Published in final edited form as:

Am J Transplant. 2013 June ; 13(6): 1617–1618. doi:10.1111/ajt.12242.

Flow Is Critical for Maintaining a Protective Phenotype in Renal Proximal Tubular Cells

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To the Editor

The cessation of blood flow is an imminent component of injury in organ transplantation and ischemia/reperfusion injury. Clinical trials have recently demonstrated benefits of hypothermic pulsatile perfusion during organ preservation supporting the concept that hemodynamic forces play a critical role in initiating organ injury (1). Nevertheless, mechanisms communicating detrimental consequences of stasis in organ preservation remain largely unknown. We have recently proposed that the expression of protective endothelial genes under conditions of pulsatile flow may serve as an explanation (2). The mechano-responsive transcription factor Kruppel-like factor 2 (KLF2) has been shown to play a critical role in integrating an anti-inflammatory, anti-thrombotic endothelial cell phenotype (3). We have previously documented that flow stasis triggers a rapid decay of KLF2 expression leading to acute endothelial dysfunction in conditions of cold storage (4). The absence of flow may thus provide an initial impetus triggering inflammation and injury in organ transplantation.

We have now collected in vitro data showing that shear stress is also able to protect tubular epithelial cells, extending our working concept that flow cessation is a critical component of organ injury. In these new experiments, we have exposed human tubular epithelial cells (HK-2) to laminar shear stress. Since tubulitis and tubular necrosis are hallmark lesions in acute kidney injury, and considering that renal proximal tubule cells have been shown to be responsive to biomechanical stimulation (5), we explored phenotypic modifications of tubular epithelial cells relevant to inflammatory processes and tested the secretion of kidney injury molecule-1 (KIM-1) as a surrogate marker for tubular injury.

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Other authors have no conflict of interests to disclose.

HK-2 cells were exposed to 24 h of physiological laminar shear stress; despite several studies focusing on the transmission of axial flow to modulate fluid reabsorption and alterations of tubule diameter in vitro, specific flow characteristics in the tubule have not been established. We therefore used a flow rate of 1 dyn/cm² in accordance with previously published data (5) recapitulating physiological conditions. Exposure of HK-2 cells to shear-stress resulted in the up-regulation of KLF2 and a significant reduction in the mRNA expression of adhesion molecules and inflammatory cytokines ICAM-1, VCAM-1, and IL-8. TNF- α mediated up-regulation of VCAM-1, but not of ICAM-1, was muted when the cells were preconditioned with laminar shear stress (Figure 1). Interestingly, the suppression of IL-8 was dependent on the expression of KLF2 (Figure 2). Moreover, exposure of HK-2 cells to laminar shear stress significantly down-regulated mRNA and protein expression of KIM-1 (data not shown).

Taken together, our data suggest that shear stress evokes a “tubulo-protective” phenotype in epithelial cells. These observations, although limited to in vitro data at this time may represent a new conceptual framework for the understanding of tubular injury in the context of renal ischemia with clinical relevance for acute kidney injury and transplantation.

Acknowledgments

Disclosure: The authors of this manuscript have conflicts to disclose according to the American Journal of Transplantation. Marc-Olivier Timsit discloses past and current research grants by AstellasPharma, Institut Georges Lopez/Waters Medical, Novartis and Astra-Zeneca. JVB is co-inventor on patents for KIM-1, which have been assigned to Partners Health Care and licensed to Johnson and Johnson, Genzyme, and Biogen Idec.

References

1. Moers C, Smits JM, Maathuis MHJ, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med*. 2009; 360:7–19.
2. Tullius SG, Garcia-Cardena G. Organ procurement and perfusion before transplantation. *N Engl J Med*. 2009; 360:78–80. [PubMed: 19118308]
3. Parmar KM, Larman HB, Dai G, et al. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. *J Clin Invest*. 2006; 116:49–58. [PubMed: 16341264]
4. Gracia-Sancho J, Villarreal G Jr, Zhang Y, et al. Flow cessation triggers endothelial dysfunction during organ cold storage conditions: strategies for pharmacologic intervention. *Transplantation*. 2010; 90:142–149. [PubMed: 20606606]
5. Duan Y, Gotoh N, Yan Q, et al. Shear-induced reorganization of renal proximal tubule cell actin cytoskeleton and apical junctional complexes. *Proc Natl Acad Sci U S A*. 2008; 105:11418–11423. [PubMed: 18685100]

Figure 1. TNF- α dependent cell surface expression of adhesion molecules is modulated by shear stress

Shear stress significantly decreased VCAM-1 but not ICAM-1 expression. Exposure to TNF α (40 nM) for 24 h led to strong VCAM-1 and ICAM-1 up-regulation. When cells were preconditioned by 24 h of flow, TNF α -dependent increase in VCAM-1 expression was less pronounced. Flow did not curtail TNF α -dependent ICAM-1 over-expression (n = 3).

Figure 2. KLF2 controls the flow-dependent regulation of IL-8 secretion and mRNA expression
 Top. (A) Transfection with siRNA targeting KLF2 leads to a 61% knock down of KLF2 mRNA expression under static and 78,2% under flow conditions. Bottom. KLF2 silencing significantly limits flow-dependent IL-8 down-regulation (B) and protein secretion (C). * $p < 0.05$ versus static control; ** $p > 0.05$ versus flow control; # $p > 0.05$ versus static control (n = 3).