The Proximal Tubule in Cystinosis: Fight or Flight?

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J Am Soc Nephrol 25: ●●●–●●●, 2014.
doi: 10.1681/ASN.2014010058

Although rare, nephropathic cystinosis is a devastating monogenic disorder leading to renal failure in the second decade of life. Fanconi syndrome, the initial renal manifestation of cystinosis in the young child, established the proximal tubule as a primary target of injury now recognized as the result of mutations in cystinosin (*CTNS*), the lysosomal cystine-proton cotransporter.[1] A major challenge in understanding the pathophysiology of cystinosis remains: what is the link between lysosomal cystine accumulation and proximal tubular dysfunction? Most of the data generated to date are based on *in vitro* studies of proximal tubular cells incubated with cystine dimethyl ester or of cells from cystinotic patients. These have resulted in the formulation of three major hypotheses regarding cystine-induced injury of proximal tubular cells: altered ATP metabolism, altered glutathione metabolism, and apoptotic cell death.[1] Mitochondria in cystinotic proximal tubules are abnormal, and cells contain decreased ATP and glutathione and undergo increased apoptosis. The interpretation of ATP metabolism in cell culture studies is hampered by the stimulation of glycolytic activity *in vitro*, whereas ATP is derived from mitochondrial oxidative phosphorylation *in vivo*.[1]

Progress has been accelerated by the recent availability of an animal model with a renal phenotype similar to human nephropathic cystinosis. The C56BL/6 *Ctns*$^{-/-}$ mouse exhibits most of the features of the human disease, including the formation of the "swan-neck deformity," a marked progressive narrowing of the proximal tubule beginning at the glomerulotubular junction.[2] Two papers in this issue of *JASN* have elucidated cellular mechanisms underlying the proximal tubular injury in the *Ctns*$^{-/-}$ mouse. Using a combination of techniques, including light, multiphoton, and electron microscopy, as well as functional endocytosis assays, Chevronnay *et al.*[3] demonstrate the evolution of lysosomal inclusions in proximal tubular cells leading to apical dedifferentiation, characterized by the loss in the S1 segment of expression of megalin, cubulin, and other transporters. This is accompanied by cellular adaptation, including expulsion of cystine crystals and tubular remodeling by downstream proximal tubular segments.

Using *in vitro* studies of human peripheral blood mononuclear cells exposed to cystine crystals, as well as *in vivo* studies of the *Ctns*$^{-/-}$ mouse model, Prencipe *et al.*[4] conclude that cystine crystals activate inflammasomes, most likely in proximal tubular cells, resulting in the release of IL-1β and IL-18. This, in turn, may contribute to the interstitial inflammation and fibrosis present in terminal phases of the human and murine *Ctns* mutants. The relative contribution of crystal-induced inflammasomes to cystinotic tubular injury remains to be determined, however. It is estimated that only 1% of intracellular cystine crystals are retained, due to lysosomal discharge of the crystals linked to endocytic recycling.[3]

The swan neck, a morphologic hallmark of cystinosis, can be viewed as a degenerative process that leaves a nonfunctional proximal tubular "atrophic" segment.[3] However, the development of extremely thin cells lining thickened tubular basement membrane may instead represent an adaptation to mitochondrial injury resulting from defective intracellular cystine processing. The proximal tubules comprise the bulk of renal mass and consume the majority of energy in reabsorption of glomerular filtrate. This tubular segment is particularly susceptible to oxidative injury resulting from a variety of stimuli, ranging from metabolic toxins (cystine accumulation) to ischemic or obstructive processes. Such oxidative injury is compounded by the generation of reactive oxidant species by damaged mitochondria.[5] Compared with the distal nephron, which has robust endogenous antioxidant defenses, the proximal tubule is more vulnerable to oxidative injury.[6] Thus, thinning of proximal tubular cells and thickening of tubular basement membrane can be viewed as an adaptive response: the machinery for cystine uptake has been eliminated by the tubular segment first exposed to cystine-rich glomerular filtrate. In this regard, megalin activity has been shown to contribute to early injury of proximal tubular cells in a model of nonselective proteinuria: loss of megalin is protective.[7] Moreover, by markedly decreasing mitochondrial content, formation of the swan neck in *Ctns*$^{-/-}$ mice reduces energy consumption and provides a structurally sound conduit to transport filtrate to functioning tubular cells downstream. This is, of course, only a temporizing measure—the transfer of function by the S1 segment of the proximal tubule to the S3 segment demonstrated by Chevronnay *et al.*[3] eventually fails to maintain nephron function, and proximal tubular cell death ensues, with the eventual formation of atubular glomeruli.[8] What begins as an effective short-term adaptation ultimately becomes maladaptive.

A useful paradigm to explain adaptations to injurious stimuli was drafted by Goligorsky, who applied Cannon's "fight

Published online ahead of print. Publication date available at www.jasn.org.

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or flight" response to tissues and individual cells.[9] The fate of a cell therefore depends on a balance between survival and death signals and is dictated by evolutionarily conserved pathways, with multiple regulatory checkpoints (fail-safes) to protect against inappropriate responses. This is illustrated by the demonstration that cystine crystal–induced IL-1β secretion requires a combination of caspase-1 activation, actin polymerization, lysosomal protease activity, potassium efflux, and the generation of reactive oxygen species.[4]

The remarkable plasticity of the proximal tubule manifested in cystinosis, with dramatic phenotypic and functional changes depending on nephron segment, is also demonstrated in AKI. In an ingenious experiment, Grgic *et al.* selectively induced acute diphtheria toxin–induced epithelial injury to the S1 and S2 tubular segments of noncystinotic mice.[10] This acute toxic injury results in successful repair and remodeling of the proximal tubule. However, repeated injury leads to maladaptive repair with interstitial fibrosis and glomerulosclerosis.[10] Because tubular basement membrane is preserved along the swan-neck segment, flattened epithelial cells may respond to therapies by remodeling and re-establishment of a functional S1 segment in the cystinotic nephron. The most commonly used animal model of CKD, unilateral ureteral obstruction, leads to massive proximal tubular cell death and the ultimate formation of atubular glomeruli.[11,12] Reduction of most proximal tubular mass reduces energy consumption, while remodeling of the Bowman capsule by sealing the urinary pole allows ongoing renin production by the juxtaglomerular apparatus.[11] These responses may also be viewed in the context of the fight-or-flight paradigm.[13] Release of obstruction can arrest the process, but this does not reverse established damage and nephron loss, possibly because of disrupted tubular basement membrane.[14]

Although a rare disorder, nephropathic cystinosis may provide unique opportunities to understand the mechanisms of response to metabolic tubular injury. The development of an animal model of cystinosis that parallels many of the characteristics of human disease has opened new avenues of investigation. In particular, this model affords an opportunity to study the evolution of the disease from birth to senescence. Progression of the tubular lesions in the $Ctns^{-/-}$ mouse is characterized by marked internephron heterogeneity in the rate of extension of the swan-neck lesion.[3] Great variability in the progression of nephron injury is reported for many types of renal disease, including polycystic kidney disease and obstructive nephropathy.[13] It is notable that these disorders (as well as many vascular, glomerular, and tubulointerstitial diseases) ultimately result in the formation of atubular glomeruli.[15] Rather than representing a mechanism of progressive renal disease, however, the formation of atubular glomeruli probably reflects decreasing proximal tubular mass, the hallmark of renal contraction in CKD. As in cystinosis, the development of significant interstitial fibrosis is also a late event in most renal disorders. In considering new therapies to preserve functional renal mass, it may be time to shift our attention from the glomerulus and the interstitium to the proximal tubule.

ACKNOWLEDGMENTS

The author is supported by a grant from the Cystinosis Research Foundation.

DISCLOSURES

None.

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