

Cystinosis Research Foundation

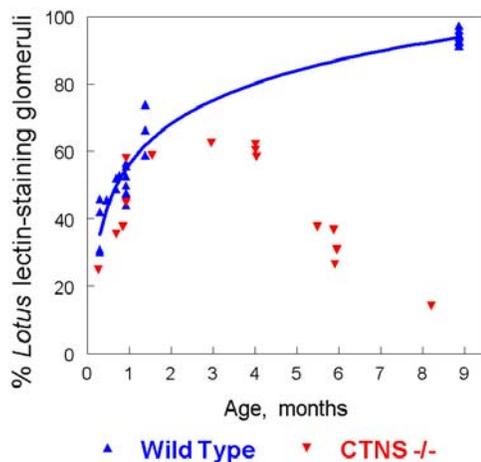
Initial Progress Report: March 2013

Oxidant injury in the progression of proximal tubular loss in cystinosis

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Preliminary studies for this grant proposal were generated from 15 *Ctns*^{-/-} mice provided by Dr. Corinne Antignac and Marie-Claire Gubler (Paris). These ranged in age from 3 to 20 months. In the initial 6 months of funding of this grant, the *Ctns*^{-/-} mouse colony established at the University of Virginia from founders provided by Dr. Cherqui (La Jolla) resulted in adequate numbers of litters to begin morphometric renal studies. These included 30 mice ranging from 8 days to 10 months of age. The fraction of *Lotus tetragonolobus* lectin-positive glomeruli in sagittal sections provides a measure of proximal tubular maturation as well as of proximal tubular injury resulting from the *Ctns* mutation.



The results for 15 male *Ctns*^{-/-} mice (red triangles) demonstrate that abnormalities of the glomerulotubular junction begin between 1 and 3 months, with rapid deterioration after 4 months.

To localize cellular oxidative stress, immunoreactive 8-OH-dG was determined in kidneys from 16 and 28-day-old as well as 3, 6, 9, 12, and 20 month-old *Ctns*^{-/-} mice. This revealed increased tubular 8-OH-dG in proximal tubules at 16 and 28 days of age, then disappearance of stain at 3-6 months, with reappearance at 9-12 months. These results indicate that oxidative stress is present in the neonatal period, then diminishes with further maturation, and recurs with progressive tubular injury in later adulthood. The results are consistent with our hypothesis for Specific Aim 1, that early intervention with mitochondria-targeted antioxidants (MitoQ) will provide optimal long-term protection from injury.

As provided to the CRF in supplementary data at the beginning of this study, intraperitoneal MitoQ administered to adult mice with unilateral ureteral obstruction (UUO) significantly reduced tubular apoptosis and preserved proximal tubular mass. These results support initiation of the studies proposed in Specific Aim 1: to administer

MitoQ vs. vehicle to *Ctns*^{-/-} and wild-type mice. As our colony of *Ctns*^{-/-} mice was not yet large enough to embark on the proposed protocol, we expanded pilot studies in the UUO model. These revealed that repeated intraperitoneal injection of either vehicle (dTPP) or MitoQ resulted in a localized inflammatory response. Use of an osmotic minipump was also not satisfactory because of precipitation of the compound in the pump. The next step was to test subcutaneous implantation of time-release capsules, which we have used successfully with other compounds in mice. Unfortunately, these also resulted in localized inflammation. We have therefore changed the route of administration of MitoQ and dTPP vehicle to adding them to drinking water in both UUO and *Ctns*^{-/-} protocols. Previous studies have demonstrated good tolerance to the compounds using this approach (500 μM MitoQ in drinking water), and after several days of habituation to the medicated water, mice gain weight normally. The protocol for *Ctns*^{-/-} mice has been modified to begin treatment at 4 weeks of age (post-weaning), and based on proximal tubular injury data (above), harvest of animals for this pilot study will be performed at 4 months of age. If significant effects are detected with this approach, it is likely that treatment beyond 4-6 months will not be necessary to confirm the importance of proximal tubular oxidative injury in the early development of cystinotic nephropathy.

Personnel

The Principal Investigator, animal surgeon, microscopist, and research associate fulfilled their roles in the first 6 months of the project as originally proposed. Dr. Galarreta, Research Associate, left June 1st to begin a pediatric residency at the University of Miami. Her work on this project convinced her to become a pediatric nephrologist. Her effort on the project has been transferred to the surgeon and microscopist, who have increased their effort accordingly.

Presentations and Publications

A summary of our preliminary results based on *Ctns*^{-/-} mouse kidney tissue obtained from Paris was presented at the Pediatric Academic Societies meeting in Boston, April 2012. Updated information including data above will be presented at the 12th International Workshop on Developmental Nephrology in Edinburgh, Scotland, June 26, 2013. A manuscript summarizing the presentation has been submitted, as requested, for publication in *Pediatric Nephrology*.