Interaction between inflammation, mineral metabolism and the renin–angiotensin system: implications for cardiorenal outcomes in chronic kidney disease

Martin H. de Borst
Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Correspondence and offprint requests to: Martin H. de Borst; E-mail: m.h.de.borst@umcg.nl; Twitter handle: @mhdeborst

Progressive renal function loss is accompanied by deregulation of several key endocrine systems in the body, including the renin–angiotensin system (RAS). RAS activation, characterized by high levels of the major effector molecules angiotensin II and aldosterone, contributes to further aggravation of kidney damage through, among others, transforming growth factor β (TGF-β). Moreover, it promotes cardiovascular disease through, among others, sodium retention and vasoconstriction, leading to hypertension. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) provide clear cardiorenal protection, strongly underlining the clinical relevance of the RAS in chronic kidney disease (CKD).

CKD–mineral and bone disorder (MBD) is characterized by the deregulation of several hormones and other circulating factors, including fibroblast growth factor 23 (FGF23), klotho, vitamin D and parathyroid hormone. Progressive hyperphosphataemia, hypercalcaemia and hyperparathyroidism, especially when combined, confer an increased cardiovascular risk [1] and are considered as treatment targets according to current guidelines [2]. Renal klotho loss and increased FGF23 levels are relatively early markers of CKD-MBD in which changes may be detectable with an estimated glomerular filtration rate of ~60 mL/min/1.73 m². A recent systematic review and meta-analysis identified 21 studies that upon meta-analysis showed that FGF23 is a strong predictor of all-cause and cardiovascular mortality both in haemodialysis patients and kidney transplant recipients [3]. Of note, a recent analysis demonstrated that individuals with CKD who displayed an increasing trend in circulating FGF23 levels over time were at high risk of premature mortality [4].

CKD is considered a state of chronic inflammation, which in itself is considered a non-traditional cardiovascular risk factor. In other populations, such as post-myocardial infarction patients, a novel monoclonal antibody–based therapy (canakinumab) targeting the pro-inflammatory cytokine interleukin-1 β (IL-1β) [5] successfully reduced the rate of cardiovascular events, compared with placebo. In contrast, in another large clinical trial in a similar population, low-dose methotrexate did not confer such risk reduction [6]. Yet inflammation seems to be a relevant target for cardiovascular protective therapy. The position of anti-inflammatory treatment, and particularly IL-1β, in the CKD patient population remains to be addressed, but given the high prevalence of chronic inflammation in CKD and the consistent associations with adverse outcomes, it seems plausible that CKD patients might benefit considerably from anti-inflammatory therapy.

INTERACTION BETWEEN KLOTHO, FGF23 AND THE RAS

The aforementioned deregulated pathways and systems have long been considered independent drivers of renal and cardiovascular damage in CKD. Over the years, however, novel insights on crosstalk between these pathways on multiple levels have challenged this view [7].

Recently Takenaka et al. [8] further unravelled the renoprotective role of klotho in an animal model of proteinuric nephropathy (adriamycin nephrosis). Rats treated with recombinant klotho protein developed much lower levels of proteinuria than vehicle-treated adriamycin rats (Figure 1A); this was accompanied by reductions in plasma and renal angiotensin II (Figure 1B) as well as plasma renin activity. Klotho treatment also strongly reduced renal TGF-β and fibronectin expression and restored E-cadherin expression (Figure 1C and D), suggesting beneficial effects were mediated at least in part by inhibition of epithelial-to-mesenchymal transition (EMT). The authors also found that klotho treatment reduced Wnt signalling, which could drive EMT in this model. This was subsequently studied in more detail by combined treatment with klotho and the 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione, an inhibitor of glycogen synthase kinase-3β (GSK3β), which plays a prominent role in Wnt-β–catenin signalling. Indeed, GSK3β inhibition reversed the klotho-induced
improvements in renal β-catenin and angiotensin II, as well as the expression of TGF-β and angiotensinogen, without affecting E-cadherin (Figure 1A–D). Thus treatment with recombinant klotho seems to provide renoprotection at least in part through correcting deregulations in the renal and circulating RAS. Moreover, the study highlights the promising role of klotho, or interventions aimed at increasing endogenous klotho expression, in renoprotective intervention in CKD. In contrast, RAS blockade is also known to upregulate klotho, as reviewed recently [9].

Upstream of klotho signalling, FGF23 has also been studied in relation to the RAS. FGF23 may induce RAS activation through suppression of 1,25-dihydroxyvitamin D, a negative regulator of renin [10]. Indeed, in patients with CKD, a higher FGF23 level has been associated with an impaired anti-proteinuric response to RAS blockade–based therapy [11]. This observation was corroborated in heart failure patients, where a higher FGF23 level was not only associated with adverse outcome, but also with less-effective RAS blockade–based therapy [12]. Subsequently, de Jong et al. [13] aimed to prospectively investigate the relationship between FGF23 and the therapy response to RAS blockade. In the rat unilateral ureteral obstruction (UUO) model, the authors found that treatment with the ARB losartan reduced renal inflammation and fibrosis. Co-treatment with recombinant FGF23 reversed some of the anti-inflammatory effects of ARB treatment, but not the anti-fibrotic effects. The combined treatment markedly reversed the changes in renal renin and ACE, ACE2 and AT1 receptor gene expression induced by ARB treatment in the contralateral (healthy) kidney. These data do support crosstalk between FGF23 and the RAS, although FGF23-induced resistance to anti-fibrotic effects of ARB treatment could not be clearly demonstrated. This could be explained by the extensive amount of kidney damage present in this model, accompanied by profound loss of klotho in the UUO kidney, whereas the effects on RAS gene expression were observed in the healthy contralateral kidney with preserved klotho expression. Interestingly, reciprocal effects have been reported very recently in cardiomyocytes obtained from patients with end-stage renal disease: treatment with angiotensin II and aldosterone induced FGF23 expression, potentially contributing to myocardial fibrosis [14].

**INFLAMMATION: DRIVER OF UPREGULATED FGF23?**

Although a growing number of studies point towards renal klotho loss and/or elevated levels of FGF23 as potential treatment targets in CKD, the pathophysiological processes driving
Emerging epidemiological data suggest that inflammation, along with deregulations in mineral metabolism, promotes progression of vascular calcification and an increased mortality risk [15, 16]. Furthermore, cohort studies suggested a link between inflammation and higher circulating FGF23 levels [17, 18]. These observations have recently been further substantiated by prospective preclinical studies. Bansal et al. studied the effect of inflammation elicited by lipopolysaccharide (LPS) on FGF23 in mice [19]. An acute-on-chronic increase in inflammation was accompanied by a marked increase in plasma C-terminal but not intact FGF23 levels in LPS mice. Interestingly, the authors found an upregulation of FGF23 gene and protein expression in the spleen but not in bone or the heart. The fact that asplenic LPS mice displayed lower C-terminal FGF23 levels points towards the spleen as a main source of upregulated plasma FGF23 levels in acute inflammation. The fact that plasma C-terminal, but not intact FGF23, was increased in LPS mice suggests that the increased FGF23 expression as shown in the spleen is paralleled by increased degradation of FGF23 into C-terminal fragments [20]. Which cells within the spleen were responsible for the increased FGF23 production and whether FGF23 was degraded into C-terminal fragments locally or elsewhere could not be demonstrated by Bansal et al. [19]. Yet this work provides an important contribution to the evidence linking inflammation with deregulated mineral metabolism.

In another very recent publication, the molecular pathways driving the connection between inflammation and CKD-MBD were further narrowed in a series of experiments highlighting a direct effect of IL-6 on the induction of FGF23 expression in bone [21]. Of note, anti-inflammatory treatment also seems to modulate the response to RAS blockade, even in experimental models of kidney disease not primarily driven by inflammation [22]. In contrast, deregulated mineral metabolism and metabolic bone disease may contribute to inflammation by affecting the bone marrow niche in CKD, although studies to support this hypothesis are still at an early stage [23].

Deregulated Klotho and FGF23 have not been fully elucidated. Although klotho may be considered an important novel target for therapy to improve deregulated mineral homoeostasis in CKD, the road for klotho treatment to reach the clinic is still long. Meanwhile, currently available interventions known to increase endogenous klotho production have been investigated. Agents activating the VDR, including calcitriol and synthetic analogues such as paricalcitol, are prominent examples of such therapies. Indeed, VDR activation has been shown to increase serum and urine klotho levels in a mouse model of CKD [24]. The VDR activator paricalcitol may provide additional renoprotection on top of RAS blockade, as shown by several small to medium-sized clinical trials [25]; whether this is partly due to upregulation of klotho has not been elucidated. On the other hand, the VIRTUE (Vitamin D Receptor activator and sodium restriction for Treatment of Urinary albumin Excretion) randomized controlled trial showed that the albuminuria-lowering effect of paricalcitol in non-diabetic CKD against the background of standardized RAS blockade and optimal antihypertensive treatment was minimal and less pronounced than the effect of sodium restriction [26]. More specific and powerful restoration or supplementation of klotho may be needed in order to provide stronger renoprotection in patients with ongoing renal damage despite optimal RAS blockade.

Beside its effects on klotho, VDR activation also modulates FGF23. In preclinical studies, FGF23 was shown to induce cardiac hypertrophy independent of klotho, through modulation of FGF receptor 4 (FGFR4)–nuclear factor of activated T cells (NFAT) signalling [27, 28]. In line, a higher circulating level of FGF23 has been associated with heart failure severity and worse outcomes in several studies in heart failure patients. Leifheit-Nestler et al. [29] subsequently studied the effect of VDR activation on cardiac FGF23 expression and activation of the downstream FGFR4–NFAT pathway, as well as left ventricular hypertrophy (LVH), in the 5/6 nephrectomy model [29]. Interestingly, the authors found that treatment with calcitriol
suppressed cardiac FGF23 expression and downstream FGFR4–NFAT signalling and also reduced LVH in 5/6 nephrectomy rats, in line with prior studies [30, 31]. These observations may have been surprising, given the fact that in other studies, VDR activation led to increased circulating FGF23 levels both in animals and humans [26, 32, 33]. Indeed, dose-dependent increased bone FGF23 expression was observed by Leifheit-Nestler et al. [29] in 5/6 nephrectomy rats upon calcitriol treatment. Thus the effects of VDR activator treatment on circulating FGF23 levels likely do not reflect the specific cardiac effects. The promising findings from this preclinical study remain in contrast with the disappointingly negative results from the PRIMO (Paricalcitol Capsule Benefits in Renal Failure–Induced Cardiac Morbidity) and OPERA (Oral Paricalcitol in Stage 3–5 Chronic Kidney Disease) trials, which could not demonstrate a clear beneficial effect of VDR activator treatment on left ventricular mass in CKD or haemodialysis patients, respectively [34, 35].

CONCLUSIONS AND FUTURE DIRECTIONS
Emerging data summarized above revealed that inflammation, deregulated FGF23 and klotho signalling and RAS activation are not independent, but rather are concertedly driving adverse cardio renal outcomes during CKD (Figure 2). Novel interventions targeting inflammation, such as IL-1β-neutralizing antibodies and interventions aimed at increasing klotho expression may hold promise to improve clinical outcomes in CKD patients. Several landmark articles recently published in NDT have been instrumental in advancing knowledge on these complex interactions and set the stage for future steps towards further innovative treatments in this field.

CONFLICT OF INTEREST STATEMENT
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REFERENCES
In this issue of Nephrology Dialysis and Transplantation, Greenwood et al. [1] examine the risk of death, cerebrovascular accident, myocardial infarction and hospitalization for heart failure among 757 patients referred to a renal rehabilitation program between 2005 and 2017. The 12-week program was modeled on conventional cardiopulmonary rehabilitation and aimed to deliver an individualized rehabilitation program incorporating combined exercise training and self-management education (Table 1) [2]. This study builds on their prior work, which showed that renal rehabilitation resulted in substantial improvement in exercise capacity and functional ability among adults at various stages of chronic kidney disease (CKD), including Stages 3–4, receiving maintenance hemodialysis and after kidney transplant [2]. The primary outcome of the earlier study was the Duke Activity Status Index (DASI), a questionnaire of functional ability across a spectrum from ability to complete activities of daily living to sporting activities [3]. Patients’ DASI scores increased by 35% on average after the program, and 60% of completers improved by at least 50 m (‘improvers’) and those who did not (‘nonimprovers’). A total of 43% completed the program, and 60% of completers improved by at least 50 m on the shuttle walking test. In multivariable analysis, patients who completed the program had a lower risk of the combined outcome [hazard ratio [HR 0.62 [95% confidence interval (CI) 0.39–1.00]], and completers who improved had a lower risk than those who did not improve [HR 0.60 (95% CI 0.36–0.98)].

Thus this program was administered by rehabilitation specialists according to traditional rehabilitation principles. The unique aspect was the concept of providing a ‘renal rehabilitation program’. Designing and implementing such a program involved recognition that functional limitations are common and severe among patients with CKD and can be addressed through exercise interventions, integration of assessment of functional