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Update on cystinuria

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Abstract

Purpose of review—Cystinuria is a rare genetic disease with increased urinary excretion of the poorly soluble amino acid cystine. It can lead to significant morbidity in affected patients due to the often large and recurrent resulting kidney stones. Treatment is focused on the prevention of stone formation. There have been few advances in the available therapeutic options for the disorder in the last 15–20 years.

Recent findings—Although no new treatments have emerged in the prevention of cystinuria in recent years, several developments hold promise for advancing the field of caring for affected patients. A new method of measuring urinary cystine and estimating potential for stone formation, called cystine capacity, may prove to be a useful tool in monitoring the disease. The discoveries of the mutations that cause cystinuria have led to a new classification system based on genotype that is more accurate than the prior phenotypic one. The finding of new compounds that inhibit cystine crystal growth *in vitro*, now being tested in animal models, may lead to new potential therapies in years to come. The Rare Kidney Stone Consortium has developed a registry and hopes to lead further efforts in dealing with cystinuria.

Summary—With several recent advances in the monitoring and treatment of cystinuria, and the gathering of clinical patient data, there are now opportunities for new management protocols and therapies.

Keywords

amino acids; cystine/analogs and derivatives; drug design; nephrolithiasis; urolithiasis

INTRODUCTION

Cystinuria is an autosomal recessive disorder characterized by failure of the proximal tubule to reabsorb cystine filtered by the glomerulus. The defective cystine transporter in the proximal tubule also leads to abnormally high urinary excretion of dibasic amino acids ornithine, arginine, and lysine. However only the loss of cystine in the urine, a poorly

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Conflicts of interest

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soluble amino acid, has clinical consequences. Cystine precipitation leads to recurrent and often large kidney stones and may be associated with a loss of kidney function.

Two genes code for the two protein components of the cystine transporter. *SLC3A1* codes for basic amino acid transport protein rBAT and *SLC7A9* codes for the functional unit, or 'light component' of the amino acid transporter ($b^{0,+}$ AT, as it transports neutral and dibasic amino acids). The two proteins are linked by a disulfide bridge; rBAT is important for trafficking of $b^{0,+}$AT to the apical membrane of proximal tubule epithelial cells. Mutations in either protein will lead to defective proximal tubular amino acid reabsorption and recurrent cystine stones.

In this review, we describe the most recent classification of cystinuria, possible improvements in monitoring therapy, and the potential for development of new drugs.

CLASSIFICATION

Traditionally, patients with cystinuria were classified into three types based on the urinary excretion patterns of their parents (obligate heterozygotes). Type I heterozygotes showed a normal urinary cystine excretion pattern (<0–100 μmol of cystine/g of creatinine), type II heterozygotes had a marked increase in excretion (>900 μmol of cystine/g creatinine), and type III heterozygotes showed a moderate increase (100–900 μmol of cystine/g creatinine). Once the underlying mutations for the disease were known, a new classification system was developed based on genetic findings. Type A cystinuria is the result of mutations in both *SCL3A1* genes and type B results from mutations in both *SCL7A9* genes. Individuals with one mutated allele of each gene (one mutation in *SCL3A1* and one in *SLC7A9*) do not have stones, because at least two mutated copies of one allele is needed to result in the phenotype, thereby ruling out 'digenic inheritance' [1]. People with the rare type AB cystinuria have two mutated alleles in the same gene in addition to a mutated allele in the other gene and so are actually AAB or ABB [1]. The older phenotypic classification system was challenged by a study which compared genotypes and phenotypes in heterozygotes [2]. The study showed that 14% of heterozygotes with a type B mutation had urinary cystine excretion that was not significantly different than normal controls. Their offspring, homozygous patients, would therefore be misclassified based on the traditional method. It is therefore preferable that genetics should be used to classify patients whenever necessary. Currently however, genotyping patients with cystinuria is not clinically necessary, as it does not have therapeutic implications. It might be useful to screen siblings, though measuring cystine in the urine is less expensive. There might also be debatable merit in screening potential mates, which we do not recommend.

LABORATORY STUDIES

Measurement of urinary cystine (a dimer of the amino acid cysteine) to date has been inaccurate for several reasons. For one, many cystine assays measure free sulfhydryl groups using colorimetric reactions. These reactions do not reliably distinguish cystine from soluble thiol–cysteine drug complexes, so they cannot reliably measure cystine in the presence of cystine binding thiol drugs (CBTDs) D-penicillamine and tiopronin. Other techniques, such as high-performance liquid chromatography (HPLC), can distinguish between the two [3].

However, the preparation of the sample for HPLC may lead to disruption of the thiol–cysteine drug complex, again leading to inaccurate measurements of cystine in the presence of CBTDs. Even in the absence of CBTDs, measurement of cystine supersaturation cannot reliably be calculated from the measurement of cystine concentration and urine pH, because of the variability of cystine solubility at different pH values [4]. These problems led to the development of a new assay called cystine capacity that directly measures the ability of a patient's urine to solubilize or precipitate cystine [5]. It is a 'solid-phase assay' in which a prefixed amount of cystine crystals are added to a patient's urine. After incubation for 48 h, the urine is spun and the supernatant removed so that the amount of solid cystine can be measured. In supersaturated urine, cystine precipitates onto the added crystals, so the solid phase that is recovered is greater than that which was added. Undersaturated urine can dissolve the added cystine crystals; such urine has a 'positive cystine capacity'. This test is now commercially available in the United States, performed by Litholink (Chicago, Illinois, USA).

In vitro studies have shown that the assay is able to accurately account for cystine whether in the presence or absence of CBTDs [5,6]. Increasing amounts of CBTDs were also shown to increase cystine capacity in *in vitro* studies [6]. In another study, the effect of CBTDs on cystine capacity in seven patients with cystinuria was determined. We compared the cystine capacity of the urine during periods on and off CBTDs. Six of seven patients had a significant increase, or improvement, in cystine capacity while on the drug [7]. These preliminary data suggest that cystine capacity may be a useful tool in monitoring response to therapy. A clinical trial is currently underway to evaluate how well this variable correlates with stone events. In this prospective study, patients with cystinuria perform semi-annual 24 h urine collections, while stone events are monitored through surveillance imaging studies every 6 months. The purpose is to determine how well cystine capacity predicts recurrent stone events in patients with cystinuria, with the hope it will help physicians guide therapy.

We also plan a dose–response study in which the effect of increasing doses of Cadets on urinary cystine capacity will be evaluated. Patients will perform 24-urine collections after taking D-penicillamine or tiopronin in doses ranging from 0 g per day up to 3 g per day. The overall goal will be to help guide therapy and ultimately minimize unnecessary side-effects caused by larger doses.

TREATMENT

Fluid therapy is one of the mainstays of treatment for all types of nephrolithiasis, including cystinuria. On the basis of urinary cystine levels, patients with cystinuria often have to drink 3–4 l per day of fluid to effectively decrease the concentration of urinary cystine below the level of saturation. There are many barriers to consistently achieving this fluid intake, including but not limited to, lack of thirst and lack of motivation. On the basis of these assumptions, it was proposed that using an antidiuretic hormone (ADH) antagonist would increase urine flow rates in patients with cystinuria who were refractory to standard therapy [8]. Two patients with cystinuria were treated with tolvaptan 15 mg daily for 5 days. Both patients had a significant increase in daily urine volume and a resultant decrease in urinary cystine concentration while taking the drug, whereas plasma osmolality was only minimally

increased. The authors propose that ADH antagonists are a potential new therapy for the prevention of cystinuria, and suggest that future studies be pursued. We disagree with their conclusions for several reasons. For one, it does not seem reasonable from a cost–benefit analysis. Tolvaptan, the only oral ADH inhibitor available in the United States today, costs approximately $200 per day. Can we justify spending $200 a day to increase urine output when the same can be accomplished by drinking several bottles of tap water? One could argue that the cost of the drug will likely decrease with time, but there are also other reasons to question the benefit of this drug in this setting. For one, the barriers to drinking fluid are often more complex than just a lack of thirst or motivation. Often, patients cannot sustain fluid intake because of occupational barriers. For example, teachers are limited in how much they can drink throughout the day because they cannot leave the classroom to go to the bathroom very often. Using an ADH antagonist would likely only cause increased thirst and potentially hyperosmolarity if they do not drink to match their urine output. The patients in the study were not reported to be on standard therapy for cystinuria, and may not actually be refractory to treatment. Finally, in studies of polycystic kidney disease, the drug was shown to cause significant liver function test abnormalities in some participants. Overall, ADH antagonists do not appear to be a reasonable alternative therapy at this time.

A recent study examined the effect of urinary pH on the efficacy of CBTDs *in vitro* [9▪]. Although alkali therapy is known to increase the solubility of cystine and is often prescribed in the absence of CBTDs, little was known about the interaction of alkali therapy with CBTDs prior to this study. CBTDs (both D-penicillamine and tiopronin) were added to samples of urine which contained an excess of solid phase cystine at varying pH values, ranging from 6.0 to 8.0. In addition to showing that the presence of CBTDs increased cystine solubility at all levels of pH, a significant difference in the time course of the effect of the drugs at the varying pH values was found. At the highest urine pH (8.0), the increase in cystine solubility was seen as early as 5 min of incubation, whereas at the lowest pH (6.0), the increase in solubility did not occur until after 60 min of incubation time. The conclusion was that the ambient pH has a significant effect on the rate of action of the drug, which could be explained by the fact that more of the drug is in a deprotonated, active form at higher pH. Although with time the drugs did reach maximum effect at the lower pH values, the clinical significance of the rate of action may be important when considering that urine only stays in the renal pelvis for some minutes, at high urine-flow rates. Further studies in human patients are necessary to elucidate the importance of this phenomenon *in vivo*.

A new alternative approach to prevention of recurrent nephrolithiasis is based on crystal growth inhibition. Ward’s group at New York University are using atomic force microscopy (AFM) to visualize the early stages of crystal formation in liquids, including the nucleation events and topographic features that play a role in crystal formation [10]. Through this technique, they can also quantify the velocity of growth of a crystal, which allows them to study the effects of varying conditions, such as different concentrations of an inhibitor, on crystal formation. Through studies of the structure and crystal formation of L-cystine *in vitro*, they were able identify potential inhibitors of growth. Examples of promising molecules tested to date are L-cystine dimethyl ester (L-CDME) and L-cystine methyl ester

(L-CME). These compounds are structural mimics of cystine in which either one carboxyl group (in the case of L-CME) or two (in L-CDME) are replaced by esterified methyl groups. When added to L-cystine *in vitro*, these molecules interfered with the formation of crystals, as seen by a roughening of the edges of the otherwise smooth hexagonal crystal structures that form in their absence. The velocity of crystal formation was also significantly decreased by these inhibitors, with an increasing effect as the concentration of inhibitor increased. The mechanism of action is thought to be steric hindrance by the drugs of additional cystine molecules being added to existing crystals.

One potential limitation of the molecules for therapeutic use is the potential for toxicity. Incubation of LLC-PK1 cells, a model for proximal tubular function, with CDME leads to the accumulation of cystine intracellularly, specifically within lysosomes [11]. Lysosomal accumulation of cystine also occurs in the autosomal recessive disease cystinosis, as a result of abnormal cystine transport. Clinically, this manifests as Fanconi syndrome and even kidney failure. The dose of the inhibitor would therefore have to be effective at doses lower than those which cause this adverse effect. The *in vitro* studies with CDME suggest that it is in fact potent enough to inhibit urinary cystine crystallization without being associated with intracellular lysosomal accumulation. In addition, Ward's group is continuing to search for other molecules that might be more effective in inhibiting cystine crystal formation or have lesser potential toxicity.

Efficacy of these compounds is being tested in animal models of cystinuria. Both *SLC3A1* and *SLC7A9* knockout mice have been developed and could be used to study potential new drugs [12–14]. Interestingly, in the *SCL3A1* knockout, only the male mice develop stones, whereas both male and female mice with the *SLC7A9* mutation develop stones. The explanation for the difference remains to be elucidated. Recently, the effect of L-CDME was determined in *SLC3A1* knockout mice [15]. CDME or water was administered to mice. The size and weight of the stones in the CDME group was significantly smaller than in the control group (0.5–3.0 mm vs. 0.5 mm–9.0 mm, and 29.2±14.8mg vs. 55.2±28.0 mg, respectively). However, there was a significant increase in the number of stones (total 181 stones vs. total of 49 stones). There was no difference in urinary cystine excretion between the CDME group and the controls, suggesting that urine was saturated with cystine in both groups. These studies suggest that CDME is as effective at inhibiting the growth of cystine crystals *in vivo* as well as *in vitro*. Further studies regarding toxicity and safety are in progress and will have to precede studies in humans. However, this holds promise as a potential new therapeutic target for cystinuria.

The Rare Kidney Stone Consortium is an international organization that focuses on research of and education for four rare inherited diseases that lead to kidney stone formation, including cystinuria (see www.rarekidneystones.org). The consortium also is studying primary hyperoxaluria, Dent disease, and dihydroxyadenine stones caused by adenine phosphoribosyltransferase (APRT) deficiency [16▪]. An international registry of people with cystinuria has been developed and enrolled more than 200 patients. Through this collaboration, which promotes the exchange of ideas and information about these rare causes of kidney stones, we hope to improve care and outcomes for patients with cystinuria and other rare kidney stone disorders.

CONCLUSION

With several recent advances in the monitoring and treatment of cystinuria, and the gathering of clinical patient data, there are now opportunities for new management protocols and therapies. Cystine capacity may be a better way to measure urinary cystine and saturation and guide therapy. New *in vitro* techniques for studying crystal growth have led to new inhibitors of crystallization. The development of a mouse model of cystinuria allows for these new crystallization inhibitors to be tested before they are applied to clinical practice. An international registry aims to collect long-term follow-up data on patients and provide new insights into prognosis and clinical care.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 496).

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KEY POINTS

- Cystine capacity may be a better way to measure urinary cystine and saturation and guide therapy.
- New *in vitro* techniques for studying crystal growth have led to new inhibitors of crystallization.
- The development of a mouse model of cystinuria allows for these new crystallization inhibitors to be tested before they are applied to clinical practice.
- An international registry aims to collect long-term follow-up data on patients and provide new insights into prognosis and clinical care.