Fibroblast growth factor 1 ameliorates diabetic nephropathy by an anti-inflammatory mechanism

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Inflammation plays a central role in the etiology of diabetic nephropathy, a global health issue. We observed a significant reduction in the renal expression of fibroblast growth factor 1, a known mitogen and insulin sensitizer, in patients with diabetic nephropathy and in mouse models implying that fibroblast growth factor 1 possesses beneficial anti-inflammatory and renoprotective activities *in vivo*. To test this possibility, we investigated the effects of chronic i.p. administration of fibroblast growth factor 1 into both the streptozotocin-induced type 1 diabetes and *db/db* type 2 diabetes models. Indeed, recombinant fibroblast growth factor 1 significantly suppressed renal inflammation (i.e., cytokines, macrophage infiltration), glomerular and tubular damage, and renal dysfunction in both type 1 and type 2 diabetes mice. Fibroblast growth factor 1 was able to correct the elevated blood glucose levels in type 2 but not in type 1 diabetic mice, suggesting that the anti-inflammatory effect of fibroblast growth factor 1 was independent of its glucose-lowering activity. The mechanistic study demonstrated that fibroblast growth factor 1–mediated inhibition of the renal inflammation *in vivo* was accompanied by attenuation of the nuclear factor κB and c-Jun N-terminal kinase signaling pathways, further validated *in vitro* using cultured glomerular mesangial cells and podocytes. Thus, fibroblast growth factor 1 holds great promise for developing new treatments for diabetic nephropathy through countering inflammatory signaling cascades in injured renal tissue.

Kidney International (2017) ■, ■–■; http://dx.doi.org/10.1016/j.kint.2017.05.013

KEYWORDS: cytokines; diabetic nephropathy; fibrosis; inflammation; mesangial cells

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Received 7 December 2016; revised 30 April 2017; accepted 4 May 2017

Diabetes mellitus has become a worldwide public health problem. Uncontrolled hyperglycemia in patients with diabetes can lead to a host of diabetic complications including diabetic nephropathy (DN), retinopathy, and neuropathy. DN is a serious and common complication of type 1 diabetes (T1D) and type 2 diabetes (T2D) and leads to end-stage renal disease in as many as 30% of individuals with diabetes.[1,2] DN is characterized by glomerular hypertrophy, thickened basement membrane, podocytopenia, increased extracellular matrix protein synthesis/deposition, and fibrosis.[1,3,4] The etiology of DN is multifactorial, with hyperglycemia, oxidative stress, advanced glycation end products, and angiotensin II as leading factors, each of which can activate nuclear factor-κB (NF-κB), the master transcription factor controlling the expression of a host of proinflammatory genes.[4–8] NF-κB regulates expression of a host of adhesion molecules, proinflammatory cytokines, and chemokines that are associated with chronic inflammation, fibrosis, and tissue remodeling in DN.[8,9] In addition, evidence indicates that stress-activated protein kinases (i.e., c-Jun NH_2-terminal kinase [JNK] and p38) of the mitogen-activated protein kinase family may contribute significantly to DN progression.[7,9,10] In particular, JNK activation is correlated with macrophage interstitial accumulation, fibrosis, and loss of renal function in human subjects with DN and in a rat model of kidney obstruction.[11–13] JNK activation is induced by various factors of the diabetic milieu, including hyperglycemia, advanced glycation end-products, angiotensin II, oxidative stress, and proinflammatory cytokines.[14–16] Hence, upregulation of proinflammatory signaling pathways is considered to be a key contributor to the progression of DN.[7,9]

As an autocrine/paracrine regulator, fibroblast growth factor 1 (FGF1) is known to be βmitogenic on cells from a variety of tissue origins including the liver, vasculature, and skin.[17–19] Recombinant human FGF1 has been clinically used to facilitate wound/burn repair and ulcer regeneration for decades. Due to its classic mitogenic activity, FGF1 has also therapeutic potential for cardiovascular disorders in coronary artery bypass graft surgery, ischemia, and nerve repair.[20–22] Recently, Jonker *et al.*[23] discovered an unexpected metabolic role for FGF1 as a critical transducer of peroxisome proliferator–activated receptor-γ signaling that mediates the

proper coupling of nutrient storage to adaptive remodeling of adipose tissue. In a follow-up study, we showed that administration of exogenous FGF1 stimulates glucose uptake in an insulin-dependent fashion and suppresses the hepatic production of glucose to achieve whole-body insulin sensitization in a mouse model of T2D.[24] Moreover, FGF1 administration suppresses plasma levels of adrenocorticotropic hormone and corticosterone, reducing lipolysis and hepatic glucose production in a rat model of T1D.[25] These findings indicate that FGF1, in addition to its mitogenic activity, is capable of impinging on multiple pathways mediating homeostatic control of normal glycemia.

Several reports have previously implicated FGF1 in kidney pathology.[26–28] Importantly, with relevance to our study, FGF1 was shown to be downregulated in renal tissue from diabetic subjects.[27] Suh *et al.*[24] observed that long-term treatment of *ob/ob* mice with FGF1 decreased serum levels of several inflammatory cytokines, including eotaxin, keratinocyte chemoattractant, macrophage inflammatory protein-1β, and interleukin (IL)-3. These findings suggest that FGF1 may have the potential to reduce the severity of inflammatory injury of DN; however, it remains unclear whether the anti-inflammatory effects of FGF1 are dependent on its glucose-lowering capability.

In this study, we investigated the hypothesis that FGF1 can attenuate the development of DN by treating mouse models of T2D (*db/db*) and T1D with recombinant human FGF1. Our findings indicated that FGF1 significantly reduced renal inflammation, morphologic damage, renal dysfunction, as well as blood glucose levels in T2D mice. Unexpectedly, FGF1 also significantly prevented the renal inflammation and dysfunction, but did not correct hyperglycemia in T1D mice. To our knowledge, this finding is the first direct evidence of the protective effects of FGF1 on DN.

RESULTS

Endogenous levels of FGF1 in diabetic human subjects and mice

Reduced levels of endogenous FGF1[27] and FGF2[29] have been previously correlated with disease progression in human T2D and T1D patients. Consistent with the previous literature, we also found that the serum FGF1 concentration (ng/ml) in T2D and T1D human subjects were, respectively, ∼45% and ∼20% lower than that in the control nondiabetic group (Figure 1a and c). We further tested the serum and kidney tissue levels of FGF1 in T2D and T1D mice. The results showed that the serum FGF1 concentration (ng/ml) in T2D and T1D mice was significantly reduced compared with the nondiabetic control, which is consistent with the observation in the human subjects (Figure 1b and d). Immunofluorescent imaging of renal tissue from T2D and T1D mice also showed reductions in FGF1 expression in both glomeruli and tubules relative to wild-type mice (Figure 1e and f), which was further confirmed by the Western blot analysis (Figure 1g–j). To find out whether the reductions in FGF1 expression is secondary to the failing kidney function, we next measured the serum FGF1 levels of experimental mice at an early stage of diabetes. The data showed that the serum FGF1 levels in both *db/db* (7 weeks of age) and streptozotocin (STZ)-induced T1D mice (4 weeks after STZ induction) were significantly lower than those in the control nondiabetic group (Supplementary Figure S1A and B). We next carried out immunofluorescent double-staining using FGF1 antibody and cell-type specific markers to identify FGF1-expressing renal cell types in normal C57L/B6 mice. As shown in Supplementary Figure S2A and B, FGF1 expression was detected in 2 major renal cells including smooth muscle actin–positive mesangial cells and Wilms tumor 1–positive podocytes in normal mice. Compared with the normal C57L/B6 mice, in STZ-induced diabetic mice, FGF1 expression was reduced in both mesangial cells and podocytes. However, FGF1 expression was markedly low in the tubular cells of both normal and STZ-induced diabetic mice (Supplementary Figure S2C). In addition, FGF1 was not observed in the infiltrating macrophages in both normal and STZ-induced diabetic mice (Supplementary Figure S2D). The *in vivo* observations were thus validated *in vitro* using mesangial cells (SV40) and conditionally immortalized mouse podocytes that were exposed to high glucose (HG). As shown in Supplementary Figure S3, HG incubation for 3 hours significantly inhibited FGF1 expression in these 2 cell lines, consistent with our *in vivo* results (Supplementary Figure S2A and B).

FGF1 prevents DN in *db/db* T2D mice

Potential protective effects of FGF1 on T2D-related kidney injury were evaluated using a treatment protocol (Figure 2a) in which FGF1 was administered by i.p. injection (0.5 mg/kg body weight) to *db/db* T2D mice for 8 weeks. The *db/db* T2D mice had significantly increased blood glucose compared with the control *db/m* mice (Figure 2b). Consistent with the previous findings,[24] FGF1 significantly reduced blood glucose levels in *db/db* mice (Figure 2b). We tested the level of FGF1 in kidneys of diabetic mice with or without FGF1 treatment. As expected, FGF1 treatment enhanced the level of FGF1 in kidneys of *db/db* mice, and the renal FGF1 level in FGF1-treated diabetic mice was similar to that in control mice (Supplementary Figure S4A and B). Because albuminuria reflects renal dysfunction at the early stage of DN,[30] we measured urinary excretion of albumin levels after treatment for 8 weeks. As shown in Figure 2c, urinary albumin excretion was markedly increased in *db/db* mice compared with nondiabetic *db/m* mice, whereas FGF1 treatment significantly decreased the urinary albumin excretion. In addition, FGF1 treatment resulted in minimal changes in body weight (Figure 2d), but significantly reversed the increased mass of kidney in *db/db* mice (Figure 2e). Serum creatinine and blood urea nitrogen are another 2 hallmarks of renal injury.[31] The mean serum creatinine and blood urea nitrogen level of the *db/db* group was higher than that of the *db/m* group; however, this diabetes-induced increase in serum creatinine and blood urea nitrogen was significantly attenuated in the FGF1-treated *db/db* group (Figure 2f and g).