis was supported by previous findings showing a beneficial effect of a combined therapy with Paricalcitol and Angiotensin receptor blockade in mice with diabetic nephropathy [9]. Interestingly, in our study, the addition of Paricalcitol in proteinuric mice on top of early ACE inhibition delayed renal failure to a better extent than monotherapy with ACE inhibition or the combination of Ramipril with Calcitriol. Levels of blood urea nitrogen as a parameter of kidney function confirmed the results on lifespan. In conclusion, Paricalcitol showed synergistic effects on top of ACE inhibition in delaying renal failure due to progressive renal fibrosis, in contrast to Calcitriol.

Low plasma levels of vitamin D are independent inverse predictors of death and disease progression in CKD [8]. Activated 1,25-dihydroxyvitamin D3 acts as a down-regulator of the RAAS [9]. Confirmatory, in our mouse model, ACE inhibition plus Paricalcitol did not only prolong lifespan until renal failure but as well had a favourable effect on proteinuria as parameter of severity of renal disease (Figure 2). High intrarenal levels of angiotensin II induce proinflammatory and profibrotic mechanisms like cytokine production, podocyte damage and up-regulation of extracellular matrix production, for example in diabetic nephropathy [10–12]. Correspondingly, Ramipril plus Paricalcitol showed a superior nephroprotective effect regarding accumulation of extracellular matrix and fibrosis (laminin and fibronectin) versus the other on top therapy and the monotherapies with Paricalcitol and Calcitriol. Suppression of the RAAS leads to a down-regulation of proinflammatory proteins like TGFβ, TNFα [18] and others, which results in a lower expression of laminin and fibronectin and thus to a delay of renal scar-tissue formation in mice with AS. CA monotherapy lowered TGFβ levels to almost normal (Figure 6a), however, CA did not show a significant effect on extracellular matrix deposition or fibrosis (Figures 4 and 5). This discrepancy might be caused by using total kidney extracts for the Western blot and by different amounts of remaining TGFβ-producing cells in the fibrotic kidneys. As a limitation, renin- and calcium-levels and blood pressure were not determined in our study; all might well be influenced by CA and PC therapy and have previously been shown to affect kidney function [6].
In our study, levels of IL6 as marker for inflammatory activity showed a net reduction in mice with renal insufficiency (treated as well as untreated animals) and significantly higher levels in healthy controls. We hypothesized that therapy should reduce the inflammatory activity and inflammation should be the lowest in healthy controls. However, IL6 was measured in whole kidney extracts, not in isolated glomeruli or podocytes. Podocytes have previously been shown to be the key players in the whole kidney extracts, not in isolated glomeruli or podocytes. The relatively high levels of IL6 in healthy wild-type mice seem to represent a ‘normal’ background inflammatory activity, whereas—in treated mice—levels of IL6 tend to diminish according to the transformation of healthy kidney tissue into scar tissue. Therefore, higher levels of IL6 found in our experiments in whole kidney extracts might correlate to the ‘better morphology and conservation of renal cells’ and thus fit with the nephroprotective results of lifespan, proteinuria and scar-tissue formation.

Paricalcitol and Calcitriol show a different potential to inhibit renin mRNA expression [13], which might contribute to the systemic role of selective vitamin D receptor activation in chronic renal disease [14]. A recent epidemiological study in patients on haemodialysis showed a beneficial effect on survival in patients receiving Paricalcitol compared with those receiving Calcitriol [15].

Our study at first demonstrates synergistic nephroprotective effects of Paricalcitol on top of ACE inhibition in a non-diabetic, non-hypertensive mouse model of progressive renal fibrosis. In contrast, parallel treatment with Calcitriol did not lead to comparable nephroprotection. These findings could be explained by the synergistic inhibition of the RAAS by ACE inhibitors and Paricalcitol, but not by Calcitriol. As our knockout mice are neither diabetic nor hypertensive, it is not likely that better blood pressure control was the predominant nephroprotective effect of Paricalcitol. The synergistic effect seems to be due to the different pathways of ACE inhibitors and Paricalcitol concerning the inhibition of RAAS and proinflammatory pathways. Beyond the established indication for Paricalcitol, hyperparathyreoidism, our findings implicate a possible role of Paricalcitol therapy because of its antifibrotic nephroprotective effects in patients with progressive renal fibrosis such as AS.

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CONFLICT OF INTEREST STATEMENT

On behalf of my co-authors, I hereby declare that the results presented in this paper have not been published previously in whole or part, except in abstract format.

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The effect of paricalcitol on renal fibrosis