Fibroblast Growth Factor 23 and α Klotho in Acute Kidney Injury: Current Status in Diagnostic and Therapeutic Applications

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Keywords

Acute kidney injury · α Klotho · Biomarker · Chronic kidney disease · FGF23 · Treatment

Abstract

Fibroblast growth factor (FGF) 23 and α Klotho are circulating mineral regulatory substances that also have a very diverse range of actions. Acute kidney injury (AKI) is a state of high FGF23 and low α Klotho. Clinical association data for FGF23 are strong, but the basic pathobiology of FGF23 in AKI is rather sparse. Conversely, preclinical data supporting a pathogenic role of α Klotho in AKI are strong, but the human data are still being generated. This pair of substances can potentially serve as diagnostic and prognostic biomarkers. FGF23 blockade and α Klotho restoration can have prophylactic and therapeutic utility in AKI. The literature to date is briefly reviewed in this article.

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duced osteomalacia, which ushered in a major hormone countering phosphate accumulation [1]. Beyond phosphate balance, FGF23 has been shown or postulated to regulate calcium and sodium homeostasis, iron metabolism, erythropoiesis, and inflammation [2].

α Klotho was cloned when serendipitous disruption of its locus led to premature multiorgan failure [3]. The list of cellular actions conferred by α Klotho encompasses anti-oxidation, anti-apoptosis, pro-autophagy, pro-stem cell, and anti-fibrosis and essentially broad-based maintenance of cell health [1].

Chronic kidney disease (CKD) is a state of FGF23 excess and α Klotho deficiency, but their levels and roles in acute kidney injury (AKI) are less well defined. Many of the functions of FGF23 and α Klotho are highly relevant to the pathobiology of AKI. AKI is a systemic syndrome triggered by a sudden loss of kidney function and brings forth significant morbidity, mortality, and burden on

Introduction

Fibroblast growth factor (FGF) 23 was cloned as a candidate gene of autosomal dominant hypophosphatemic rickets and a differentially expressed gene in tumor-in-

Javier A. Neyra and Ming Chang Hu contributed equally.

Contribution from the AKI and CRRT 2020 Symposium at the 25th International Conference on Advances in Critical Care Nephrology, Manchester Grand Hyatt, San Diego, CA, USA, February 24–27, 2020. This symposium was supported in part by the NIDDK funded University of Alabama at Birmingham–University of California San Diego O'Brien Center for Acute Kidney Injury Research (P30DK079337).

multiple organ systems [4, 5]. Even when one evades the acute high mortality, survivors are at risk for CKD, end-stage kidney disease, and cardiovascular disease [6–9].

For a common serious syndrome with dire short- and long-term consequences, progress in diagnosis, prognosis, and therapy has been modest. We need means to diagnose AKI early, foresee the time course of recovery, and predict the risk of progression to CKD. For therapeutics, we need effective prophylaxis of AKI in high-risk situations, accelerate recovery in established AKI, prevent extrarenal complications, and forestall AKI-to-CKD transition. We will explore if FGF23 and α Klotho can fulfill these roles.

FGF23 and AKI

Multiple factors increase FGF23 production, including high blood pressure, phosphate loading, iron deficiency, hypoxia, and inflammation [10]. FGF23 actions are transduced by the FGF receptor 1 (FGFR1) [11, 12] and its coreceptor transmembrane α Klotho [13]. FGF23 can also exert action through binding to FGFR4 in the heart and liver, independently of α Klotho [14, 15]. Metabolism of FGF23 includes cleavage of bioactive intact FGF23 (~30 kDa) into C-terminal (~12 kDa) and N-terminal (~18 kDa) fragments. FGF23 seems to be also cleared by the kidneys, possibly through filtration and/or catabolism [16]. Circulating FGF23 includes the intact hormone (iFGF23) and the N- and C-termini. In states such as iron deficiency and inflammation, increased production of FGF23 is matched by FGF23 cleavage, with high levels of FGF23 fragments in circulation [17]. Two assays used to measure circulating FGF23 levels are “C-terminal” (cFGF23) which detects both C-terminal fragments and the full-length peptide and “intact” (iFGF23) which measures only intact FGF23.

Elevated FGF23 levels have been observed in multiple studies of human AKI [10]. Plasma cFGF23 levels were 5.6-fold higher in patients with AKI versus age-matched patients without AKI [18]. Note that increased FGF23 levels detected with the cFGF23 assay (intact + C-term) may reflect increased FGF23 production and/or impaired clearance but do not inform about FGF23 bioactivity. Few studies have used both cFGF23 and iFGF23 assays in conjunction. In adult patients undergoing cardiac surgery, plasma cFGF23 levels were markedly increased (~100-fold) postoperatively in patients who did versus did not develop AKI, while iFGF23 levels were modestly (~2-fold) higher postoperatively in AKI versus non-AKI patients [19]. Similar findings were seen in a folic acid ne-

phropathy mouse model [20]. Therefore, both production and clearance of FGF23 may be affected in AKI.

In 250 adult patients undergoing cardiac surgery, plasma cFGF23 levels differentially increased at the end of cardiopulmonary bypass in patients with versus without postoperative AKI, predating changes in other mineral metabolites [19]. Performance evaluation of utility of cFGF23 for early AKI diagnosis was modest (AUC = 0.78) but superior to urinary kidney injury biomarkers [19]. Similar observations were derived from cohorts of critically ill patients in which cFGF23 was measured in plasma and urine within 24–48 h of ICU admission [21, 22].

FGF23 levels may also have prognostic utility in AKI. In a large post hoc analysis ($N = 1,527$ patients), increased risk of 60-day mortality was noted in patients with the highest versus lowest quartiles of cFGF23 (~3.8-fold) and iFGF23 (~2.0-fold) [23].

The canonical mineral metabolism regulators of FGF23 production in bones seem not to play a major role in AKI [20, 24]. Furthermore, contribution of FGF23 production from other organs in acute injury/inflammation settings is not fully understood. FGF23 may augment myofibroblast activation and fibrosis via TGF- β -related pathways, opening an angle for therapeutic FGF23 blockade [25, 26]. However, off-target effects of FGF23 such as impairment of immune [27, 28], endothelial [29], and cardiac functions [15] are contenders and require further investigation. Therefore, it is still unclear if anti-FGF23 antibody (burosumab), C-terminal FGF23 (experimentally shown to interfere with FGF23 signaling at supra-physiological concentrations) [30], or specific FGFR4 inhibition have clinical applications in attenuation of incident AKI or its consequences.

α Klotho in AKI

Diagnosis

α Klotho deficiency is universal in AKI animal models including ischemia-reperfusion injury (IRI) [31], unilateral ureteric obstruction [32] (UUO), sepsis induced by lipopolysaccharide (LPS) injection or cecal ligation and puncture (CLP) [33, 34], and nephrotoxins including cisplatin [35] (CP) and folic acid [36] (FA), indicating that α Klotho downregulation in the kidney is a general phenomenon after acute kidney insults (Table 1). In rats, α Klotho mRNA started to fall 6 h post-IRI-AKI and returned to baseline around 3–4 days [37]. α Klotho protein in the kidney fell at 3 h while neutrophil gelatinase-associated lipocalin (NGAL) did not increase until 5 h, so the

Table 1. α Klotho levels in animal models of AKI [32–37, 40, 46, 47]

Animal model	Blood α Klotho	Urine α Klotho	Kidney α Klotho assay	Kidney function	Kidney histology	Comments	Citation
Hypovolemic phlebotomy (4 mL), Wistar rat	ND	ND	No change in mRNA with Northern blot from 30 to 120 min	ND	ND	Prerenal insult did not change α Klotho mRNA within 120 min after phlebotomy (up to 15% blood volume/body weight) No information about Bp, renal function, and renal α Klotho protein	[34]
LPS (10–20 mg/kg), mice	ND	ND	↓ protein with IB and ↓ mRNA with qPCR at 3 h	↑ Scr and ↑ NGAL at 24 h		Dose-dependent decrease of α Klotho in the kidney	[34, 46]
Cecal ligation and puncture, mice	ND	ND	↓ protein with IB	↑ BUN ↓ urinary volume	↑ kidney tubular damage scores	CLP caused lower survival, impaired renal function, impaired hepatic function, greater oxidative stress, inflammatory pathways (at the systemic and kidney tissue levels) in <i>kl/+</i> mice than <i>WT</i> mice	[33]
IRI (60 min), Wistar rat	ND	ND	↓ mRNA with qPCR ↓ protein with IB	↑ Scr	ATN	1. α Klotho mRNA ↓ at 6 h after IRI 2. Scr ↑ at 6 h after IRI 3. α Klotho protein started to rise at 3 days and back to normal at 10 days	[37]
IRI (30 min), mice	↓ with IB-IP	↓ with IB	↓ protein with IB	↑ Scr	ATN	1. <i>kl/+</i> mice with lower α Klotho had severer kidney damage and <i>Tg-kl</i> mice with higher α Klotho had milder kidney damage 2. α Klotho decline started 3 h after IRI and early than elevation of NGAL	[40]
IRI (20–35 min), mice	ND	ND	↓ mRNA with qPCR ↓ protein with IB	↓ ClCr ↑ ACR	Renal fibrosis at 20 weeks	1. Longer ischemia reduced more renal α Klotho 2. ↓ kidney α Klotho is associated with ↑ kidney fibrosis 3. ↑ dietary Pi causes ↓ kidney α Klotho	[40]
Cisplatin (10 mg/kg) once, mice	ND	ND	↓ mRNA with qPCR ↓ protein with IB starting 1 day and stayed at low levels for 1 week	↑ Scr and ↑ ACR	1. Acute phase: ATN 2. Chronic phase: kidney fibrosis	1. Old mice had more kidney fibrosis and higher Scr with less kidney α Klotho 2. <i>kl/+</i> mice had worse kidney function and severer AKI and CKD 3. <i>Tg-kl</i> mice had better kidney function and less AKI and less CKD	[35, 36, 47]
Folic acid, IP, (250 mg/kg) once, mice	ND	ND	↓ mRNA with qPCR ↓ protein with IB at 1 day after folic acid	↑ Scr	↑ AKI scores ↑ macrophages in the kidney	1. ↓ α Klotho mRNA and protein expression with more AKI pathological scores and macrophages in the kidney at day 1 2. TWEAK and TNF α reduce kidney α Klotho	[36]
UUO, mice	ND	ND	↓ protein with IB	ND	↑ renal fibrosis at up to 5 days	1. More fibrosis in <i>kl/+</i> than <i>WT</i> mice 2. Lower kidney α Klotho is associated with more kidney fibrosis	[32]

ACR, albumin/creatinine ratio; AKI, acute kidney injury; ATN, acute tubular necrosis; Bp, blood pressure; BUN, blood urea nitrogen; CKD, chronic kidney disease; ClCr, creatinine clearance; eGFR, estimated glomerular filtration rate; IB, immunoblot; IRI, ischemia-reperfusion injury; *kl/+*, heterozygous α Klotho deficient mouse; LPS, lipopolysaccharide; NGAL, neutrophil gelatinase-associated lipocalin; ND, not done; qPCR, quantitative PCR; Pi, phosphate; Scr, serum creatinine; *Tg-kl*, transgenic α Klotho overexpressing mouse.

Table 2. αKlotho protein and gene therapy in animal models of AKI [35, 37, 40–45, 48, 49]

Animal model	αKlotho manipulation	Blood αKlotho and assay	Urine αKlotho and assay	Kidney αKlotho and assay	Comment	Citation
LPS injection (10 mg/kg), mice	αKlotho (0.01–0.02 mg/kg, R&D) IP 60 min before LPS injection, once	ND	ND	ND	αKlotho reduced kidney damage in the dose-dependent manner	[48]
IRI (60 min), Wistar rat	Adenovirus encoding the mouse αKlotho gene (ad-kl) (1.6×10^{10}) 3 days before IRI	ND	ND	ND	1. Ad-Kl ↑ αKlotho in the liver, but not in the kidney prior to IRI 2. Ad-Kl ↓ kidney damage and apoptosis	[37]
IRI (30 min), mice	Recombinant αKlotho (lab made) (0.01 mg/kg BW) was IP given after IRI, once	Less ↓ with IB-IP	Less ↓ with IB	Less ↓ with IB	1. αKlotho given immediately after IRI led to better kidney recovery 2. αKlotho given at 60 min after IRI did not have better recovery	[40]
IRI (30 min), mice	αKlotho protein (lab made) (0.01 mg/kg) for 4 consecutive days starting 24 h after Bi-IRI	Less ↓ with IB			1. αKlotho ↑ renal αKlotho at 20 weeks after IRI 2. αKlotho did not change C/Cr at 3 and 7 days, but ↑ it at 8 and 20 weeks 3. αKlotho ↓ kidney fibrosis	[40]
Uni-IRI (30 min) + contralateral Npx for only 24 h, mice	Minicircle carrying 20-μg αKlotho gene was given using the hydrodynamic tail-vein injection 2 days prior to AKI induction	Less ↓ with ELISA (Cusabio)	ND	More with IHC	1. Minicircle carrying 20-μg αKlotho gene ↑ plasma αKlotho compared with αKlotho plasmid 2. Minicircle carrying 20-μg αKlotho gene ↓ (1) necrosis, (2) apoptosis	[41]
CP (10 mg/kg) + 2.0% Pi diet starting 2 weeks after CP injection for 18 weeks, mice	At 4 weeks after cisplatin injection, recombinant αKlotho protein (0.3 mg/kg body weight/month) was given for 16 weeks via osmotic minipumps	ND	ND	Less ↓ with IB and qPCR	αKlotho mitigated cisplatin-induced AKI-to-CKD progression, improved mineral homeostasis, and ameliorated cardiac remodeling and vascular calcification.	[35]
CP (15 mg/kg) IP once, mice	AAV-dgαKlotho gene was IV injected to Cas9 mice 8 days prior to cisplatin injection	Less ↓ with ELISA (Cusabio)	ND	ND	A CRISPR/Cas9 system sufficiently delivered αKlotho ↑ serum αKlotho, prevented kidney from cisplatin nephrotoxicity, and ↑ survival rate	[42]
UUO up to 7 days, mice	Soluble αKlotho (0.01–0.02 mg/kg BW) was IP given immediately after UUO q.o.d. up to 7 days				αKlotho treatment did not affect the severity of hydronephrosis, but ↓ kidney fibrosis probably through binding to TGFβR2	[49]
UUO up to 14 days, mice	αKlotho protein (R&D systems) (0.02 mg/kg BW) was given immediately after UUO q.o.d up to 14 days	ND	ND	ND	αKlotho ↓ kidney fibrosis, and ↓ TGF-β1/Smad signaling activity	[44]
UUO up to 10 days, mice	Upon completion of UUO surgery, retrograde ureteral infusion of αKlotho-sgRNA with the Cas9 system was performed from the proximal site to deliver enzymes for the demethylation of αKlotho gene	ND	ND	Less ↓ mRNA with qPCR	1. Hydroxymethylation of αKlotho gene ↑ renal αKlotho mRNA 2. Hydroxymethylation of αKlotho gene ↓ (1) kidney fibrosis, and (2) fibroblast and collagen I in the kidney of UUO mice	[43]

Table 2 (continued)

Animal model	α Klotho manipulation	Blood α Klotho and assay	Urine α Klotho and assay	Kidney α Klotho and assay	Comment	Citation
UUO up to 10 days, mice	Minicircle carrying 20- μ g α Klotho gene was given using the hydrodynamic tail-vein injection 2 days prior to UUO	Less $\downarrow$ with ELISA (Cusabio)	ND	ND	1. Minicircle carrying 20- μ g α Klotho gene $\uparrow$ plasma α Klotho 2. Minicircle carrying 20- μ g α Klotho gene $\downarrow$ (1) renal fibrosis, (2) apoptosis, and (3) fibrotic markers in the kidney	[41]
Rhabdomyolysis, IM 50% glycerol (8 mg/kg), mice	On day 1 after injury, mice received a tail vein injection of 2.0×10^8 EVs containing α Klotho protein in 100 μ L or 1 pg/mouse	ND	ND	$\uparrow$ protein with IB	1. EV containing α Klotho $\downarrow$ kidney damage and $\uparrow$ kidney recovery 2. $\sim$ 200-fold lower α Klotho than direct IP injection of recombinant α Klotho is required for AKI animal	[45]

AAV, adeno-associated virus; Ad, adenovirus; AKI, acute kidney injury; CKD, chronic kidney disease; C/Cr, creatinine clearance; CP, cisplatin; dg α Klotho, dead single-guide α Klotho; EMT, epithelial mesenchymal transition; FGF23, fibroblast growth factor 23; EV, extracellular vesicle; IB, immunoblot; IHC, immunohistochemistry; IP, intraperitoneal; IRI, ischemia-reperfusion injury; IP-IB, immunoprecipitation followed by immunoblot; LPS, lipopolysaccharide; ND, not done; Pi, phosphate; qPCR, quantitative PCR; Scr, serum creatinine; sg α Klotho, single-guide α Klotho; UUO, unilateral ureteral obstruction.

kidney α Klotho protein is an early marker of AKI [38] (Table 1). Urine and plasma α Klotho paralleled changes in kidney α Klotho [38], decreasing dramatically at 3 h, started to recover at $\sim$ 48 h, and reached normal levels by 7 days. Soluble α Klotho may be a surrogate for kidney α Klotho.

The first human AKI study showed significantly lower urine α Klotho at the time of AKI diagnosis when compared with healthy controls [38]. A larger prospective ICU study showed that AKI patients had lower urinary α Klotho within 48 h of AKI diagnosis when compared with ICU controls [39]. Urine α Klotho concentration and urine α Klotho-to-Cr ratio inversely correlated with hospital and mechanical ventilation days. Each 1-fold higher urine α Klotho/Cr was associated with an 83% lower risk of major adverse kidney events at 90 days [39].

Prognosis

Heterozygous α Klotho-deficient (*kl/+*) mice subjected to IRI, CP, sepsis, and UUO had lower α Klothoprotein and more kidney damage than wild-type (WT) mice [34, 35, 37, 38] (Table 1). Longer ischemia time caused more severe α Klotho deficiency and kidney fibrosis. After recovery of kidney function, lower α Klotho associated with more chronic fibrosis and kidney dysfunction [40], suggesting α Klotho level can predict risk of AKI-to-CKD transition (Table 1).

Prophylaxis

Mice with genetically high α Klotho before AKI had milder kidney damage and a better renal outcome in IRI [38] and CP-induced AKI [31]. Adenoviral α Klotho gene delivery increased circulating but not kidney α Klotho prior to IRI, ameliorated injury, and kidney dysfunction [37]. α Klotho-bearing minicircle vector increased plasma α Klotho, prevented kidney damage induced by IRI, and attenuated kidney fibrosis in an UUO model [41]. α Klotho overexpression through CRISPR-Cas9 prevented kidney damage and alleviated kidney fibrosis in CP nephrotoxicity [42] and in UUO [43] (Table 2).

Post-AKI Treatment

Clinical utility mandates efficacy if given after AKI. Recombinant α Klotho protein given to mice immediately after IRI reduced kidney damage [38], but the benefit diminishes dramatically starting 1 h after the insult. α Klotho administration immediately after UUO followed up to 14 days reduced renal fibrosis, but not hydronephrosis severity [44]. α Klotho-carrying extracellular vesicles [45] promoted kidney recovery in rhabdomyolysis-induced

Fig. 1. Protracted natural history of AKI and translational opportunities. The various stages of AKI are shown on top corresponding to different levels of kidney function. Dx¹, identification of susceptible patients with impending kidney injury; Dx², early diagnosis of AKI prior to onset of detectable clinical parameters; Px¹, prognostic predictor of recovery-phase onset and speed of recovery; Px², predictor of probability, rapidity, and severity of AKI-to-CKD progression; PPx¹, prevention of onset of AKI in susceptible patients; PPx², prevent, delay, or slow down AKI-to-CKD progression. Application in combination with Px²; Rx¹, promote or accelerate recovery from AKI; Rx², independent of its effect on kidney function, treatment can be directed to the amelioration of extrarenal complications with cardiovascular complications being a major target. AKI, acute kidney injury; CKD, chronic kidney disease. Dx, diagnosis; Px, prognosis; PPx, prophylaxis; Rx, treatment.

Table 3. Potential modalities for FGF23-αKlotho therapy in AKI

Decrease FGF23 bioactivity		Increase αKlotho bioactivity	
mechanism	description	mechanism	description
Decrease synthesis	Phosphate restriction	Increase endogenous protein	Phosphate restriction ACE inhibition PPARγ activator Recombinant αKlotho itself
Increase degradation	GALNT3 inhibitor	Integrated transgene	Successful in animals
Neutralization	Monoclonal antibody	Gene delivery	Successful in animals
Blockade of receptor binding	Experimental development	Recombinant exogenous protein	Successful in animals
Blockade of receptor signaling	Experimental development		

FGF23, fibroblast growth factor 23; AKI, acute kidney injury.

AKI. At 24 h after IRI, when kidney injury was fully established, serum creatinine had peaked, and endogenous αKlotho was at its lowest levels, αKlotho protein injection for 4 consecutive days preserved endogenous kidney αKlotho levels, accelerated recovery, suppressed kidney fibrosis, and protected against AKI-to-CKD transition [40] (Table 2).

Late Treatment

In 2 CKD models, αKlotho protein was given 4 weeks after CKD induction and sustained for 12 weeks [46], or

up to 16 weeks [35]. Plasma αKlotho protein levels, kidney function and fibrosis, and cardiac remodeling/cardiomyopathy were improved possibly via both direct effects and secondary effects due to renoprotection (Table 2).

Summary

The database of FGF23 is currently composed largely of human studies, which are strong but only associative in nature. In contrast, the αKlotho database is populated by com-

elling animal experiments but the clinical data are still in early stages of development. Regarding this pair of proteins with mineral-regulating properties and a plethora of other actions, one can envision many potential applications in human AKI (Fig. 1; Table 3). The potential applications are immense, and further research should be directed at the pathobiology of FGF23 in AKI in preclinical studies to dissect whether it is pathogenic or a biomarker (or both), that is, interventional experiments. Prospective longitudinal clinical studies using simultaneous iFGF23 and cFGF23 assays will enrich our database. Standardization of soluble α Klotho assays will enrich the human database and make it generalizable. The extensive preclinical data in α Klotho are in dire need of human translation in both diagnostics and therapeutics but one faces various hurdles in this effort.

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Acknowledgements

The authors are supported by the National Institutes of Health (R01-DK091392, R01-DK092461, and R01-DK092461-S1 to OWM and MCH), the George O'Brien Kidney Research Center (P30-DK-07938 to OWM), the Charles and Jane Pak Center Innovative Research Support (to OWM and MCH), and Endowed Professor Collaborative Research Support (to OWM and MCH). JAN is a recipient of an Early Career Pilot Grant from the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR001998. The authors are grateful to the excellent secretarial assistance provided by Ms. Yesenia Aguirre.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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