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Intra-tubular hydrodynamic forces influence tubulo-interstitial fibrosis in the kidney

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Abstract

Purpose of review—Renal epithelial cells respond to mechanical stimuli with immediate transduction events (e.g., activation of ion channels), intermediate biological responses (e.g., changes in gene expression), and long term cellular adaptation (e.g., protein expression). Progressive renal disease is characterized by disturbed glomerular hydrodynamics that contributes to glomerulosclerosis, but, how intra-tubular biomechanical forces contribute to tubulo-interstitial inflammation and fibrosis is poorly understood.

Recent findings—*In vivo* and *in vitro* models of obstructive uropathy demonstrate that tubular stretch induces robust expression of transforming growth factor β -1 (TGF β -1), activation of tubular apoptosis, and induction of NF- κ B signaling which contribute to the inflammatory and fibrotic milieu. Non-obstructive structural kidney diseases associated with nephron loss follow a course characterized by compensatory increases of single nephron glomerular filtration rate and tubular flow rate. Resulting increases in tubular fluid shear stress (FSS) reduce tissue-plasminogen activator and urokinase enzymatic activity which diminishes breakdown of extracellular matrix. In models of high dietary Na intake, which increase tubular flow, urinary TGF β -1 concentrations and renal mitogen activated protein kinase activity are increased.

Summary—In conclusion, intra-tubular biomechanical forces, stretch and FSS, generate changes in intracellular signaling and gene expression that contribute to the pathobiology of obstructive, and non-obstructive kidney disease.

Keywords

Hydrodynamic forces; tubule; inflammation; fibrosis; signaling

Introduction

Temporally and spatially dynamic biomechanical forces contribute to adaptive and maladaptive cellular responses in the kidney. For example, increases in hydraulic intra-glomerular pressure and single nephron glomerular filtration rate (GFR) associated with nephron loss is an adaptive response to maintain GFR; however, chronically this response leads to glomerulosclerosis[1]. Intra-tubular biomechanical forces can also generate maladaptive epithelial responses that produce inflammation and fibrosis. Tubular stretch in obstructive

uropathy[2] and epithelial fluid shear stress (FSS) in subtotal nephrectomy[3] and dietary Na loading[4] models illustrate how intra-tubular hydrodynamic forces effect the pathobiology of these respective renal disease models.

Intra-tubular hydrodynamic factors

Based on extrapolation from a wealth of studies performed in endothelial cells (ECs), and more limited data from renal epithelial cells, we propose that renal tubules experience three major mechanical forces (Fig. 1); (A) circumferential stretch, (B) apical wall fluid shear, and (C) hydrodynamic bending moments along the cilium. In turn, these biomechanical forces generate intracellular signals that, via unique signaling pathways, activate target-specific genes and proteins.

Obstructive uropathy is the classic example in which there is an increase in intra-tubular pressure and tubular stretch[2]. In the unilateral ureteral obstruction (UUO) rodent model, transforming growth factor β -1 (TGF β -1), a potent modulator of renal fibrosis, is upregulated and induces epithelial mesenchymal transition (EMT) and tubulo-interstitial fibrosis through downstream activation of tubular SMAD3[5]. To emulate these *in vivo* conditions *in vitro*, renal epithelial cells were mechanically stretched for 24 and 48 hrs to induce EMT; however, this effect was prevented by incubating the cells with a neutralizing anti-TGF β -1 antibody or stretching renal epithelial cells deficient in SMAD3, suggesting that mechanical stretch induces tubular TGF β -1 secretion and downstream SMAD3 mediated EMT[5]. Other studies have shown that stretching of tubules activates mitogen activated protein kinases (MAPKs) which lead to phosphorylation of phospholipase A2 and generation of arachidonic acid metabolites [6], and caspase activation and apoptosis[7], respectively. On the other hand, human proximal tubular cells, grown in a plastic flask and pressurized using a sphygmomanometer to 60 mmHg [8,9], rapidly increase expression of inducible nitric oxide synthase through a mechanism dependent on epidermal growth factor receptor and NF- κ B signaling in the absence of mechanical stretch[10]. In sum, two important consequences of increased intra-tubular pressure consist of (1) epithelial compression and (2) epithelial stretch. Each of these consequences of elevated intra-tubular pressure generates unique intracellular signals which contribute to the pathologic sequella of obstructive uropathy.

Fluid shear stress (FSS) generates a force parallel to the apical membrane and induces hydrodynamic bending moments along the antennae-like central cilia that decorate the apical surface of virtually all renal epithelial cells. In the nephrology literature, the cilium is believed to be the primary renal epithelial mechanosensor. Praetorius et al. demonstrated that bending the cilium of MDCK cells, either with a micropipette or by FSS, induces an increase in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$)[11]. This flow-induced $[\text{Ca}^{2+}]_i$ response was abolished if the cilium was removed by chloral hydrate treatment of the monolayer [11,12]. Human and murine models of polycystic kidney disease (PKD), associated with mutations of cilia-associated proteins, are linked with either absent or aberrant FSS-induced $[\text{Ca}^{2+}]_i$ responses, supporting the role of the central cilium as a mechanosensor [13–17]. It should be noted that many of the cilia-associated proteins mutated in PKD are expressed in other mechanosensitive protein complexes. For example polycystin-1 (PC1), a protein mutated in 85% of autosomal dominant PKD patients, is normally expressed in cilia and focal adhesion complexes of renal epithelia[18]. Targeted deletion of PC1 abrogates the shear-induced $[\text{Ca}^{2+}]_i$ response in renal epithelia, and this is presumably due to the lack of PC1 in the cilium; however, we propose that the absence of PC1 in mechanosensitive focal adhesion complexes may also contribute to this phenotype.

In ECs which archetypically do not express cilia, though this assumption has come under some scrutiny[19,20], FSS activates non-ciliary mechanosensors, including the glycocalyx[21],

integrins[22,23] and G-protein coupled receptors[24], that initiate the intracellular signaling process and alter gene-specific mRNA and protein expression. Acute exposure to FSS rapidly activates MAPKs (eg. p42/p44, c-jun N-terminal kinases[JNK])[25,26], focal adhesion kinases [23], and protein kinase C[27] in ECs which, in turn, alter the mRNA and protein expression of inflammatory genes such as monocyte chemoattractant protein-1 (MCP-1)[28] and fibrosis-associated genes like TGF β -1[29]. In Figure 2 we illustrate the some of the mechanosensitive signaling pathways expressed in ECs and renal epithelial cells as a composite diagram within a cilia expressing renal epithelial cell.

As implied by our figure, ECs and tubular renal epithelial cells can respond to physiologic levels of FSS (which differ between the vasculature and tubule) or flow with similar increases in shear sensitive systems. For example, FSS induces physiologic increases in nitric oxide (NO) production from ECs which, in turn, leads to paracrine activation of cGMP-induced vasodilation. *In vitro* exposure of cultured collecting duct (CD) cells to FSS and *ex vivo* microperfusion of microdissected thick ascending limb of the loop of Henle (THAL) both demonstrate flow-inducible renal epithelial NO production [30–32]. In THAL and CD, NO inhibits NaCl transport and also reduces water permeability in the CD, thereby, serving as an autocrine/paracrine modulator of Na and water transport [33–35]. In this case, FSS and flow regulate NO production in ECs and renal epithelial cells that regulates tissue specific functions of the respective cell type.

Though much of the nephrology literature has focused on the cilium as the sole renal epithelial mechanosensor, other mechanosensors exist as mentioned above. In proximal tubule (PT) microvilli are hypothesized to be the primary mechanosensor regulating “glomerular-tubular” Na balance[36,37]. Du et al., utilizing PT microperfusion and mathematical modeling, showed that changes in PT microvillar torque led to congruent changes in transepithelial Na transport while non-toxic disruption of the actin cytoskeleton inhibited the flow-induced Na response [36]. In a murine PT cell culture model in which cilia are absent, FSS induced robust changes in PT cytoskeletal structure again suggesting a critical role for mechanosensitive microvilli [38]. CD intercalated cells, devoid of cilia (though this also is controversial[39]), respond to increases in tubular low rate with an increase in $[Ca^{2+}]_i$, identical to that observed in adjacent principal cells which express cilia[40,41]. In studies of murine kidney epithelial cells (MEK), a renal epithelial cell line derived from CDs of embryonic mouse kidney, basolateral integrins, the actin cytoskeleton, and the intracellular microtubular network each contributed to the cell’s ability to raise $[Ca^{2+}]_i$ in response to an increase in FSS[42]. In sum, the central cilium is an important mechanosensory organelle of renal epithelia; however, other mechanosensitive organelles and protein complexes, including microvilli and focal adhesion complexes, respectively, contribute to FSS-induced signaling.

Models of high tubular flow and tubulo-interstitial inflammation-fibrosis

High Na diets are associated with high urinary flow rates, high tubular flow rates, and, presumably, high tubular FSS. C57BL/6 mice fed a “normal” NaCl diet (0.25%) form a hypertonic urine (2035±254 mosm) and a 24 hr urine volume of 1.35±0.15 mL. A high NaCl diet (10%) reduced the urine osmolality to 969±12 mosm, and increased the 24 hr urine volume by ~6 fold to 8.94±0.83 mL [43]. Though the effects of chronically high dietary Na ingestion are believed to be mediated by increases in urine protein and the development of hypertension [44], we speculate that high tubular flow rates, independent of hypertension and proteinuria, contribute to tubulo-interstitial inflammation and/or fibrosis through activation FSS-regulated signaling processes.

To investigate this question we need to study a diuretic model in which blood pressure and urine protein excretion are unaffected. To this end, Ying et al. demonstrated that dietary Na

loading of less than 4 days did not affect blood pressure or proteinuria in rats[4]. He discovered that after four days of a high Na diet (8%), urinary TGF β -1 concentration increased compared to that measured in animals fed a control (0.3%) Na diet[45]. In addition, steady-state abundance of phospho-p38, phospho-p42/44, and phospho-JNK in the cortex and medulla was greater in rats fed a high Na diet than those fed the control Na diet and that inhibition of these MAPK pathways reduced expression of TGF β -1 in glomeruli of high Na fed rats[4]. Prior to sacrifice, treatment of the high Na fed rodents with tetraethylammonium, a K channel inhibitor which also abrogates the activity of the flow-stimulated maxi-K channel, reduced MAPK activity, suggesting a role for the maxi-K channel in MAPK activation and TGF β -1 production [4]. We speculate these early events, before the establishment of albuminuria and hypertension lay the foundation for progressive renal disease.

The subtotal nephrectomy models of CKD are also associated with increases in both PT and distal nephron (DN) flow rates. In rat PT flow rates, as measured by micropuncture, increase by 2–3 fold after subtotal nephrectomy (15 nL/min in normal rat kidney to 30–45 nL/min after subtotal nephrectomy)[46,47]. DN flow similarly increases by ~3 fold after subtotal nephrectomy[46,47]. Under normal conditions the DN flow rate is between 3–5 nL/min, but may increase to 9–18 nL/min after nephrectomy[46,47]. In a subtotal nephrectomy model, Ota et al. showed that tubulo-interstitial fibrosis at 2 weeks and tubulo-interstitial macrophage infiltration at 4 weeks after-subtotal nephrectomy was greater than that observed in sham treated control animals[48]. Immunohistochemical evidence of tubulo-interstitial MCP-1 protein expression was also first seen at 4 weeks post-nephrectomy while absent in sham-treated animals[48]. Proteinuria did not develop until after 8 weeks, suggesting these early inflammatory and fibrotic changes are independent of proteinuria[48]. We speculate that the hydrodynamic changes, which occur within days after nephrectomy, contribute to these early inflammatory/fibrotic renal events.

Few, if any, human studies directly address the role of intra-tubular hydrodynamic forces on the progression of CKD. However, certain human studies suggest that tubular flow rate, and hence, FSS contribute to progressive CKD. In a retrospective observational study to determine the effect of high Na diet on the progression of CKD, high NaCl (>200 mEq/day) and low NaCl (<100 mEq/day) dietary groups with CKD were identified and observed over 3 years. At baseline creatinine clearance was lower and proteinuria higher in the low dietary NaCl group, but blood pressure was similar to high dietary NaCl group[49]. Though the low NaCl group was at greater risk for progressive CKD (lower creatinine clearance and higher proteinuria), the GFR decline in this group was slower than that observed in the high NaCl group, suggesting that Na, independent of its effects on blood pressure and proteinuria, contributes to the progressive loss in GFR[49].

In a retrospective study of human kidney biopsies, De Borst et al. demonstrated phospho-c-jun, the transcription factor activated/phosphorylated by JNK, is highly expressed in the tubulo-interstitium of inflammatory and non-inflammatory glomerular disease compared to normal human kidneys[50]. Of interest is that tubulo-interstitial phospho-c-jun expression did not correlate with proteinuria, but instead correlated with decreasing GFR, suggesting the possibility that increasing single tubule flow rates associated with falling GFR may activate MAPKs. Moreover, Hebert et al. showed that graded increases in urinary flow rate, as measured by 24 hr urine volume collections, are associated with greater declines in renal function in patients with CKD compared to individuals who excrete less urine, suggesting that altered tubular flow rate may contribute to declines in renal function[51].

Identifying the role of FSS in progressive kidney disease

Essig et al. first reported that mouse PT cells subjected to FSS of 0.17 dynes/cm² exhibited reduced tissue-plasminogen activator (t-PA) and urokinase mRNA by Northern blot analysis and reduced cellular fibrinolytic activity compared to static cells[3]. There appeared to be a threshold effect, such that flow rates less than 1 mL/min (FSS<0.17 dynes/cm²) did not effect the expression of t-PA compared to static controls. However, at 1 mL/min (FSS~0.17 dynes/cm²) there was significant repression of t-PA mRNA expression[3]. These findings have been recently reproduced in cultured NRK-52E rodent PT cells in which the rodent PT cells were exposed to FSS of 5 to 10 dynes/cm² for 1 to 6 hrs, and t-PA and urokinase mRNA measured using semi-quantitative PCR[*52]. These investigators also found a magnitude and time-dependent reduction in t-PA and urokinase mRNA expression in sheared PT cells[*52]. It is hypothesized that reduced fibrinolytic activity of t-PA and urokinase produces an imbalance in the remodeling of the extracellular matrix and favors the deposition of extracellular matrix in the tubulo-interstitium[53].

To recapitulate these findings *in vivo*, these investigators chose to utilize the subtotal nephrectomy model of kidney disease which leads to ~3 fold increase in single nephron GFR, and, subsequent increases in PT FSS[3]. In C57BL/6 mice (a strain relatively resistant to proteinuria and hypertension after subtotal nephrectomy[54]), these investigators demonstrated that urokinase specific fibrinolytic activity was reduced by 35% in nephrectomized mice compared to sham-operated control mice. mRNA expression of urokinase was similarly reduced in the nephrectomy model[3]. In addition the PTs of nephrectomized mice showed actin reinforcement along the brush border and terminal web, which was identical to that observed in PT cells exposed *in vitro* to FSS[3]. In sum, these data suggest an important role of FSS, independent of proteinuria and hypertension, on the development of tubulo-interstitial fibrosis.

Conclusion

Our understanding of how intra-tubular biomechanical forces contribute to progression of kidney disease is still in its infancy. The evidence that intra-tubular pressure and stretch contribute to the inflammation and fibrosis associated with obstructive kidney disease is relatively strong[2]. However, much less is known about the effects of tubular FSS on progressive kidney disease. In this manuscript, we propose that high tubular flow rates and subsequent changes in FSS contribute to inflammation and fibrosis of CKD, as observed in animal models. The contribution of tubular FSS to inflammation and fibrosis is a new area of investigation with compelling direct and circumstantial evidence to posit its importance in CKD; however, further studies will be required to confirm its participation in the pathogenesis of CKD.

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Figure 1. Intratubular hydrodynamic forces in the kidney

Dynamic changes in tubular flow rate (e.g., urine flow rate) exert hydrodynamic forces on the tubular epithelium, specifically, (A) circumferential stretch and (B) fluid shear stress. (C) Apical fluid shear generates hydrodynamic bending moments along the length of cilia in renal epithelia.

Figure 2. Composite diagram of EC and renal epithelial cell signaling pathways illustrated in a tubular epithelial cell

(A) *MAPKs*. In ECs FSS activates G-protein coupled receptors (GPCR) [24] that induce Ras dependent activation/phosphorylation of p42/p44 and JNK through their respective MAPK signaling pathways, which in turn, phosphorylates c-fos and c-jun to generate activated protein-1 (AP-1), an important transcription factor that induces immediate early genes, including MCP-1 [26,28,55]. (B) $[\text{Ca}^{2+}]_i$. Bending of the apical cilium by flow permits mechanical gating of the polycystin-1/polycystin-2 (PC1/PC2) complex which initiates extracellular Ca^{2+} entry, the initiating Ca^{2+} signal [13]. In turn, phospholipase C (PLC) generates inositol triphosphate (IP3) which binds the IP3 receptor (IP3R) and releases Ca^{2+}

from its intracellular stores[11]. Alternatively, the initial rise in $[Ca^{2+}]_i$, due to mechanical activation of polycystin, mediates ryanodine receptor-induced Ca^{2+} release from endoplasmic reticulum (ER) Ca^{2+} stores[13]. (C) *NF- κ B*. In PT cells FSS initiates the reorganization of the actin-cytoskeleton which releases NF- κ B from its IKK regulatory protein and permits translocation of NF- κ B to the nucleus and binding to shear stress responsive elements in DNA [3].