Klotho Deficiency and the Cardiomyopathy of Advanced CKD

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Advancing CKD is associated with a progressive and massive increase in morbidity and mortality, particularly from cardiovascular causes. In patients with type 2 diabetes, for example, the risk of dying from cardiovascular disease vastly exceeds the risk of reaching ESRD. In contrast with nonrenal patients, where most cardiac deaths result from coronary artery disease, patients with advanced CKD or on dialysis mostly die from sudden cardiac death.1 Cardiac magnetic resonance imaging identified evidence of myocardial fibrosis (subendocardial or diffuse) in almost 30% of 134 Scottish patients on dialysis, and in most of these cases, this macroscopically detectable fibrosis was associated with left ventricular hypertrophy (LVH).2 Uremic cardiomyopathy, more correctly termed cardiomyopathy of advanced CKD, is further aggravated by capillary rarefaction, local ischemia (particularly during phases of systemic hypotension), and vascular as well as intramyocardial calcifications. Importantly, there is evidence that this cardiomyopathy is potentially reversible and can improve, for example, after kidney transplantation.3 This indicates that the identification of uremia-associated factors that drive the cardiomyopathy associated with advanced CKD might potentially result in novel therapeutic approaches.

The few facts described above already implicate a large number of pathogenetic mechanisms likely in sudden cardiac death, LVH, and cardiomyopathy in advanced CKD. Nevertheless, there is hope to find some master switches that lend themselves to therapeutic intervention. One such factor, namely fibroblast growth factor 23 (FGF23), was recently described in rodents.4 Circulating FGF23 levels are massively increased in CKD and correlate with LVH. FGF23 caused hypertrophy of rat cardiomyocytes independent of Klotho, a coreceptor for FGF23. In mice, the administration of FGF23 resulted in LVH, and in a rat model of CKD, treatment with an FGF receptor (FGFR) blocker reduced LVH.4 A related pathogenetic system is described in this issue of JASN: phosphate and Klotho. In an elegant and very comprehensive series of experiments, Hu et al.5 have characterized cardiac hypertrophy and fibrosis associated with Klotho deficiency. First, Hu et al. describe that even heterozygous Klotho deficiency results in early LVH in mice and that this precedes subsequent marked myocardial fibrosis. Second, Hu et al. induced secondary deficiency of circulating Klotho in mice by hyperphosphatemia plus CKD and again, noted an association with LVH and myocardial fibrosis. In a number of experimental approaches, Hu et al. then identify the joint effects of low Klotho and high phosphate on the heart. Such effects were particularly pronounced in aging mice. By mathematical modeling of all experimental data, Hu et al. conclude that Klotho and phosphate are independent promoters of cardiac remodeling and that FGF23 is a third contributor but only if Klotho levels are decreased. Finally, in vitro exogenous Klotho dampened the profibrotic response of rat cardiac fibroblasts and reduced the activation of rat cardiomyocytes in response to various stimuli.

In their seminal paper, Kuro-o et al.6 described the biologic function of a newly discovered gene, termed Klotho. Disrupting Klotho gene expression in mice resulted in a syndrome that resembled human aging, including short lifespan, infertility, arteriosclerosis, and osteoporosis. Kuro-o et al.6 concluded that the Klotho gene product may function as part of a signaling pathway that suppresses aging and age-related diseases in vivo. Soon, it became clear that three Klotho family members exist (termed α-, β-, and γ-Klotho) that are part of a distinct endocrine complex consisting of different FGFs, FGFRs, and Klotho molecules regulating various metabolic pathways.7 Because FGFRs are expressed ubiquitously in most cells, tissue-specific expression of Klotho determines the target organ specificity of endocrine FGFs. In the kidney and parathyroid glands, the membrane-bound α-Klotho forms complexes with several FGFRs, serving as the high-affinity receptor for the phosphatonin FGF23. It is secreted by osteoblasts, partly in response to hyperphosphatemia and/or oral phosphate loading. The FGF23–FGFR–Klotho complex stimulates phosphaturia by reducing the expression and activity of sodium–phosphate cotransporters in renal proximal tubular cells. In addition, it decreases the activity of the renal α-1-hydroxylase and increases the activity of the 24-hydroxylase, resulting in a decline of active vitamin D (calcitriol) blood levels, which in turn, reduces gastrointestinal.
phosphate adsorption. In concert, these actions lead to a decrease in overall body phosphate load.

Other than the membrane-bound αKlotho acting as a coreceptor for FGF23, a shorter-length protein is generated through alternative splicing of the Klotho gene and released from cells. In addition, αKlotho is subjected to proteolytic cleaving, and therefore, the entire extracellular domain is released into blood and urine. Currently, it is not clear how far circulating αKlotho levels reflect (renal) tissue αKlotho expression. It is also unknown whether this extracellular αKlotho protein can act as a coreceptor for FGF23 or whether it functions as a soluble factor independent of FGF23. Nonetheless, given that αKlotho expression is largely confined to the kidney and parathyroid glands, most extrarenal effects would be expected to be mediated by soluble αKlotho. On the basis of experimental data in various animal models, the hypothesis has been put forward that CKD is a state of general deficiency, because significant downregulation of αKlotho mRNA and protein in renal tissue was documented together with low levels of soluble αKlotho in blood and/or urine. Vice versa, maintenance of higher αKlotho levels by genetic manipulation in a CKD animal model protected kidney function and reduced soft tissue calcification.

Clinical data supporting the above concept of CKD being a state of Klotho deficiency are scarce. In early diabetic kidney damage, renal expression of the Klotho gene decreased markedly. Recently published clinical studies using a commercially available ELISA for the measurement of soluble αKlotho in blood mostly confirmed low αKlotho levels in CKD but yielded conflicting results with respect to the predictive power for cardiovascular and renal events. Sakan et al. evaluated αKlotho expression in kidney biopsy samples and also measured levels of soluble αKlotho in serum and urine from 239 patients with CKD. Sakan et al. found reduced αKlotho and elevated FGF23 serum levels in early CKD stages. Renal αKlotho expression was an independent determinant of soluble αKlotho. In children with CKD stages 1–5D and after kidney transplantation, only a weak association between soluble αKlotho and eGFR was detected, whereas the correlation was stronger in adults with CKD. In the latter study, the decrease in soluble αKlotho preceded the increase in FGF23 levels, but the correlation between eGFR and FGF23 was much stronger than that of eGFR and αKlotho in both studies. In a post hoc analysis of a prospective study, serum αKlotho levels were associated with more severe CKD stage, and baseline αKlotho independently predicted the renal outcome after adjustment for several variables, including eGFR and FGF23. In contrast, in a larger study comprising 444 well characterized patients with CKD stages 2–4, we failed to observe a strong relationship between eGFR and soluble αKlotho. Of note, although we confirmed a weak association between age and soluble αKlotho, we did not find any correlation of soluble αKlotho with parameters of calcium–phosphate metabolism, progression, or cardiovascular end points. Instead, FGF23 levels turned out to be the best parameter of calcium–phosphate metabolism for predicting adverse outcomes.

Presuming that the above ELISA test indeed reliably measures soluble αKlotho, these variable clinical data obviously must be reassessed in large epidemiologic studies. This point is particularly pertinent if one considers the use of recombinant soluble αKlotho protein for the prevention of progression and/or cardiovascular morbidity related to CKD to avoid overtreatment and potentially, deleterious circulating αKlotho levels. Until then, we are left with the usual: give an angiotensin-converting enzyme inhibitor and thereby, increase renal Klotho expression.

DISCLOSURES

REFERENCES
Endotoxin and AKI: Macrophages Protect after Preconditioning

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Sepsis remains a major cause of morbidity and mortality in hospitalized patients, with AKI serving as an ominous prognostic factor. According to the current literature, the pathogenesis of AKI-induced sepsis, albeit being poorly understood, is thought to be multifactorial. Consequently, therapeutic interventions to curtail deterioration in kidney function in the setting of sepsis have been mostly supportive and, at best, modestly successful. In this issue of JASN, Hato et al. present data that demonstrate a renoprotective effect of macrophages in a model of endotoxin preconditioning and sepsis-induced AKI. The concept of disease tolerance as a possible host defense strategy against infection was recently highlighted in animal immunity. New data suggest that tissue protection by being “tolerant” to an insult may play an important role in sepsis.

Endotoxin preconditioning, or “tolerance,” is achieved by pretreatment with a low dose of endotoxin and has been shown in various models to alleviate the adverse effects of a large dose of endotoxin, as would be present in Gram-negative sepsis. Endotoxin preconditioning not only protects the host from damage caused by secondary exposure to endotoxin, but also mediates protection from other insults (cross-tolerance) such as ischemia-reperfusion injury and other Toll-like receptor (TLR) agonists. Endotoxin preconditioning has shown promise in improving outcomes of sepsis in various organs, including the kidney. A more profound understanding of the underlying mechanisms of protection by endotoxin preconditioning is key to developing therapeutic interventions in sepsis.

In previous work, the authors demonstrated that S1 proximal tubular epithelial cells acquire systemically administered endotoxin via a TLR4-dependent mechanism, subsequently initiating oxidative stress in S2 and S3 segments downstream. These findings established that endotoxin damage to renal tubular epithelial cells is a local renal event. Furthermore, the application of 2-photon (2P) intravital microscopy allowed the authors not only to interrogate the temporal and spatial location of endotoxin uptake in the kidney but also to assess the outcome of endotoxin uptake. Oxidative stress, directly visualized in vivo by fluorescent markers, was shown to occur in the S2 and S3 tubules.

In this issue of JASN, Hato et al. continue this work by investigating whether endotoxin preconditioning protects the kidney in vivo against future sepsis-induced AKI and elucidate the mechanism of endotoxin preconditioning in the mouse kidney. They first show in bone marrow chimeric mice that are reconstituted with TLR4 bone marrow that endotoxin-mediated renal injury is independent of hematopoietic cells and their produced cytokines. Furthermore, they demonstrate that exposure to low-dose endotoxin is protective not only against high-dose endotoxin but also in cecal ligation and puncture and live Escherichia coli injection models of sepsis. This was evident by decreased kidney injury markers (kidney injury molecule-1, neutrophil gelatinase-associated lipocalin), preserved kidney function, and decreased oxidative stress detected by intravital imaging of renal proximal tubules. Notably, protection occurred despite the increased uptake of endotoxin by S1 tubular cells in preconditioned mice. On the basis of the evidence established by the authors, it was thus likely that endotoxin preconditioning was also a local renal event independent of hematopoietic cells. However, experiments using bone marrow chimeric mice revealed that TLR4-expressing hematopoietic cells are required for the renoprotective effects of endotoxin preconditioning. Likewise, bone marrow chimeric mice demonstrated that the LPS coreceptor CD14 on hematopoietic