TITLE: Use of tolvaptan, a vasopressin antagonist, to increase urine dilution and reduce cystine urolithiasis among patients with homozygous cystinuria: a pilot investigation

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Boston Children's Hospital

A. Specific Aims/Objectives

The goal of this research project is to establish that in short-term use, tolvaptan is a safe and potentially effective new therapy for cystinuria, by conducting a short-term pilot study of the safety and tolerability of this drug, and assess impact on urinary stone risk parameters, among adolescent and young adult patients with clinical cystinuria.

Specific Aim 1) To characterize the short-term safety profile of tolvaptan therapy in adolescent patients with cystinuria.

Hypothesis 1a: Tolvaptan will be well tolerated with low adverse effects, minimal disruption of serum electrolytes, and no detectable alterations in blood pressure and other cardiovascular parameters

Specific Aim 2) To characterize the short-term effect of tolvaptan on urinary risk factors for urolithiasis in adolescent patients with cystinuria.

Hypothesis 2a: Tolvaptan therapy will result in >25% increased urine output, decrease in urinary osmolality of >25%, and increase in urinary cystine capacity of >25%

B. Background and Significance

Cystinuria pathophysiology. Cystinuria is a rare disorder of amino acid metabolism that leads to high levels of cystine in the urine. (Cystine is a dimeric amino acid formed from two cysteine amino acid residues linked by a disulfide bond.) Patients with cystinuria have mutations in one of two genes (SLC3A1 and SLC7A9) that encode subunits of the amino acid transporters that are responsible for reabsorbing filtered cystine and dibasic amino acids (ornithine, lysine, and arginine) from the lumen of the proximal renal tubule into the tubular cells. Although in normal individuals virtually 100% of the filtered load of amino acids is reabsorbed from the proximal tubule, homozygotes (and some heterozygotes, depending on the mutation) are unable to reabsorb these amino acids. These individuals therefore excrete large amounts of these amino acids into the urine. As cystine is relatively insoluble in urine, it precipitates into crystals and dense stone stones, resulting in clinical urolithiasis. The condition is rare; homozygous cystinuria occurs in about 1/15,000 persons in the United States (~20,000 affected individuals nationwide)(1). Age at presentation is variable, but significant numbers of patients present during childhood or adolescence with clinical stone disease, with over 80% presenting before age 20(2).

Urolithiasis in patients with cystinuria is a difficult problem to manage. Stones tend to be large and recurrent, form rapidly, and require repeated surgical treatments. This often has devastating effects on renal function over the long-term, such increased probability of nephrectomy, high incidence of decreased GFR, and progression (in some rare cases) to end-stage renal disease(2, 3).

Contemporary cystinuria management. Metabolic strategies for preventing cystine stones focus on increasing solubility of cystine in the urine, through dilution, alkalinization, or chelation.

Hydration therapy is the underlying foundation of all therapy for cystinuria. Hydration therapy is based on an idea that, given a relatively constant amount of cystine being excreted in the urine each day, adequate hydration will result in dilution of the fixed cystine load to a concentration below that at which crystallization typically occurs. Target concentrations are usually set at about 250 mg/L, as stone formation rates increase significantly at concentrations above this. While simple in concept, and although there is longstanding evidence that hyperhydration can be effective in reducing stone recurrence(4), in practice adequate oral hydration is difficult to achieve for many patients. The target cystine concentration requires a urine volume of over 3 L per day, which in turn requires fluid intake of greater than 3.5-4 L per day. Many patients find that they cannot sustain this level of intake voluntarily.

A complementary strategy for reducing cystine crystallization is to chemically alter the solubility of cystine. Below pH of 7.0, the solubility of cystine is relatively constant at about 250 mg/L. However, as pH rises above 7.0, solubility increases to about 500 mg/L at 7.5(1). Thus, alkalinization has been used to increase urinary pH to a range that permits significantly more cystine in solution in the urine, regardless of volume. Potassium citrate is the agent most commonly used for this purpose, is well tolerated, and avoids the potential negative effects of using the sodium salts, as sodium intake appears to increase cystine excretion(1, 5).

Dietary modification to reduce cystine intake is attractive, in theory. Reduction in dietary animal protein intake has been found to reduce the excretion of cystine and other amino acids(6). Such reduction is also associated with increase in urine pH. However, exogenous protein is not the only source of cystine in the body; the amino acid is produced as part of metabolic processes. Thus, the potential impact of reduced intake is blunted

Chelation therapy for cystinuria. For a large proportion of cystinuria patients, oral hydration and alkalinization are insufficient to adequately manage urinary cystine levels. For such patients, chelation therapy is utilized as an adjunct. The drugs most commonly used for this purpose are d-penicillamine and the newer agent α --mercaptopropionylglycine (tiopronin, thiola). The mechanism for both agents is similar: both molecules contain a thiol group, which reduces the disulfide bond in the cystine molecule, splitting it into two cysteine residues. The cysteine residue then bonds to the thiol group of the drug, forming a drug-cysteine compound that is 50 times more soluble than the cysteine-cysteine dimer (cystine)(7).

Treatment with these agents is limited by the substantial toxicity observed in both drugs. Adverse reactions include aplastic anemia, agranulocytosis, leukopenia, thrombocytopenia, Goodpasture's syndrome or myasthenia gravis, severe gastrointestinal distress (nausea, vomiting, diarrhea), arthralgia, and lupus-like symptoms(8, 9). Therapy discontinuation rates are between 30-70% due to side effects(10). Thus, the value of the currently-available therapeutic agents is lowered by these toxic effects, and many patients are unable to achieve adequate urinary cystine control, resulting in recurrent stone disease with all its attendant morbidity.

Chelation has also been reported using the ACE inhibitor captopril. Like d-penicillamine and α-MPG, captopril contains a thiol group and can complex with cysteine to improve solubility. However, clinical studies have reported conflicting results(11-14) and there are no randomized controlled trials (RCTs) demonstrating efficacy or improved outcomes.

The combination of oral hydration, alkalinization, and chelation represents a multifaceted approach to stone prevention in cystinuria. Unfortunately, few patients achieve adequate results with current medical therapy. In one study, only 15% of medically-managed patients durably maintained adequate urinary cystine concentrations(15). Clearly, new therapies are urgently needed for this

devastating illness. However, since the introduction of α-MPG in the 1980's there have been few new options

Measurement of urinary cystine. Cystine levels can be measured simply as the quantity of cystine per volume of urine, but as many factors affect cystine solubility, this is a poor predictor of clinical stone formation. Therefore, more complex measures have been developed. Cystine supersaturation is the ratio of the measured urinary cystine concentration to the empirically-determined saturation concentration of the patient's urine. Values above 1 indicated that the urine is supersaturated with cystine, and target values are typically less than 0.6(16). However, because the thiol-containing drugs commonly used to manage cystinuria (e.g. thiola) can interfere with accurate measurement of cystine levels, the cystine capacity test was developed to account for such drugs(17). This proprietary test (Litholink Corp., Chicago IL) is reported as a value in mg/L above or below zero. Positive values indicated that the urine is undersaturated with cystine, while negative values indicate that the urine is supersaturated with cystine. Target values during treatment are positive, preferably about 150 mg/L.

Tolvaptan: mechanism and effects. Tolvaptan (Samsca) is a selective arginine vasopressin V2-receptor antagonist. Activation of arginine vasopressin V2-receptors in the distal nephron increases the number of aquaporin channels in the apical membrane of collecting duct cells, thereby increasing water absorption from the tubular lumen into the interstitium. Conversely, inhibition of V2-receptors reduces the number of aquaporin channels, increasing urinary excretion of water, with minimal changes in electrolyte excretion. Tolvaptan has been used clinically for treatment of hyponatremia(18), congestive heart failure(19), syndrome of inappropriate antidiuretic hormone(20) and autosomal dominant polycystic kidney disease(21). It appears to have a good safety profile with few adverse reactions. Although most clinical studies have been in adults, examples of use in the pediatric population do exist (19, 22).

Tolvaptan dosing. In published studies dosing of tolvaptan has varied considerably. Most studies in adults have used doses of 15-120 mg once daily, in either fixed or increasing dosages, with increases usually limited by tolerance. In the SALT trials, dosing was 15-60 mg once daily, adjusted for effect on serum sodium levels. This translated to a dose of 0.2-0.8 mg/kg. In the 3-year RCT by Torres et al., dosing was twice daily, with morning and afternoon doses of 45-90 mg and 15-30 mg respectively(21), which translates to doses of 0.6-1.1 mg/kg in the morning, and 0.2-0.4 mg/kg in the afternoon (total daily doses 0.8-1.5 mg/kg). In the pediatric heart failure study by Regen et al., dosing ranged from 0.1-1.3 mg/kg (median 0.3 mg/kg).

Tolvaptan safety. In short-term studies of tolvaptan effects in healthy Japanese, Korean, and Caucasian men, tolvaptan was generally well-tolerated with few apparent adverse reactions(23-26). The most common side effect was thirst, presumed to be an effect of the pharmacologic action of the drug. Similar adverse event profiles were noted in 7-day RCTs among adult patients with congestive heart failure (27, 28). A single-dose study in a series of pediatric patients with heart failure also identified no significant adverse effects(19).

Findings have generally been favorable in longer-term trials, as well. The SALT-1and SALT-2 trials were RCTs examining 30-day outcomes of tolvaptan among patients with hyponatremia(18). (The investigators hypothesized that the aquaresis associated with tolvaptan would increase serum sodium levels and thereby improve clinical outcomes.) In this seriously ill population (hyponatremia was due to chronic heart failure, liver cirrhosis, or syndrome of inappropriate antidiuretic hormone (SIADH) secretion), the rates of adverse events were similar in treatment and placebo groups. The most common tolvaptan-associated effect was thirst and dry mouth. In an open label extension of the SALT studies, 111 adult patients with hyponatremia continued to take tolvaptan for a mean of

701 days(29); again, adverse reactions related to aquaresis were observed, but the drug was otherwise well tolerated.

The longest-term RCT of tolvaptan was the TEMPO 3:4 trial, comparing tolvaptan to placebo during a 3 year period among patients with autosomal dominant polycystic kidney disease (ADPKD)(21). As in other studies, the most common adverse effects in the tolvaptan group were those related to aquaresis (thirst, polyuria), and these led to drug discontinuation in 8.3% of patients. However, in this trial the tolvaptan group also had a significantly higher incidence of elevated liver function enzymes; elevated alanine aminotransferase was seen in 4.9% of tolvaptan patient vs. 1.2% of placebo patients. Overall, 1.2% of patients in the tolvaptan group discontinued the study drug due to abnormal liver tests. Abnormal liver tests returned to normal in all cases, either before or after discontinuation of study drug, and there were no persistent sequelae reported.

For all female subjects in the study, we will be conducting a pregnancy test prior to drug administration. If a positive pregnancy test is found, they would be withdrawn from the study. Medical staff will also notify the subject of their positive pregnancy and the CTSU nurses/ clinicians will give them standard guidance with their pregnancy status.

C. Preliminary Studies

We reviewed our cystinuria experience at the Boston Children's Hospital Pediatric Kidney Stone Clinic and, using the data warehouse, the i2b2 search query tool, and nephrology and urology department records, we were able to identify approximately 30 patients seen over the past 5 years with a diagnosis of cystinuria and cystine stones. We are currently in the process of performing a retrospective review of these patients to describe such characteristics as age at presentation, number of surgical treatments required, recurrence of stones, etc.

Tolvaptan in cystinuria. We have not yet treated any pediatric cystinuria patients with tolvaptan. We believe that, given the uncertainty regarding safety and efficacy of this treatment in this population, the treatment should be administered within the framework of a research study with careful monitoring. The only reported instance of using tolvaptan for treatment of cystinuria was reported by de Boer et al. in two adult patients(30). Each of these patients was treated for 5 days with tolvaptan; both exhibited a significant increase in urine output, decrease in urine cystine concentration, and a minor increase in plasma osmolality. Both patients experience minor thirst symptoms but otherwise tolerated treatment well. The authors concluded that this was a promising potential treatment for cystinuria (and in fact any chronic lithogenic condition), and that further studies of this agent were warranted.

D. Design and Methods

(1) Study Design

We will conduct a single-arm, unblinded, short-term prospective evaluation of tolvaptan in patients with cystinuria. The goal will be to obtain safety and short-term effectiveness data on Tolvaptan in adolescent to young adult patients, to demonstrate tolerability and favorable impact on urinary parameters associated with cystine urolithiasis risk.

(2) Patient Selection and Inclusion/Exclusion Criteria

Sample population

For this pilot study 16 patients with cystinuria will be recruited for 8 complete participants. Subjects will be recruited from our existing database of cystinuria patients seen in the

Boston Children's Hospital Pediatric Kidney Stone Clinic, and from new patients diagnosed during the research period who present to Boston Children's Hospital. We will also consider recruitment through advertising in other outlets e.g. International Cystinuria Foundation, Rare Kidney Stone Consortium.

Inclusion criteria:

Males and females age 12 – 25 years
Weight ≥ 25kg
Confirmed cystinuria via:
Documented cystine stones (stone >75% cystine on chemical analysis),
Cystine crystals in urine (pathognomonic hexagonal crystals),
Urinary cystine levels greater than 250 mg/L or
Genetic screening.
Estimated GFR greater than 80 ml/min/1.73 m2

Exclusion criteria:

Concurrent non-renal disease that might increase risk of complications due to aquaresis.
Liver or biliary disease (chronic or acute)
Malabsorption syndrome or other gastrointestinal condition that may interfere with response to therapy
Non-cutaneous malignancy within last 5 years
History of adverse reaction or allergy to Tolvaptan or other arginine vasopressin V2-receptor antagonists
Positive pregnancy test
Currently breastfeeding
Inability or unwillingness to consent/assent

(3) Description of Study Treatments or Exposures/Predictors

Starting at between 10AM and 2PM on Day 1, Tolvaptan will be administered orally. See dose chart below:

T-CUPS:

Tolvaptan use in Cystinuria and Urolithiasis: A Pilot Study

DOSING SCHEDULE by WEIGHT

Weight (kg)	Day 1-4	Day 5-8
12.5 kg -37.49 kg (mean=25kg) *For illustration: We will not recruit anyone weighing less that 25kg.*	7.5mg (.6mg/kg -.2mg/kg)	15mg (1.2mg/kg - .4mg/kg)
37.5kg-62.49kg (mean =50kg)	15.0 mg (.4mg/kg - .24 mg/kg)	30mg (.8mg/kg - .48mg/kg)
62.5 kg -87.49kg (mean 75kg)	22.5 mg (.36mg/kg - .26mg/kg)	45.0mg (.72mg/kg - .52mg/kg)
87.5 kg – 112.49kg (mean 100kg)	30.0 mg (.34mg/kg - .27mg/kg)	60.0mg (.68mg/kg -.54mg/kg)
112.5kg – 137.5kg (mean 125kg)	37.5 mg (.33 mg/kg - .27mg/kg)	60.0mg (.66mg/kg - .54mg/kg)

This daily dose will continue for a total of 4 doses, through Day 4. On Day 5, the dose will be increased per chart, up to a maximum does of 60 mg. If tolerated, the higher dose will be continued for a total of 4 doses through Day 8. No drug will be given on day 9 or 10.

Tolvaptan is produced commercially as 15 mg triangular tablets and 30 mg round tablets.

Patients currently on chelation therapy will NOT discontinue these medications during the study period.

(4) Definition of Primary and Secondary Outcomes/Endpoints

The primary outcome will be urinary cystine levels as measured by cystine capacity (Litholink Corp; mg/L). Four 24-hour urine samples will be obtained during the study: one at baseline 3-6 days prior to the measurement, one on day 3-4 of the dosing period, one on day 7-8 of the dosing period, and one 3-6 days after the washout period. Each 24 hour urine sample will be sent individually for analysis by Litholink Corp, which performs the cystine assays.

Secondary outcomes will include daily urine output and urine osmolality. Additional data points to be collected include serum sodium and other electrolyte levels, serum osmolality, plasma AVP concentration, and urinary electrolytes and osmolality. Urinary symptoms will be measured with the AUA symptom score.

(5) Data Collection Methods, Assessments, Interventions and Schedule (what assessments performed, how often)

All information will be collected and reported onto a paper case report forms (CRFs), which will later be entered into REDCap. 24 hour urines and clinical (vital signs, weights, fluid intake and output, thirst and dry mouth) and laboratory data (urine cultures/analysis, serum electrolytes and liver function tests) will be collected throughout the duration of the patient's participation in the research study. The following table indicates the procedures that will be done each day the patient is involved with the research study:

3-6 days before	Day 0	Day 1	Day 2	Day 3

Day 0				
24 hour urine collection.	CTSU-Outpt. Physical exam. Blood sample. Urine sample.	CTSU – admitted. Tolvaptan given orally. Urine sample.	CTSU – discharged Tolvaptan given orally. Blood sample. Urine Sample	CTSU – outpatient. Tolvaptan given orally. 24 hour urine collection.

Day 4	Day 5	Day 6	Day 7	Day 8
CTSU – outpatient. Tolvaptan given orally. Blood sample.	CTSU – admitted. Tolvaptan given orally. Urine sample.	CTSU – discharged. Tolvaptan given orally. Blood sample. Urine Sample	CTSU – outpatient. Tolvaptan given orally. 24 hour urine collection.	CTSU – outpatient. Tolvaptan given orally. Blood sample.

Day 9	Day 10	3-6 days after Day 10
CTSU – outpatient. No medication given. .Urine sample	CTSU – outpatient. No medication given. Blood sample.	24 hour urine collection.

(6) Study Timeline (as applicable)

The study will be conducted through the Clinical and Translational Study Unit at Boston Children's Hospital. The treatment period will for 8 days. Subjects will be spend two 24=hour periods under inpatient observation; the remainder of the period will be under outpatient observation, with daily visits to the CTSU. The day prior to the treatment period (Day 0) will be used to obtain baseline clinical and laboratory data. Study drug will be administered starting on Day 1 through Day 8, with a 2 day washout period on days 9 and 10. Patients will be monitored during the period with 24-hour urine collection for cystine stone risk profile, daily weights, vital signs, blood draws, fluid intake and output, and assessment of subjective outcomes.

E. Adverse Event Criteria and Reporting Procedures

Subjects will be monitored for drug tolerability and safety. Patients will be observed in the inpatient CTSU setting for 24 hours after the initial dose (Day 1) and also for 24 hours after the increased dose (Day 5). During each of these 24 hour periods, subjects will undergo around the clock monitoring of vital signs and daily input and outputs.. On Day 2-4 and Day 6-8 subjects will be monitored on an outpatient basis, coming in each day to the CTSU. At each outpatient visit vital signs will be checked. Serum Electrolytes will be checked on days 0, 2, 4, 6, 8,and 10. Liver function tests will be obtained at baseline, Day 4, and Day 8, and Day 10. Thirst and dry mouth will be assessed daily with visual analog scales. (31-33).

Criteria for cessation of treatment will include abnormal serum sodium (>5 mmol/L above or below normal range), potassium (>0.2 mmol/L above or below normal range), or CO2 (>2 mmol/L above or below normal range). Other criteria will include unstable vital signs (e.g. tachycardia, hypotension), intolerable subjective symptoms of thirst or voiding symptoms, or other symptoms subjectively attributed to the medication.

Subjects that experience adverse reaction at the higher dose level will be reduced to the lower dose level; if adverse effects persist at the lower dose level, the study drug will be discontinued.

For other potential adverse events not listed above, the investigators will decide on a case-by-case basis whether treatment should be continued, taking the subject's short-term and long-term health and safety as the primary factor for consideration.

F. Data Management Methods

Clinical and laboratory data will be maintained in a secure REDCap database.

G. Quality Control Method

Trained research personnel will be collecting data and entering it into REDCap. There also will be data checks within the REDCap system.

H. Data Analysis Plan

In this pilot study, the primary analysis will be description of the change from baseline during treatment of cystine stone risk profile. This will be demonstrated graphically. With respect to the primary endpoint, differences in cystine capacity between baseline and low-dose treatment phase, and between baseline and the high-dose treatment phases, will be compared with paired t-tests. Data regarding thirst and dry mouth will also be recorded and demonstrated graphically.

I. Statistical Power and Sample Considerations

As a pilot study with very low numbers of subjects, statistical analysis will not be feasible for adverse effects. Adverse effects will be classified with respect to whether they resulted in the subject discontinuing study medication or requiring additional monitoring, testing, or treatment, or not. For electrolyte and cardiovascular measures, we will analyze these outcomes according to proportion of subjects experiencing at least one outlier measurement (defined as a clinically abnormal value based on the standard normal range for each test) for each parameter (each of the serum chemistry values, and cardiovascular measures).

J. Study Organization

Data for this study will be managed via the REDCap web-based data management system at Boston Children's Hospital. This database will store patient-level variables and clinical outcome data. PHI on individual study participants will be maintained in a password protected database. Data within the PHI database will be linked to the clinical data in the primary database via study identification numbers.

K. References

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