

Cystinosis Research Foundation Progress Report

Title: ***Cysteamine effects on extracellular matrix accumulation in chronic kidney disease.***

Grant number: 412560090101

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Funding Period: July 1, 2008 to June 30, 2011

Progress Report: January 1, 2009 to June 30, 2009

OVERVIEW

The project is progressing according to the proposed research plan. The three mouse lines are all actively breeding (*Ctns*^{-/-}, *vanin*^{-/-} and *Ctns*^{-/-}*vanin*^{-/-} double knockouts). The animal work for the first cysteamine *in vivo* study has been completed and tissue analyses are in progress, with exciting initial findings.

1) **Aim #1:** To perform preliminary studies to develop an optimal model and drug delivery strategy to investigate the effect of cysteamine in a mouse chronic kidney disease model.

a) **Searching for a mouse model of cystinosis-associated nephropathy.**

Based on our hypothesis that *Ctns*^{-/-} mice have an essentially normal phenotype due to the expression of endogenous cysteamine synthesized via an enzymatic pathway that is encoded by the *vanin* gene, we are generating a colony of *Ctns*^{-/-} *vanin*^{-/-} double knock-out mice (Figure 1). To date 5 male and 5 female mice have been born and confirmed using PCR-based genotyping to be double knockouts. This first cohort of 10 mice will be placed in metabolic cages at 3 months of age (08/09). 24h urine collections will be tested for evidence of tubular dysfunction. Repeat 24h studies are planned at 6 and 9 months of age. Two cages of double knockout mice are actively breeding to expand the number of mice in this study. A cohort of *Ctns*^{-/-} will be bred to compare the renal phenotype with the *Ctns*^{-/-} *vanin*^{-/-} double knock-out mice when they are sacrificed at 9 months of age.

Findings from an initial study of the unilateral ureteral obstruction (UUO) model in 8-week old male mice, comparing the degree of fibrosis between wild-type (*vanin*^{+/+}) and *vanin*^{-/-} mice, suggests that *vanin*-deficiency alone does not alter the degree of kidney fibrosis (Figure 2). However, the numbers are still small (4 *vanin*^{+/+} and 3 *vanin*^{-/-} mice on day 21; n=4/group at day 14). The colony is breeding to add additional mice to this study.

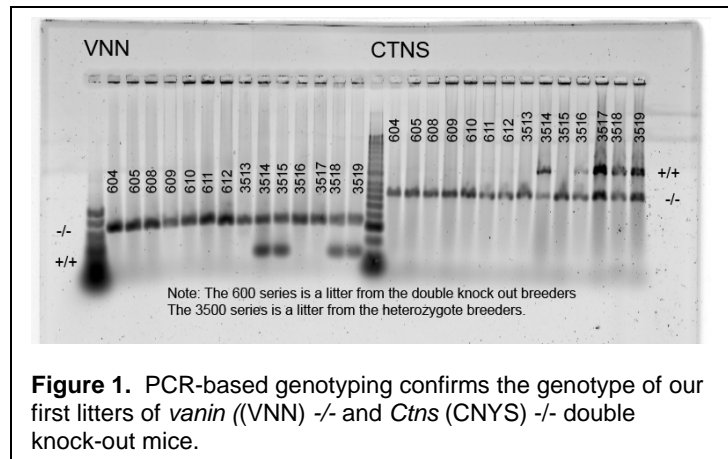


Figure 1. PCR-based genotyping confirms the genotype of our first litters of *vanin* (VNN) ^{-/-} and *Ctns* (CNYS) ^{-/-} double knock-out mice.

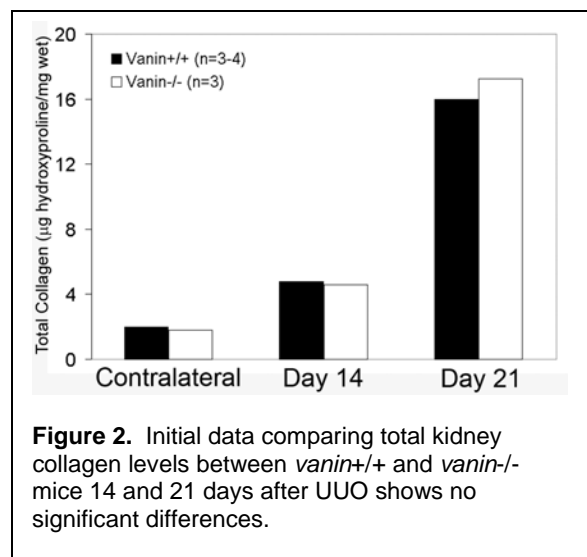


Figure 2. Initial data comparing total kidney collagen levels between *vanin*^{+/+} and *vanin*^{-/-} mice 14 and 21 days after UUO shows no significant differences.

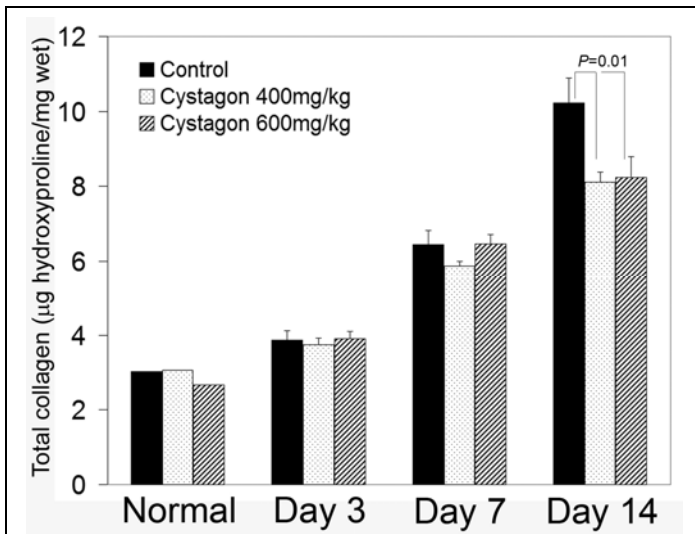


Figure 3. Cystagon® significantly reduces total kidney collagen levels after 14 days of UUO. We are planning to perform a 21 study.

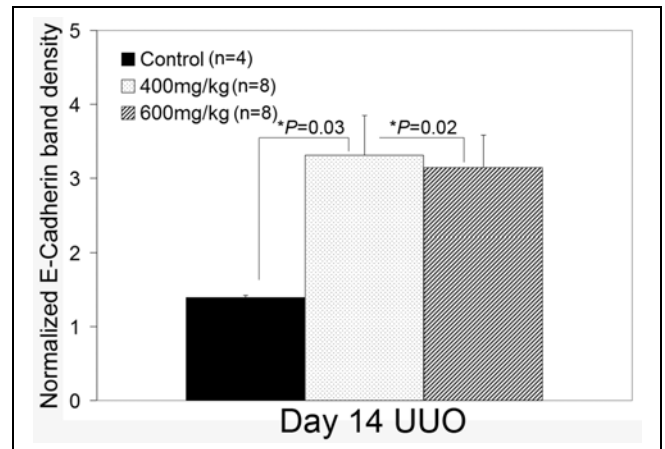


Figure 4. By immunoblotting kidney E-cadherin levels are significantly higher in the Cystagon®-treated mice after 14 days of UUO, indicating better preservation of intact tubules.

2) **Aim #2:** To investigate the efficacy of cysteamine therapy for interstitial renal matrix protein reduction in chronic kidney disease and to determine its mechanism of anti-fibrotic action.

Based of the findings from a pilot study, the effect of cysteamine (administered as Cystagon® added to the drinking water that was made freshly every 24h) on the degree of renal fibrosis was investigated using two doses: 400 and 600 mg/kg/day. Groups of mice (n = 8 at each time-point) were studied 3, 7 and 14 days after the onset of chronic injury induced by UUO. Both doses were shown to significantly reduce kidney collagen levels by day 14 (Figure 3). We plan to extend these observations to a 21-day UUO study. Using loss of the renal tubular cell adherens junction protein E-cadherin as a marker of the degree of tubular damage, immunoblotting studies demonstrate that renal tubular integrity is significantly improved in the Cystagon®-treated UUO mice (Figure 4).

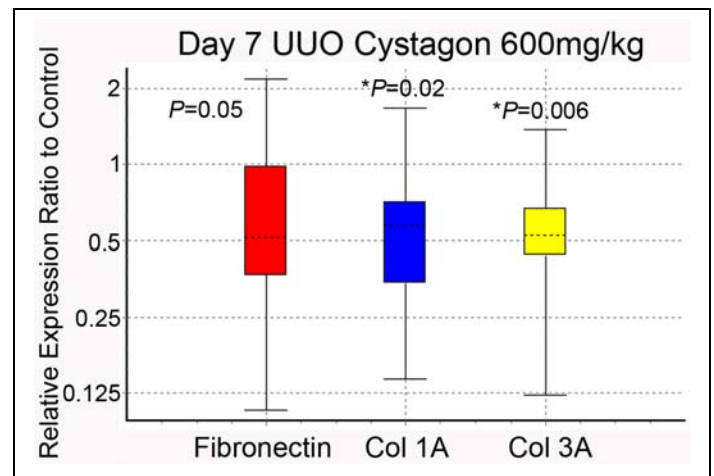


Figure 5. Kidney extracellular matrix protein mRNA levels measured by real-time RT-PCR are significantly lower after UUO in Cystagon®-treated mice, especially at the higher dose of 600 mg/kg/day. Data are shown as means of the relative expression ratios (treated versus untreated) (n=8/group).

Kidney tissue studies are now focusing on elucidating the mechanism by which Cystagon® reduces kidney fibrosis. One potential pathway may be its ability to reduce collagen synthesis rates. Measured by real-time RT-PCR (qRT-PCR), kidney procollagen I mRNA levels were significantly lower in the mice treated with 600 mg/kg/day (Figure 5). However, it appears that other mechanisms are involved as this effect was not seen at the lower Cystagon® dose and fibronectin and procollagen III levels were unchanged at either dose on day 14.

We will next be investigating effects on inflammation, oxidative stress and transglutaminase-mediated matrix cross-linking activity.

3) Aim #3: To investigate the effect of cysteamine on apoptosis of renal tubular epithelial cells, oxidant stress, and other novel target pathways of chronic kidney disease. These studies have been initiated using the kidneys from the mice in the second specific aim. Kidneys from the *Ctns*^{-/-} *vanin*^{-/-} double knockout mice will become available over the next 6 months and will be compared to wild-type kidneys and age/gender-matched kidneys from the *Ctns*^{-/-} mice. As originally proposed, the effects on apoptosis, glutathione activity and novel cysteamine targets will be the primary focus of these studies

In summary, early data from the first Cystagon® treatment study establishes its significant anti-fibrotic effects. Ongoing studies are planned to identify how this effect is achieved and to determine if it is sustained when chronic kidney damage persists for longer periods of time. Over the next 6 months the first data will become available on the renal phenotype of the *Ctns*^{-/-} *vanin*^{-/-} double knock-out mice

BUDGET EXPENDITURES

January 1, 2009 to June 30, 2009

Category	Award *	Prior expenditures **	Expenditures this period *** (01/01/09 – 06/30/09)	Anticipated expenditures ****	Total expenditures this period	Total expenditures to date	Remaining Balance
Personnel	\$58,615	\$20,373	\$22,087	\$3,894	\$25,982	\$46,355	\$12,260
Animals/ Maintenance	\$5,500	\$2,650	\$3,279	\$325	\$3,604	\$6,254	\$(754)
Supplies	\$13,000	\$13,212	\$5,158	\$1,650	\$6,808	\$20,020	\$(7,020)
Travel	\$1,500	0	0	0	0	0	\$1,500
Subtotal	\$78,615	\$36,236	\$30,524	\$ 5,869	\$36,393	\$72,629	\$5,986
IDC	\$7,862	\$3,624	\$3,055	\$587	\$3,642	\$7,266	\$596
Total	\$86,477	\$39,859	\$33,579	\$6,456	\$40,035	\$79,895	\$6,582

* Award amount for 07/01/08-06/30/09

** Prior expenditures: 07/01/08-12/31/08

*** Expenditures this period: 01/01/09 - 06/14/09

*** Anticipated expenditures: 06/15/09 - 06/30/09

8% budget remaining