Urinary cystine excretion and capacity in patients with cystinuria

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The treatment of cystinuria is hampered by methods used to measure urinary lithogenicity. Most cystine assays cannot reliably distinguish cystine from soluble thiol drug–cysteine complexes. We used a solid-phase assay of urinary cystine capacity in a large sample of patients with cystinuria. A known amount of solid-phase cystine is added to urine. In supersaturated urine, cystine precipitates onto added crystals, so the solid phase recovered after incubation will be greater than that added. We studied the effect of cystine-binding thiol drugs (CBTD) to solubilize cystine and determined correlates of cystine capacity in patients who were and were not taking CBTD. Increasing concentrations of D-penicillamine, tiopronin and captopril dissolved cystine in urine with similar efficacy. A general linear model in which 24 h cystine excretion was the dependent variable showed that creatinine, urea nitrogen, and sodium excretions were associated with cystine excretion ($P<0.02$, all three). Urine volume, pH, and cystine excretion strongly correlated with cystine capacity ($P<0.001$). Tiopronin had no effect on supersaturation in a cross-sectional analysis. A subset of supersaturated samples, with negative cystine capacity, occurred mainly among women not taking CBTD. For this subset, capacity differed significantly between CBTD users and non-users; use of CBTD avoided extremes of supersaturation. Female enrichment in the supersaturated group was accounted for in part by underprescription of CBTD to women. This assay of cystine capacity was reliable in the presence of CBTD. It should be useful in monitoring patients' response to dietary interventions and administration of fluid, citrate, and CBTD.

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The treatment of cystinuria is currently hampered by the available methods used to measure the lithogenicity of urine from patients with cystinuria. One problem is that measurement of cystine excretion is complicated by artifactually low values when cystine solubility is poor. Cystine is least soluble at pH 5–7, a range frequently found in human urine. Adding acid to 24 h urine collections was used in the past to assure that all excreted cystine remained in solution. However, acidification during collection prevents measurement of urine pH, a critical factor in determining cystine solubility. This problem can be surmounted by alkalinization of the sample after the urine collection is completed and pH measured.[1]

Another problem is that many cystine assays do not reliably distinguish cystine from soluble thiol drug–cysteine complexes. Colorimetric reactions measure the amount of free sulfhydryl group. In the presence of thiol-containing drugs, this no longer remains an accurate estimate of cystine concentration; the drugs themselves may be detected by the colorimetric assay. High-performance liquid chromatography and other chromatographic techniques can distinguish thiol drug from cystine and cysteine but often the sample preparation leads to disruption of thiol drug–cysteine complex.[2,3] The result is that clinicians cannot estimate the extent to which the thiol drug has reduced the relevant cystine saturation. Even in patients not treated with thiols, supersaturation cannot be accurately predicted from measurement of pH and cystine concentration, as the effect of pH on cystine solubility varies among patients.[4,5] Finally, no studies demonstrate correlation of rates of cystine stone formation or growth with any measure of urine cystine excretion or concentration.

We sought to overcome these problems with an assay of cystine supersaturation (CSS). Such an assay would directly measure the ability of an individual patient's urine to solubilize or precipitate cystine, called cystine capacity. Measurements of total cystine are not critical, and the solubilizing effects of cystine-binding thiol drugs (CBTD) can be accurately measured. Previously, the efficacy of CBTD could be measured only with the passage of time and estimates of stone growth or dissolution. We report our initial measurements of cystine capacity in patients with cystinuria and the effect of CBTD on urinary cystine capacity.

RESULTS

Performance of solid-phase assay in urine

We demonstrated the reliability of the solid-phase assay by studying its ability to account for all dissolved cystine from urine (Figure 1). Cystine crystals were added to urine of patients with cystinuria taking CBTD ($n=10$) and not taking CBTD ($n=12$), and stirred for 48 h at 37°C. When greater amounts of cystine were added to urine, dissolved cystine in the supernatant reached a plateau at 1.4 mM. As more solid-phase cystine was added and not dissolved, the amount of cystine remaining in the solid-phase rose proportionately. The sum of the supernatant and solid phases at each point rose appropriately, indicating that cystine recovery was complete and reliable.

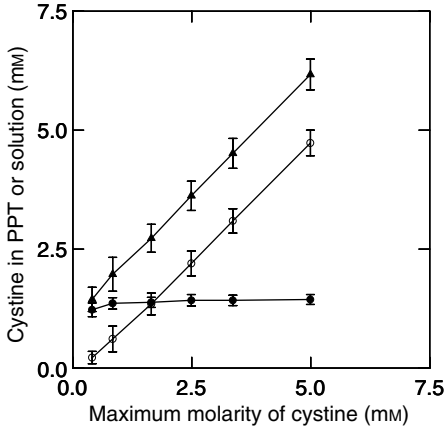

Figure 1 | Performance of solid-phase assay. Cystine crystals were added to urine from patients taking CBTD ($n=10$) and not taking CBTD ($n=12$) and stirred for 48 h at 37°C. As the result did not vary whether patients were taking or not taking CBTD, the two groups were combined. Filled circles indicate that when greater amounts of cystine were added to urine, dissolved cystine in the supernatant reached a plateau at 1.4 mM, the point of saturation. (Error bars = ±2 s.e.m.). Open circles indicate that the amount of cystine remaining in the solid phase (precipitate, PPT) rose proportionately as cystine dissolution plateaued at saturation. Triangles represent the sum of the supernatant and solid phases and indicate that cystine recovery was complete and reliable.

Effects of CBTD on cystine capacity

Figure 2 shows the ability of increasing concentrations of D-penicillamine, tiopronin, and captopril to dissolve solid-phase cystine in urine. The slopes of all three lines are similar, indicating that all three have similar efficacy in causing dissolution of preformed cystine crystals. From the slope, we estimate that CBTD are able to bind cysteine (each cystine yields two cysteine) at a molar drug:cysteine ratio of 1.25–1.0 in these *in vitro* conditions. In clinical use, captopril levels in urine do not approach those of tiopronin and penicillamine, limiting its efficacy.

Determinants of cystine excretion

For this analysis, urine samples from subjects taking captopril or D-penicillamine were excluded, as these CBTD affect cystine measurement in the cystine assay used; tiopronin does not. Thus, 79 urine samples are included in this analysis. Using a general linear model in which 24 h urine cystine excretion was the dependent variable and all standard urine chemistries, gender, and tiopronin use were included, 24 h urine creatinine, urea nitrogen, and sodium excretions were independently associated with cystine excretion ($P<0.02$ for all three; r^2 for the complete model was 0.56). There was a modest effect of gender (adjusted least square excretions were 3.3 vs 3.8 mmol/day, male vs female, $P=0.038$). Use of tiopronin was without significant effect ($P=0.06$). The correlation of urine cystine excretion with urine creatinine (Figure 3, upper right panel) was strong, although there was a significant difference between men and women ($r^2=0.36$, $P<0.001$ for men; $r^2=0.52$, $P<0.001$ for women). The cystine excretion was lower for a given creatinine among men than women. Urine cystine excretion was correlated with urine sodium excretion ($r^2=0.38$, $P<0.001$; Figure 3, upper left panel) and urine cystine per gram creatinine was correlated with urine sodium per gram creatinine (Figure 3, upper middle panel; gender had significant effect, $P<0.01$; $r^2=0.15$, $P<0.01$ for men, $r^2=0.27$, $P<0.001$ for women). The correlation of urine urea nitrogen and cystine excretion was

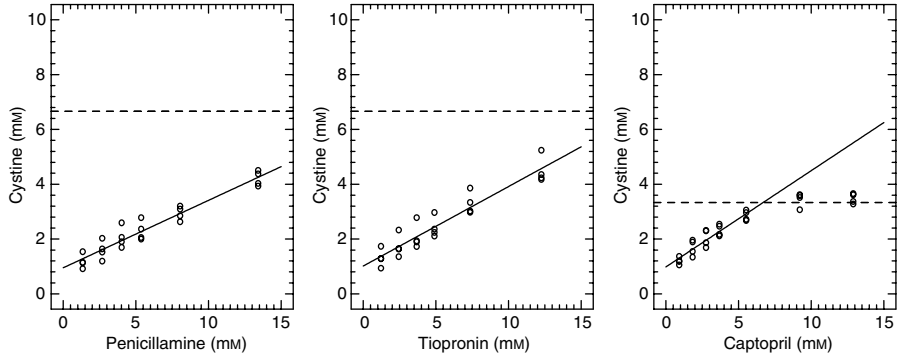

Figure 2 | Effects of CBTD on cystine capacity. Increasing concentrations of CBTD (D-penicillamine, tiopronin, and captopril) in urine dissolved more solid-phase cystine, shown on *y* axis. The slopes of all three lines are similar. The dotted lines represent the maximum amount of cystine added to each sample. Less cystine was added to study the effect of captopril than for D-penicillamine and tiopronin because captopril achieves lower urinary concentrations than the other two.

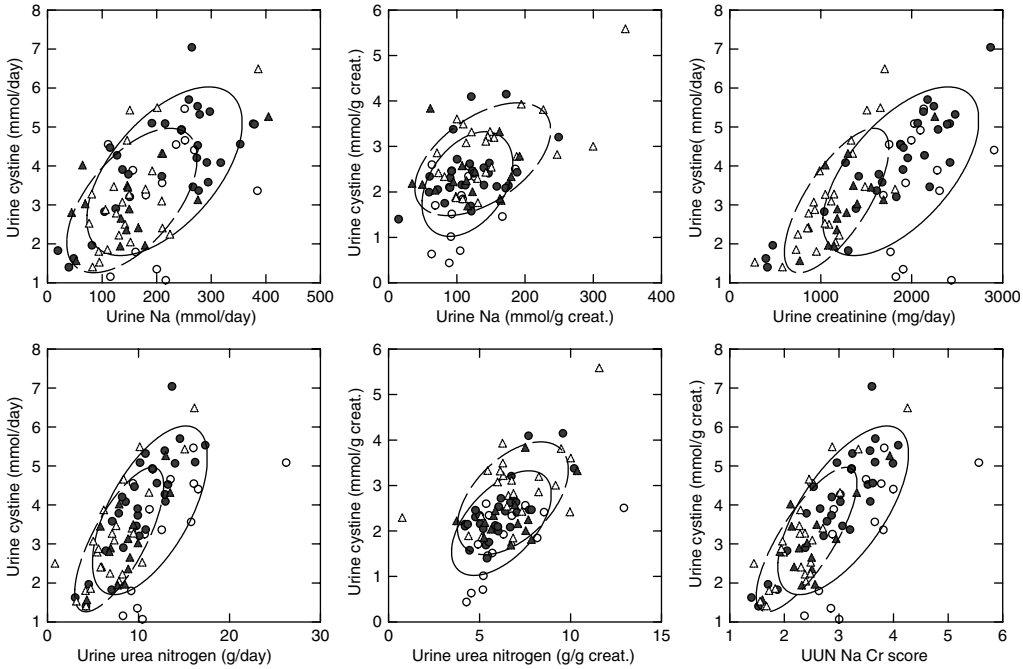

Figure 3 | Variables associated with cystine excretion. Increasing 24 h urine urea nitrogen (UUN), sodium and creatinine excretions were independently and strongly associated with increasing cystine excretion ($P<0.01$ for all). Circles: males; triangles: females; open symbols: no CBTD; closed symbols: CBTD. Ellipses enclose 1 standard deviation from the mean; solid line: males; broken line: females.

also strong (Figure 3, lower left panel; $r^2=0.49$, $P<0.001$; gender was not significant). When urine urea nitrogen was factored for urine creatinine, the correlation persisted although gender differences were significant (Figure 3, lower middle panel, gender effect $P<0.01$; $r^2=0.30$, $P<0.001$ for men, $r^2=0.25$, $P=0.001$ for women). Finally, a multivariable score created using pH, urine urea nitrogen, and creatinine correlated very strongly with cystine excretion (Figure 3, lower right panel, $r^2=0.54$, $P<0.001$). Calcium, citrate, and uric acid excretion were not independently associated with cystine excretion.

Cystine solubility

We used a general linear model in which 24 h urine cystine solubility was the dependent variable and all standard urine chemistries, initial cystine concentration, and use of tiopronin were included. The solubility of cystine as determined by the concentration of cystine in urine after incubation with an excess of solid phase was correlated with both urine pH and initial cystine concentration of the urine (Figure 4, upper panels; $r^2=0.14$, $P=0.002$ for urine pH and $r^2=0.18$, $P<0.001$ for initial cystine concentration). As cystine concentration is the result of 24 h cystine excretion and urine volume, both variables were tested: the urine cystine excretion had a modest correlation with solubility ($r^2=0.06$, $P=0.03$) whereas the urine volume did not correlate significantly with solubility ($r^2=0.03$, $P=0.1$). Use of tiopronin did not have a significant interaction in the general linear model and gender did not have an influence on cystine solubility.

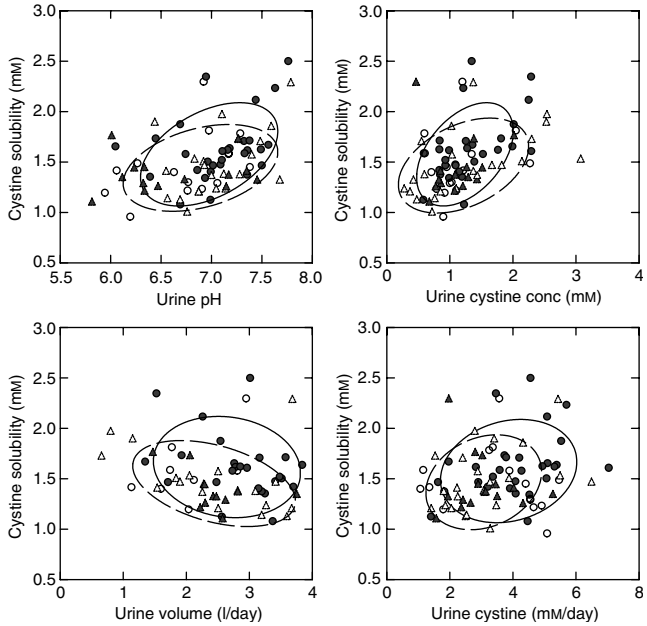

Figure 4 | Variables associated with cystine solubility. Cystine solubility was strongly correlated with both urine pH and initial cystine concentration of the urine. CBTD had no effect on cystine solubility. Circles: males; triangles: females; open symbols: no CBTD; closed symbols: CBTD. Ellipses enclose 1 standard deviation from the mean; solid line: males; broken line: females.

Cystine saturation

We used cystine uptake from an excess of solid phase (cystine capacity) to gauge urine saturation. Negative values for cystine capacity represent urine supersaturated with cystine;

positive values represent undersaturation. In a general linear model, we used solid-phase uptake as the dependent variable and included all standard urine chemistries, initial cystine concentration and use of CBTD and alkali. Urine samples collected from patients taking captopril and/or D-penicillamine are included, as the measurement of cystine capacity is not affected by these medications. Like solubility, saturation was controlled mainly by the initial urine cystine concentration and by urine pH (Figure 5, left hand panels, top and bottom; $r^2 = 0.48$ for cystine concentration and $r^2 = 0.19$ for urine pH, $P < 0.001$ for both). The effect of cystine concentration on cystine capacity was more striking for men than women, as evidenced by the more narrow ellipse of containment (Figure 5, top left panel). Of interest, virtually all urine samples with an initial cystine concentration below 1 mM were undersaturated. As cystine concentration is the result of 24 h cystine excretion and urine volume, we tested both variables (Figure 5, upper middle and right panels): both variables significantly correlated with cystine capacity ($r^2 = 0.07$, $P = 0.02$ for cystine excretion, $r^2 = 0.24$, $P < 0.001$ for urine volume). A score using urine volume, cystine excretion, and urine pH strongly correlated with cystine capacity, (Figure 5, lower middle panel, $r^2 = 0.5$, $P < 0.001$) and cystine solubility accounted for only a small amount of variation in solubility ($P = 0.01$, $r^2 = 0.09$).

Effect of CBTD on cystine capacity

Use of tiopronin did not have a significant interaction in the general linear model for determinants of cystine solubility (Figure 4). Tiopronin also had no apparent effect on mean values for CSS, and was clearly of little consequence in either gender (Figure 5). Mean values of cystine capacity were 0.29 ± 0.1 mM in CBTD users and 0.11 ± 0.1 mM in non-users ($P =$ nonsignificant (NS)). Although means for CBTD users did not differ from those of non-CBTD users, a group of supersaturated urine samples, with negative cystine capacity, mainly among women not taking CBTD, can be seen in Figure 5 (upper left panel, open symbols). For this subset of 29 urine samples, values for cystine capacity differed significantly between CBTD users and non-users (-0.56 vs -0.27 mM uptake, no CBTD vs CBTD, $P = 0.019$). There were more women than men among patients with supersaturated urine. We attribute this female preponderance to a general underprescription of CBTD to women (24 of 37 men vs 12 of 32 women were given CBTD, $\chi^2 = 5.15$, $P = 0.023$). This disparity in prescribing patterns differs markedly from the more uniform prescription of alkali (23 of 37 men vs 23 of 32 women were given alkali, $\chi^2 = 0.73$, $P =$ NS).

DISCUSSION

The management of cystinuria consists of increasing fluid intake, alkalinizing urine, and using CBTD. Judging the

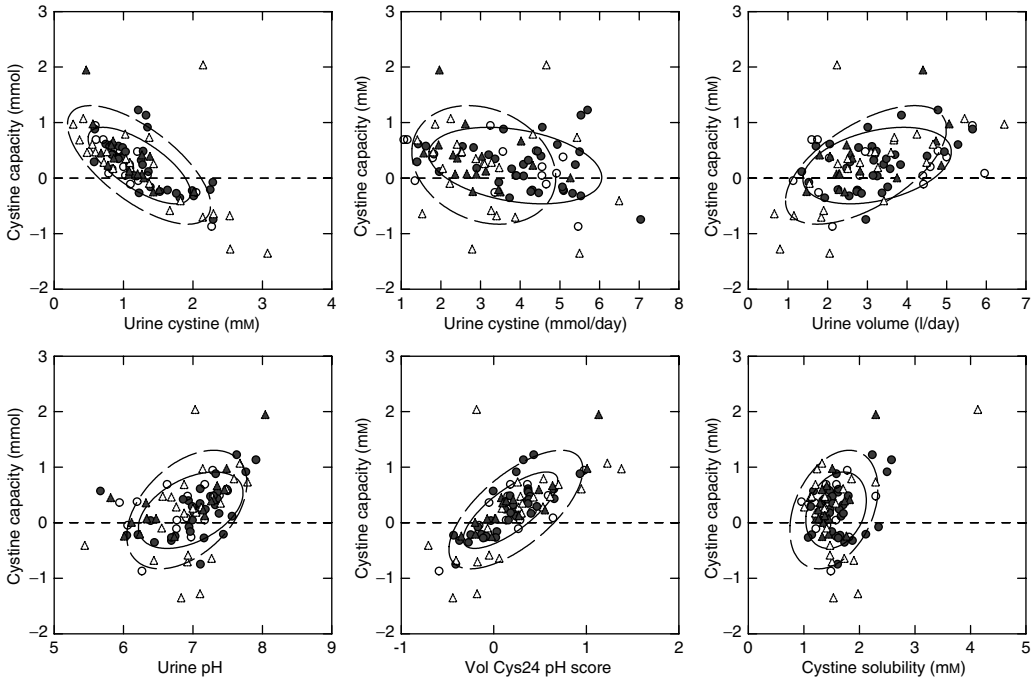

Figure 5 | Effect of CBTD on cystine capacity. Cystine capacity was strongly correlated with the initial cystine concentration of the urine and with urine pH ($P < 0.001$ for both). Increasing cystine concentration was associated with more negative cystine capacity, in both CBTD users and non-users. The more supersaturated women not taking CBTD are seen as the tail of open triangles outside the ellipses. Daily cystine excretion was not correlated and urine volume and cystine solubility were modestly correlated with cystine capacity ($P < 0.05$). A model incorporating urine volume, cystine excretion and urine pH shows an excellent correlation with cystine capacity ($P < 0.001$). Circles: males; triangles: females; open symbols: no CBTD; closed symbols: CBTD. Ellipses enclose 1 standard deviation from the mean; solid line: males; broken line: females. Dashed horizontal line represents cystine saturation, with values above the line representing undersaturated urine and values below the line representing supersaturated urine.

success of medical therapy based on changes in urinary variables such as cystine excretion and pH has not been entirely satisfactory however.[6] Because of substantial inter-patient variability, cystine solubility in individual urine samples is not reliably calculated from nomograms employing cystine concentration and urinary pH data alone.[4,5] Further complicating management, measurements of cystine excretion cannot be interpreted in a straightforward manner. First, cystine excretion and supersaturation may be underestimated in samples due to cystine precipitation.[5] Second, measurement of cystine is complicated by the presence of CBTD. Many cystine assays use preparation steps that break thiol–cysteine bonds, leading to release of free cysteine, which recombines with itself to form the insoluble dimer cystine. CBTD themselves, active because they contain the active thiol group similar to that of cysteine, variably interfere with the measurement of cystine as well, so that the drugs and amino acids are not entirely or reliably distinguished.[1] The result is a lack of useful values to correctly titrate CBTD doses, minimize their frequent drug-induced side effects, and judge success of therapy. Lacking a reliable predictor of response to therapy, only the passage of time with serial assessment of stone formation can realistically assess adequacy of treatment.

Our solid-phase assay of urinary cystine, which leads to direct measures of urinary CSS and cystine capacity, is reliable in the presence of CBTD.[1] We demonstrated its utility in directly measuring supersaturation in urine of patients with cystinuria.[5] The current report applies this assay to a large cohort of patients including those taking and not taking CBTD. The solid-phase assay was successful in accounting for added cystine whether in the presence or absence of CBTD (Figure 1). The assay also demonstrated that increasing concentrations of D-penicillamine, tiopronin, and captopril caused expected increases in *in vitro* cystine capacity. Given these operating characteristics, we believe that the assay will be of clinical utility in measuring urinary cystine capacity in patients taking CBTD. The assay could help find an optimally efficacious dose of CBTD. A satisfactory increase in cystine capacity could allow physicians to prescribe a minimal dose and limit side effects of the medications.

The ability of restriction of dietary animal protein and salt intake to lower urinary cystine excretion and diminish stone-forming activity has been debated. Our data demonstrate strong relationships of urinary sodium and urea excretion to cystine excretion (Figure 3). That salt restriction reduces cystine excretion has been demonstrated in relatively small studies in both adults and children.[7,8] These studies reported acute effects (after several weeks) of changes in sodium intake to alter cystine excretion. Our data confirm this finding in a larger sample and in a cross-sectional analysis. How salt excretion affects cystine excretion is not known. Both sodium chloride and sodium bicarbonate administration cause increased urinary cystine excretion.[9] The cystine transport process affected by the disease-causing mutations in SLC3A1 (rBAT) and SLC7A9 ($b^{0,+}AT$) is sodium-independent.[10] The effect of increasing sodium intake to increase cystine excretion could occur due to an unexplained influence of sodium transport on cystine transport in the proximal tubule or via more indirect effects of sodium intake on angiotensin II or aldosterone metabolism if levels of these substances are relevant to renal cystine handling. Also not known is whether increases or decreases in salt intake mediate changes in net cystine balance or cystine metabolism. *In vitro* studies suggest that the deleterious effect of increasing sodium intake to increase cystine excretion could be offset by a favorable effect of ionic concentration on cystine solubility in urine.[4] However, in our data, sodium excretion was not an independent variable determining cystine solubility in either direction. No prospective studies have demonstrated changes in stone activity as the result of sodium-restricted diets.

Protein restriction has been prescribed for patients with cystinuria based on the premise that reduced ingestion of methionine, cystine's dietary precursor (and cystine itself), will lower cystine excretion. One study demonstrated that diminished intake of cystine's dietary precursor methionine reduces urinary cystine excretion.[11] However, the ability of such diets to cause clinically meaningful effects and of patients to adhere to such regimens has been questioned. We show a tangible relationship between increased urinary urea excretion, a marker of protein ingestion, and cystine excretion (Figure 3). Creatinine excretion also correlated with cystine excretion. This could result from people with larger muscle mass ingesting more methionine-containing animal protein and excreting more cystine. Alternatively, it implies that a significant proportion of urinary cystine derives from endogenous muscle turnover.

We cannot explain the correlation between cystine solubility and cystine concentration of urine. As the range of cystine solubility in human urine is large and cannot be fully accounted for by urine pH, there must be other factors that control solubility. Further work is needed to identify them. As expected, cystine capacity independently correlated with cystine concentration of urine as well as its pH. In contrast, an unanticipated finding was the lack of an independent effect of CBTD on values of cystine capacity for the entire study population. Many papers have reported successful treatment of cystinuria with CBTD, both tiopronin[12,13] and D-penicillamine,[14] and one would expect that this success would require a reduction in CSS. Most of the patients studied here were taking tiopronin and lack studies off and on CBTD; we cannot be certain if the drugs effectively lowered CSS levels in most patients. The mean values of cystine capacity for patients taking and not taking CBTD were indistinguishable. However, extremes of supersaturation occurred almost exclusively in patients not taking CBTD (Figure 5, left panel). The most negative cystine capacity values occurred in seven women and one man not taking CBTD and one man taking CBTD, constituting the best evidence in this study for a benefit of CBTD. Whether the higher proportion of women in this subgroup with high supersaturation values occurred because women were less

willing to take CBTD, or because physicians prescribed the drugs less frequently to women cannot be determined. We previously demonstrated the beneficial effect of CBTD on urinary cystine capacity in seven patients who did 24 h urine collections while on and off CBTD.[15] Six of seven patients had an increase in cystine capacity on CBTD compared to off drug.

A limitation of our data is that we do not know the dose of CBTD patients were taking. The lack of an effect on mean values of saturation could result from inadequate doses or poor compliance. On the other hand, as the physicians referring the patients were urologists and nephrologists (including ourselves) with active stone clinics and relatively sizeable populations of patients with cystinuria, we suspect that CBTD were prescribed in appropriate dosages. Our observations regarding the effects of CBTD are also influenced by the possibility that the patients who are prescribed CBTD are the most active stone-forming patients and have higher initial supersaturations than those not prescribed drugs, and once treated have cystine capacity values similar to those not treated with CBTD.

It may be that not all patients achieve benefit in reducing cystine capacity or supersaturation from CBTD although the reasons for such differences are currently not known. In our previous study of cystine capacity on and off CBTD, patients with the most saturated urine (negative capacity values) had greater effects than patients with relatively less saturated urine (more positive capacity).[15] That study's paired design has greater efficacy in demonstrating the potential benefit of CBTD as compared to the current cross-sectional comparison. Our assay may therefore be put to best use in determining responses of individual patients to therapeutic maneuvers such as prescription of citrate, CBTD and fluid and dietary manipulations.

The stone-preventing efficacy of CBTD has not been demonstrated in a randomized controlled trial, although uncontrolled reports of efficacy have been published since the 1960s for D-penicillamine and since the 1980s for tiopronin. The relative advantage of CBTD as compared with urinary alkalinization and/or fluid intake has not been directly established. Only anecdotal reports using small numbers of historical controls have demonstrated the drugs' impact.[14] There is certainly a need for controlled clinical trials of available medical therapies. The relative roles of the various determinants of cystine capacity need to be explored in such studies, so that measurements of urinary cystine capacity could be correlated with clinical benefit. It would then be possible to select patients with greater predicted responses from CBTD and for the response to therapy to be gauged in a more reproducible method than what is currently available.

MATERIALS AND METHODS

We measured urinary cystine using a nitroprusside colorimetric assay and cystine solubility and capacity using a solid-phase assay. Cystine excretion is the amount of cystine excreted in a timed collection, expressed as millimoles per day or as millimoles per gram of urinary creatinine. Urine was incubated with cystine crystals, the 'solid phase', for 48 h. Cystine solubility is the final concentration of dissolved cystine at the end of the incubation period, when cystine in the solid phase and in urine are at equilibrium, in millimoles per liter. At equilibrium, urine is saturated with cystine: higher concentrations represent supersaturation, lower concentrations, undersaturation. Cystine capacity is measured using 25 ml samples and expressed as the change in size of the solid phase, in millimoles. The uptake of solid-phase cystine by urine occurs in the case of undersaturated urine and is expressed as positive cystine capacity; the giving up of cystine to solid phase occurs in the case of supersaturated urine and is expressed as negative cystine capacity.

Cystine assay

The assay was identical to that reported previously.[1,5,16] A 100 μl portion of urine was diluted with 400 μl H_2O and 1 ml phosphate-buffered saline buffer, pH 7.4 (Sigma-Aldrich Co., St Louis, MO, USA). A 300 μl portion of a 10% sodium cyanide solution was added and the mixture incubated at room temperature for 20 min. Addition of 100 μl of a 20% sodium nitroprusside solution initiates a colorimetric reaction linearly related to the concentration of cystine present. Absorbance was measured within 20 s of the nitroprusside addition at 521 nm using a Beckman DU 650 spectrophotometer and concentration calculated from a standard curve run with each assay. The intra-assay coefficient of variation is 2.9% and the inter-assay coefficient of variation 2.6% in this laboratory.

Cystine capacity assay

To 25 ml of urine, maintained at original pH, a known amount of solid-phase cystine (Sigma-Aldrich Co.) was added and incubated for 48 h at 37°C with constant stirring. Solid phase was harvested by centrifugation at 3800 r.p.m. for 20 min at room temperature. The supernatant was removed and the remaining pellet dissolved in 25 ml of high-pH buffer (0.1 M sodium carbonate, pH 9.9). Cystine concentration was determined in the supernatant and high-pH buffer as described above. The sum of cystine in the supernatant and in the residual solid phase should equal the sum of the amount of cystine added and the amount of cystine in the original urine. The assay was performed using urine from patients who were and were not using CBTD. Undersaturated urine will dissolve some solid-phase cystine, so the amount of solid-phase cystine recovered after 48 h of incubation will be lower than that originally added. In supersaturated urine, cystine precipitates from solution onto added cystine crystals; the amount of solid phase recovered after incubation will be greater than that originally added.

Effects of CBTD on cystine capacity

To determine the effects of CBTD on the cystine capacity assay, we added increasing amounts of D-penicillamine, tiopronin or captopril to 25 ml of urine. Four patients with cystinuria not taking CBTD participated, and the mean of the results of four experiments was taken. A fixed amount of cystine was added: for tiopronin and D-penicillamine, 40 mg, to achieve a concentration of 6.67 mM; for captopril, 20 mg, to achieve a concentration of 3.33 mM. The lower amount of solid-phase cystine in the captopril assay reflects the lower urine concentration attainable clinically with this drug. Tiopronin and D-penicillamine are usually prescribed in doses of 5–15 mmol/day compared to captopril at 0.5–1 mmol/day. Incubation was carried out as in the preceding section. After 48 h, pellet and supernatant were separated and the pellet processed in high-pH

buffer as above. Cystine concentration was determined in the recipient high-pH buffer to calculate the amount of cystine dissolved in urine.

Characterization of urine of patients with cystinuria

We analyzed a total of 91 24 h urine collections from 69 stone-forming patients (37 male and 32 female patients) with cystinuria (defined as excretion greater than 250 mg of cystine per 24 h). Patients were taking medications and following diets as prescribed by their physician. Forty-four urine samples were collected while patients were taking tiopronin, four of whom were also taking captopril. Four urine samples were collected while patients were taking D-penicillamine and four were collected while patients were taking only captopril. Thirty-three urine samples were collected from patients not receiving alkali therapy and 58 urine samples were collected while patients were taking alkali. Of the latter, 35 were taking concomitant CBTD. Sixteen samples were collected from patients taking neither CBTD nor alkali. Seven of the 69 patients were children and they contributed 10 urine collections.

Urine was collected from patients for 24 h at home. Urine samples were not refrigerated. An antibacterial preservative and a volume marker were added to the collection container at the beginning of the collection. Patients removed a 50 ml aliquot and alkalinized the rest of the collection with Na_2CO_3 to bring any precipitated cystine into solution.[5] They removed a 50 ml aliquot from the alkalinized collection, and sent the aliquots of the original urine and the alkalinized sample to the laboratory by overnight delivery. Cystine concentration was measured on both samples. The higher value was used to calculate CSS. CSS is the total concentration of cystine in urine divided by the concentration of cystine at saturation after incubation with solid-phase cystine. Cystine capacity is defined as the change in the amount of cystine dissolved after addition of solid-phase cystine to an alkalinized sample in mmol/l: a positive number indicates an undersaturated sample that took up cystine from solid phase; a negative number indicates a supersaturated solution that precipitates cystine onto solid phase.

Urine samples were also analyzed by standard analytic techniques for concentration of sodium, potassium, citrate, chloride, urea nitrogen, creatinine, pH, calcium, oxalate and phosphate.[17] Clinical data included total number of stones, stones formed in the last 2 years and drugs taken at the time of the urine collections, including CBTD and citrate supplementation. Data regarding stone activity were not of sufficient quality to permit an analysis correlating them with urinary cystine measurements. Data for body weight were not available.

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