**Effects of oral cysteamine treatment on *Ctns* knockout rats**

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**Lay Abstract**

Cystinosis is a rare disease that results in the build-up of cystine in all cells of the body. Cystine is a protein building block and normally the excess is moved out of cells. In cystinosis the transporter for cystine does not work and cystine builds up inside the cells and forms crystals which cause damage to all organs and muscles. The only treatment available is cysteamine, which removes excess cystine from the cells. However, even if taken regularly and from birth, the kidneys will stop working eventually. Other organs will also eventually fail. To improve the lives of cystinosis patients there needs to be new therapies to reduce damage to the kidneys (as cysteamine does not stop this). We have shown in cells in the lab that using a drug called everolimus, the damage to the kidneys can be addressed. Using cysteamine and everolimus together all of the problems associated with cystinosis are corrected in these cell models. To test this new combination treatment we have made a rat model of cystinosis that is very good at replicating the disease seen in humans. Before we test the combination treatment we must test each drug individually first. We fed the cystinotic rats with cysteamine twice a day for 6-months and took a number of measurements. We found that at the end of the study, the cystinotic rats had improved growth, were excreting less protein and nutrients (Fanconi syndrome) and had better kidney appearance. However, they were not as good as wild-type controls showing that like in humans cysteamine can delay the onset of Fanconi syndrome but not prevent it.

**Scientific Abstract**

The lysosomal storage disease cystinosis results from mutations in *CTNS*, encoding a cystine transporter, and initially causes kidney proximal tubule dysfunction followed by kidney failure. Patients receive the drug-based therapy cysteamine from diagnosis, however, despite long-term treatment, patients still progress to kidney failure with the need for transplant inevitable. As such, there is an urgent need for alternative treatments. In previous work using stem cell and kidney organoid models, we discovered that a drug combination of cysteamine and the mTOR inhibitor everolimus can correct the cystinotic cell phenotype. To evaluate the therapeutic potential of this therapy *in vivo*, we generated a cystinotic rat model that faithfully recapitulates the human disease phenotype. These rats display hallmark characteristics of cystinosis within 3-6 months as seen by: failure to gain weight, excessive thirst (polydipsia) and urination (polyuria), cystine accumulation, Fanconi syndrome and kidney dysfunction. Slit lamp examination of the eyes of 3-month old animals revealed the presence of cystine crystals, and histology showed the presence of swan neck lesions in kidney tissues. We have begun evaluating the effects of cysteamine and everolimus individually in *Ctns*-/- rats, as a necessary prerequisite before testing the cysteamine/everolimus combination therapy.

Oral treatment with 30mg/kg cysteamine for 6-months delivered twice daily via ‘jelly pills’ resulted in weight gain and significantly reduced polydipsia and polyuria. Gross kidney morphology and kidney weights were improved compared to untreated kidneys, but were not fully rescued to that of wildtype appearance. In depth urine analysis revealed significantly less protein and glucose were excreted at 6-months in cysteamine treated animals while urea and creatinine were significantly increased. Although some parameters were improved, the levels were not rescued to wild-type levels suggesting that Fanconi syndrome is delayed in the cystreamine treated cystinotic rats but not prevented.