Interleukin-6 contributes to the increase in fibroblast growth factor 23 expression in acute and chronic kidney disease

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The high serum fibroblast growth factor 23 (FGF23) levels in patients with acute kidney injury (AKI) and chronic kidney disease (CKD) are associated with increased morbidity and mortality. Mice with folic acid–induced AKI had an increase in bone FGF23 mRNA expression together with an increase in serum FGF23 and several circulating cytokines including interleukin-6 (IL-6). Dexamethasone partially prevented the increase in IL-6 and FGF23 in the AKI mice. *IL-6* knock-out mice fed an adenine diet to induce CKD failed to increase bone FGF23 mRNA and had a muted increase in serum FGF23 levels, compared with the increases in wild-type mice with CKD. Therefore, IL-6 contributes to the increase in FGF23 observed in CKD. Hydrodynamic tail injection of IL-6/soluble IL-6 receptor (sIL-6R) fusion protein hyper IL-6 (HIL-6) plasmid increased serum FGF23 levels. Circulating sIL-6R levels were increased in both CKD and AKI mice, suggesting that IL-6 increases FGF23 through sIL-6R–mediated *trans*-signaling. Renal IL-6 mRNA expression was increased in mice with either AKI or CKD, suggesting the kidney is the source for the increased serum IL-6 levels in the uremic state. HIL-6 also increased FGF23 mRNA in calvaria organ cultures and osteoblast-like UMR106 cells in culture, demonstrating a direct effect of IL-6 on FGF23 expression. HIL-6 increased FGF23 promoter activity through STAT3 phosphorylation and its evolutionarily conserved element in the FGF23 promoter. Thus, IL-6 increases FGF23 transcription and contributes to the high levels of serum FGF23 in both acute and chronic kidney disease.

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Fibroblast growth factor 23 (FGF23) is an endocrine member of the FGF family, expressed by osteocyte and osteoblast cells in bone. FGF23 levels are increased markedly in chronic kidney disease (CKD), correlating with increased mortality.[1,2] The primary physiological action of FGF23 involves regulation of bone and mineral metabolism through a bone–kidney–parathyroid axis. FGF23 inhibits proximal tubular phosphate re-absorption and $1,25(OH)_2$ vitamin D (1,25D) biosynthesis.[3,4] In addition, FGF23 acts on the parathyroid to decrease parathyroid hormone (PTH) gene expression and parathyroid cell proliferation.[5–7] In CKD, however, the parathyroid resists the action of FGF23 owing to a down-regulation of the FGF23-receptor complex, klotho–FGF23-receptor 1.[8,9] FGF23 is a 30-kDa protein that may be cleaved into an 18-kDa N-terminal fragment and a 12-kDa C-terminal tail.[10,11] Many factors are associated with the high FGF23 levels in CKD. These include phosphate retention, high serum PTH levels, acidosis, vitamin D treatment, calcium, and FGF-receptor activation by low-molecular-weight FGFs.[12–18] Inflammation and iron deficiency also stimulate FGF23 production.[19,20] Acute inflammation induced by single injections of heat-killed *Brucella abortus* or interleukin (IL)-1β decreased serum iron and increased FGF23 mRNA levels and serum levels of carboxy-terminal FGF23 (cFGF23), with no changes in intact FGF23 (iFGF23).[19] IL-1β injection increased FGF23 mRNA and C-terminal FGF23 levels similarly in wild-type and Col4a3ko mice, a model for CKD, but markedly increased iFGF23 levels only in the CKD mice.

Acute kidney injury (AKI) is associated with high mortality and accelerated progression of CKD. AKI initiates processes of necrosis and apoptosis in renal tubular epithelial cells, as well as injury that releases immunologic signals activating the innate immune system. AKI resulting from both ischemic acute renal failure and bilateral nephrectomy is associated with an increase in multiple serum cytokines, including IL-6, IL-1β, tumor necrosis factor–like weak inducer of apoptosis (TWEAK) 9 and 10 and members of the tumor necrosis factor superfamily cytokines.[21] Bilateral nephrectomy also results in a rapid increase in serum iFGF23 levels, suggesting that the kidney is important in FGF23 homeostasis by regulation of its plasma levels and metabolism.[22] Experimental folic acid–induced nephropathy is a model of renal injury that shares with human AKI the development of extensive cell death, interstitial

inflammatory infiltrates, and tubular cell proliferation that restores tubular cell mass. Christov *et al.*[23] showed an early increase in circulating FGF23 levels in a folic acid–induced AKI model in mice, 1 hour after AKI induction. The increase in FGF23 levels was independent of PTH, dietary and serum phosphate, 1,25D and vitamin D receptor activation. In experimental uremia, FGF23 binds to hepatic FGF23-receptor 4, resulting in increased expression of inflammatory cytokines such as C-reactive protein (CRP), IL-6, and tumor necrosis factor-α.[24] In patients with CKD, increased FGF23 levels are associated independently with higher levels of inflammatory markers including IL-6, CRP, and tumor necrosis factor-α, and predict poor clinical outcome as in the Chronic Renal Insufficiency Cohort study.[25–28] In addition, higher levels of IL-6, CRP, and FGF23 all were associated independently with an increased risk of death. Thus, increased levels of IL-6, CRP, and FGF23 are independent risk factors for mortality in CKD.[29] There is also a close correlation between IL-6 expression and AKI. In an ischemic AKI animal model, IL-6 transcription and signaling were increased locally and systemically after bilateral kidney ischemia. Similarly, in nephrotoxin-induced AKI, IL-6 expression was enhanced dramatically in the kidney, predominantly in renal tubular epithelial cells, and strongly correlated with renal injury. Intriguingly, stimulation of IL-6 trans-signaling significantly mitigated renal damage and preserved renal function via an underlying anti-oxidative stress mechanism.[30] A similar observation was reported in an ischemia-reperfusion–induced AKI model, which proposed that IL-6 trans-signaling may play a protective role by protecting the kidney from further injury.[31,32] IL-6 is a pleiotropic cytokine, a member of the glycoprotein 130 (gp130) family that uses gp130 as a common signal transducer.[33] IL-6 binds to the IL-6 receptor (IL-6R) and the complex of IL-6–IL-6R associates with gp130, which dimerizes and initiates intracellular signaling. Upon binding to gp130 and after ligand-induced dimerization of the IL-6R, the associated Janus kinase (JAK) kinases, JAK1 and JAK2, cross-phosphorylate tyrosine residues of adjacent gp130 subunits of the complex, leading to activation of the signal transducer and activators of transcription (STAT), mitogen-activated protein kinase, and the phosphatidylinositol-3'-kinase/protein kinase B pathways.[34] IL-6 activation of STAT3 by phosphorylation at tyrosine 705 leads to STAT3-induced transcription regulation of downstream genes.[35,36] A soluble form of IL-6R (sIL-6R), comprising the extracellular portion of the receptor, binds to IL-6 with a similar affinity as the membrane-bound IL-6R and interacts directly with gp130 in cells regardless of IL-6R expression by trans-signaling.[37,38] Osteoblasts produce IL-6 and are its main source in bone. IL-6 stimulates osteoclast generation and activity indirectly through its effect on osteoblasts.[39] Mice with total-body IL-6 gene deletion (*IL-6*$^{-/-}$) have a remarkable resistance to mercuric chloride (HgCl2) toxicity and by that to AKI.[30] The roles of cytokines, in particular IL-6, in the increased FGF23 of uremia have not been shown.

In this study, we show that IL-6 is an important regulator of FGF23 in both acute and chronic kidney failure. In addition, IL-6 increases FGF23 expression *in vivo* and *in vitro* in calvaria organ cultures as well as in cells in culture.

RESULTS

Inflammatory cytokines are increased in folic acid–induced AKI

We induced AKI in mice by a single injection of folic acid. Folic acid led to the expected increase in serum blood urea nitrogen (BUN) and phosphate within 3 hours, which increased progressively for the 26 hours of the experiment (Table 1). Serum calcium levels increased at 3 and 6 hours after folic acid and then decreased (Table 1). Serum PTH increased after 3 hours, which may have contributed to the early increase in serum calcium levels at 3 and 6 hours after folic acid. The decrease in serum calcium levels at 26 hours may have been owing to the high serum phosphate levels complexing with calcium. The folic acid–induced AKI led to an increase in calvaria FGF23 mRNA and both serum iFGF23 and cFGF23 (Figure 1a–c). To study a possible role for cytokines in this process we measured serum levels of selected cytokines after folic acid administration. At 3 hours, folic acid led to significant increases in IL-5, IL-10, interferon-γ, tumor necrosis factor-α, CXC chemokine ligand 1/keratinocyte chemoattractant, and IL-6 (Figure 1d). There was no change in any of the measured cytokines at 1 hour (not shown). IL-6 levels also remained high at 6 hours (Figure 2e).There was no change in serum IL-1α, IL-1β, IL-2, IL-4, IL-17, and granulocyte-macrophage colony-stimulating factor levels at 3 hours (not shown).

Dexamethasone prevents the increase in serum FGF23 in AKI

To determine the role of inflammation in the increased FGF23 levels in AKI, we injected the anti-inflammatory agent dexamethasone 1 hour before a single injection of folic acid (Figure 2a). At 6 hours after folic acid administration, serum

Table 1 | Serum biochemistry and PTH levels in folic acid–induced AKI

	3 h		6 h		26 h	
Parameter	Control	Folic acid	Control	Folic acid	Control	Folic acid
BUN (mg/dl)	17 ± 1	33 ± 1.3[a]	14 ± 0.8	59 ± 2.5[a]	13 ± 1	144 ± 6[a]
Phosphate (mg/dl)	9.8 ± 0.3	18 ± 1.3[a]	10 ± 0.5	19 ± 0.8[a]	8 ± 0.6	32 ± 5.5[a]
Calcium (mg/dl)	8 ± 0.2	9 ± 0.1[a]	9 ± 0.2	11 ± 0.3[a]	8.7 ± 0.3	6 ± 0.5[a]
PTH (pg/ml)	338 ± 46	2106 ± 430[a]	323 ± 60	2014 ± 309[a]	231 ± 20	1931 ± 281[a]
n	6	6	6	7	6	7

AKI, acute kidney injury; BUN, blood urea nitrogen; PTH, parathyroid hormone.
Mice received a single i.p. injection of folic acid or vehicle. Serum was analyzed at 3, 6, and 26 hours. Results are presented as means ± SE.
[a]$P < 0.05$, compared with control.