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ORIGINAL PAPER

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# Cystine analyses of separate day and night urine as a basis for the management of patients with homozygous cystinuria

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**Abstract** Based on previous observations of the diurnal variation of urinary cystine excretion, the use of separate day and night urine collections was proposed. To improve the medical treatment of patients with cystinuria, this strategy was performed to guide the fluid intake and the administration of SH compounds (tiopronin, D-penicillamine,and MESNA).Twenty-six patients (19 treated with SH compounds and seven with alkalinization and hydration only) were followed during two 3.5-year periods. During *Period 1,* 24-h urine was collected and during *Period 2,* separate day and night urine was collected.There were 56 episodes of high urinary cystine supersaturation (>1,200 μmol/l) during *Period 2*, 47% of which would have evaded detection with 24-h urine analysis. In comparison with *Period 1,* the urinary cystine concentration was lower ($P < 0.05$), and the urinary volume was higher ($P < 0.05$) during *Period 2*. Patients treated with tiopronin had reduced cystine excretion ($P < 0.05$) and at the end of *Period 2,* an increased dose of tiopronin, reflecting a more aggressive treatment. Furthermore, a reduced number of stone episodes and need of active stone removal ($P < 0.05$) was noted in the whole group of patients. Analyses of separate day and night urine samples can be used advantageously to reveal episodes of high supersaturation with cystine not detected in 24-h urine samples. Such a procedure might be useful for optimizing the treatment of patients with cystinuria.

**Keywords** Cystinuria · Cystine · Diurnal variation · Tiopronin · D-penicillamine

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## Introduction

Homozygous cystinuria is an inherited disorder affecting the cellular transport of cystine and the dibasic amino acids. Because of low solubility of cystine in urine, the increased excretion results in stone formation, first described by Wollaston in 1810 [38]. One of the first Swedish reviews of the natural history of cystinuria was presented by Morner in 1932 [31], and Bostrom and Hambreaus extended this study in 1964 [2]. Treatment is designed to avoid supersaturation by reducing the concentration of free cystine in urine and by increasing the urinary solubility of cystine. This goal can be achieved by an increased fluid intake and alkalinization of the urine as shown by Dent and Senior in 1955 [12]. Their 10-year follow-up, presented in 1965, showed that, in many patients, this basic treatment did not satisfactorily reduce cystine stone formation [13]. In 1963, Crawhall et al. described the use of D-penicillamine to reduce the amount of free urine cystine [7]. The use of D-penicillamine, however, has been limited by the high frequency of side effects [8, 17, 20]. Later tiopronin (2-mercapto-

propionylglycine) was introduced as a preferred and effective alternative to D-penicillamine because of its less pronounced side effects [10, 19, 23, 24, 25, 26, 30, 33, 35]. MESNA (mercaptoethane sulfonate) has also been used and is comparable to tiopronin [14], but proteinuria seems to be a very common side effect [9]. All of these SH substances (tiopronin, D-penicillamine, and MESNA) bind to cystine to form a more soluble disulfide compound.

When we evaluated the long-term treatment with tiopronin in 1995, we observed that stone formation was arrested in 2/3 of the patients. In the remaining patients, recurrent urinary stones appeared despite a 24-h urinary cystine concentration below 1,200 μmol/l and a urine pH above 7 [27]. Studies on the diurnal variation in urinary cystine excretion revealed transient periods of high supersaturation not detected in 24-h urine samples [28]. From results of a preliminary study, it was proposed that separate analyses of cystine in day and night urine samples would increase the chance of detecting episodes of urinary cystine supersaturation [11]. The treatment should subsequently be adjusted according to these observations. In this paper, we report the results of such a therapeutic strategy compared with the standard procedure based on 24-h analysis. To our knowledge, no long-term follow-up study has been presented in which the treatment of homozygous, stone-forming, cystinuric patients has been based on analyses of divided day and night urine samples.

## Patients and methods

### Patients

Twenty-six patients, 14 men and 12 women, age 28–72 years (mean 48 years) with homozygous cystinuria were included in this study. The age at clinical presentation of the disease ranged from 12–44 years (mean 23 years) and the duration of the disease ranged from 5–52 years with a mean of 25 years. The diagnosis of homozygous cystinuria was confirmed by an increased urinary excretion of cystine and the dibasic amino acids arginine, lysine and ornithine. All of the patients had a history of cystine stone formation. Twenty-one of the patients had had stone surgery, and nine had undergone extracorporeal shockwave lithotripsy (ESWL) since the clinical onset of the disease. Three patients were unilaterally nephrectomized because of stone disease, and one patient had unilateral renal aplasia. The individual glomerular filtration rates are shown in Table 1. Seven patients had a moderately impaired renal function [15], but none of the patients had a glomerular filtration rate (GFR) below 40 ml/min. The group consisted of patients with cystinuria managed at the Department of Urology, Linköping University Hospital and the Department of Nephrology and Transplantation, Malmö University Hospital during the period studied. The usual interval between visits at the outpatient clinic was 6 months.

Basic treatment consisted of hydration (aiming for more than 2 l during the day and at least 1 l during the night) and alkalinization of the urine. Sodium bicarbonate (23.8–143 mmol daily) or potassium citrate (40–80 mmol daily) was used for urine alkalinization. The aim, thereby, was to attain a urinary pH of at least 7. Nineteen patients were treated with SH compounds during the study. In this group, 12 patients were treated with tiopronin (Thiola), five with D-penicillamine (Cuprimine), one with MESNA (Uromitexan). One patient entered the study while being treated with MESNA was successfully switched to treatment with tiopronin during the first period of the study because of proteinuria. The indication for starting treatment with SH compounds was the demonstration of 24-h urinary cystine concentrations exceeding 1,200 μmol/l in spite of hydration or active cystine stone formation in spite of basic treatment. Seven patients were not treated with SH compounds because of adverse effects. The prescription of Thiola was licensed for each individual patient by the Swedish National Board of Health and Welfare.

One female patient was excluded because of poor compliance (not following tiopronin prescription). The compliance of the remaining patients treated with tiopronin and D-penicillamine was confirmed by urine analysis of the mixed disulfides tiopronin-cysteine and D-penicillamine-cysteine and by asking the patients. No measurement was made of the disulfide between cystine and MESNA. Patient characteristics in terms of sex, age, time since clinical onset, history of stone surgery and ESWL, known renal stones at start of the study, GFR, single kidney and type of SH-treatment are given in Table 1.

### Study design

Patients were followed for 3.5 years, during which time the treatment was designed based on the results of separate day and night urine samples (*Period 2*). Collection of night urine was started at bedtime and the daytime sampling in the morning. A comparison of the biochemical and clinical course was made to a previous 3.5 year period, with treatment based on 24-h urine analysis (*Period 1*). Mean values for each patient were used for comparison of the two periods.

High urinary cystine supersaturation was defined as a cystine concentration exceeding 1,200 μmol/l [12]. A renal stone episode was defined as an event of typical renal colic or passage of a stone. Active stone removal was carried out with ESWL or endoscopic stone extraction. None of the patients was treated with open surgery during the study period. The patients were followed annually with plain x-ray films of the urinary tract. The local ethics committee approved the study.

### Analytical methods

Free urinary cystine was measured at least every 6 months with ion-exchange chromatography at the Department of Clinical Chemistry, University Hospital, Malmö [10, 21]. The urine samples were frozen without preservatives within 24 h after delivery and transported to the laboratory as frozen samples. Prior to chromatography, an internal standard of aminoethylcysteine was added to the samples, and urinary proteins were precipitated with sulfosalicylic acid. Separation and detection of analyzed constituents were carried out with an amino acid analyzer (Biochrom 20, Pharmacia Amersham Biotech, Uppsala, Sweden). The ninhydrin complexes of amino acids were detected spectrophotometrically at 570 nm and their concentrations calculated by EZChrom integration software (Scientific Software Inc., USA). The mixed disulfides cystein-thiola and cystein-penicillamine were also separated in the same chromatogram.

### Statistical analysis

Since the number of patients was small and results of the variables were not clearly of normal distribution, non-parametric tests were used for group comparisons. The Wilcoxon signed-rank test was used in all cases except the comparison of renal stone episodes and active stone removal, where the Mantel-Haenszel test (adjusted for individual patients) was used because these observations were of a nominal character. A $P$ value $< 0.05$ was considered statistically significant.

**Table 1** Patient characteristics of 26 cystinuric patients with and without treatment with SH compounds. *GFR* glomerular filtration rate, *ESWL* extracorporeal shockwave lithotripsy, *SH-treatment* tiopronin, D-penicillamine and MESNA

| Patient no | Sex | Age (years) | Age at diagnosis (years) | Duration of clinical disease (years) | History of stone formation | History of stone surgery | History of ESWL | Known renal stones at study start | GFR[a] (ml/min/ 1.73m$^2$) | Single kidney | SH-treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 49 | 28 | 21 | Yes | Yes | Yes | No | **70** [d] | No | Tiopronin |
| 2 | M | 43 | 25 | 18 | Yes | Yes | No | No | 111 [f] | No | Tiopronin |
| 3 | F | 36 | 18 | 18 | Yes | Yes | No | Yes | **77** [e] | No | Tiopronin |
| 4 | M | 62 | 13 | 49 | Yes | Yes | No | No | **59** [d] | Yes b | Tiopronin |
| 5 | M | 52 | 18 | 34 | Yes | Yes | No | No | **59** [d] | Yes c | Tiopronin |
| 6 | F | 32 | 13 | 19 | Yes | Yes | Yes | Yes | 100 [d] | No | Tiopronin |
| 7 | M | 39 | 34 | 5 | Yes | No | Yes | No | 100 [f] | No | Tiopronin |
| 8 | M | 72 | 27 | 45 | Yes | Yes | No | Yes | **45** [e] | Yes b | Tiopronin |
| 9 | M | 69 | 32 | 37 | Yes | Yes | Yes | No | 79 [d] | No | Tiopronin |
| 10 | F | 37 | 18 | 19 | Yes | Yes | No | No | 101 [d] | No | Tiopronin |
| 11 | F | 58 | 34 | 24 | Yes | Yes | No | No | **45** [d] | No | Tiopronin |
| 12 | M | 71 | 19 | 52 | Yes | Yes | Yes | Yes | 64 [f] | No | Tiopronin |
| 13 | F | 31 | 18 | 13 | Yes | Yes | Yes | Yes | 137 [d] | No | MESNA/ Tiopronin [h] |
| 14 | F | 47 | 19 | 28 | Yes | No | No | Yes | 73 [d] | No | MESNA |
| 15 | F | 43 | 20 | 23 | Yes | Yes | No | Yes | **60** [d] | No | D-penicillamine |
| 16 | M | 71 | 25 | 46 | Yes | Yes | No | Yes | 68 [d] | No | D-penicillamine |
| 17 | M | 39 | 18 | 21 | Yes | No | No | No | 89 [f] | No | D-penicillamine |
| 18 | M | 71 | 44 | 27 | Yes | Yes | No | No | 73 [f] | No | D-penicillamine |
| 19 | F | 47 | 23 | 24 | Yes | Yes | Yes | Yes | 88 [f] | No | D-penicillamine |
| 20 | F | 35 | 12 | 23 | Yes | Yes | Yes | Yes | 93 [d] | Yes b | No |
| 21 | F | 38 | 15 | 23 | Yes | No | No | No | 110 [d] | No | No |
| 22 | M | 37 | 22 | 15 | Yes | Yes | No | Yes | 113 [d] | No | No |
| 23 | M | 56 | 14 | 42 | Yes | Yes | No | No | 115 [g] | No | No |
| 24 | M | 28 | 16 | 12 | Yes | Yes | Yes | Yes | 101 [d] | No | No |
| 25 | F | 44 | 36 | 8 | Yes | No | No | Yes | 75 [e] | No | No |
| 26 | M | 49 | 34 | 15 | Yes | Yes | No | No | 97 [d] | No | No |

[a] Values in bold type faces are below normal range for the method with respect to the patient's age
[b] Nephrectomy because of stone disease
[c] Unilateral renal aplasia
[d] $^{51}$CrEDTA-plasma clearance
[e] $^{99m}$Tc DTPA-plasma clearance
[f] $^{99m}$Tc DTPA-plasma clearance estimated from external gamma-camera measurements of $^{99}$Tc- MAG III [16]
[g] 24 h endogenous creatinine clearance
[h] Patient switched from MESNA to tiopronin during *Period 1*

## Results

As shown in Fig. 1, 56 episodes of urinary cystine supersaturation were recorded during *Period 2*. Twenty one (47%) had not been detected with 24-h samples. On 11 occasions, cystine concentrations were above 1,200 μmol/l in all measurements (day, night and 24-h urine samples). This explains the difference obtained when the total number of episodes of supersaturation in day and night collections (56 episodes) were compared with the total sum of missed and observed episodes of supersaturation in the 24-h samples (45 episodes). There was no difference in the distribution of supersaturation episodes between day and night urine.

In the patients treated with SH compounds, 23 supersaturation episodes were detected, 12 (52%) of which would have remained undetected with analysis of 24-h urine only. In the patients who did not recieve SH compounds, 33 episodes of supersaturation were recorded. Nine of these episodes (27%) had not been detected with a standard 24-h urine analysis. Individual data of urinary cystine concentration and excretion as well as urinary volumes and high cystine supersaturations are shown in Table 2.

As can be seen in Fig. 2 a, the median cystine concentration for all patients was 653 μmol/l (10th percentile 441 μmol/l; the 90th percentile 1,036 μmol/l) during *Period 2*. During *Period 1*, the median urinary cystine concentration was 812 μmol/l (10th percentile 441 μmol/l; 90th percentile 1,389 μmol/l). This difference was statistically significant ($P < 0.001$). When *Periods 1* and *2* were compared in patients treated with SH compounds (Fig. 2b) there was also a significant difference ($P < 0.05$). Median values were 741 μmol/l for *Period 1* and 623 μmol/l for *Period 2*. In patients not treated with SH compounds, there was a tendency of lower cystine concentration during *Period 2*, but this numerical difference was not statistically significant ($P = 0.063$).

The median urinary volume in the whole group of patients was 2,520 ml during *Period 2* (10th percentile 1,730 ml and 90th percentile 3,497 ml), a volume that was significantly higher than that recorded during *Period 1,* when the median was 2,142 ml (10th percentile

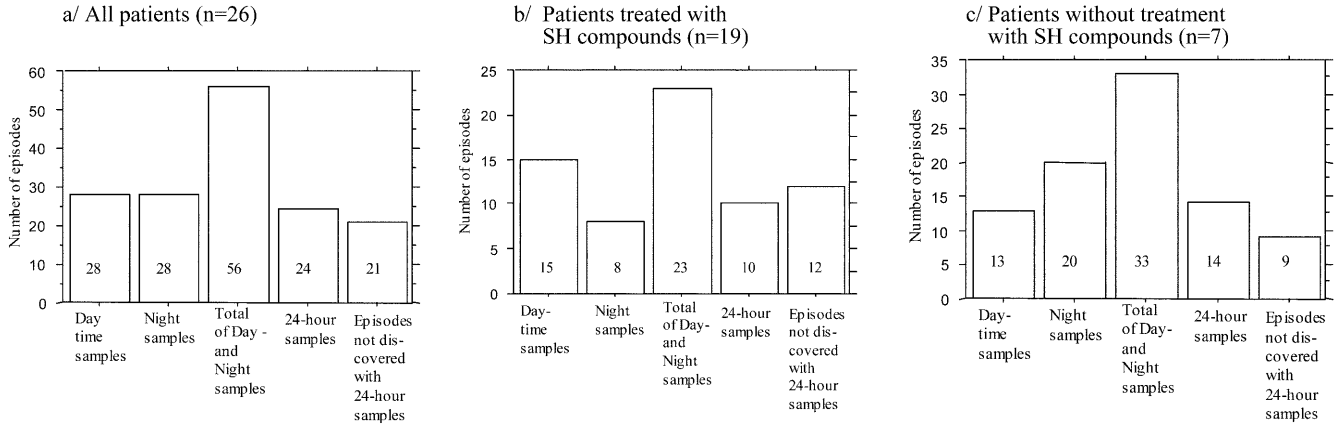



**Fig. 1a–c** Episodes of high urinary cystine supersaturation in 26 patients with and without treatment with SH compounds (tiopronin, D-penicillamine and MESNA)

1,366 ml and 90th percentile 3,265 ml); $P < 0.05$. There was also a significant increase in urinary volume in the patients treated with SH compounds as well as in patients on conservative treatment when comparing *Periods 2* and *1*; $P = 0.03$ and 0.02, respectively (Fig. 3 a–c, Table 3).

As shown in Fig. 4, the amount of free cystine excreted in urine by patients treated with SH compounds was significantly decreased when the results of day and night urine was used to guide the treatment ($P < 0.05$). The median urinary cystine excretion during *Period 1* was 1,431 μmol and during *Period 2* 1,257 μmol. When the tiopronin dose was compared between the two periods, more tiopronin had been administered at the end of *Period 2* than at with the end of *Period 1*. The median doses were 2,125 mg and 2,250 mg, respectively, $P < 0.05$ (Table 3, Fig. 5). No statistical difference was noted in patients treated with D-penicillamine ($n = 5$) and MESNA ($n = 1$). The patient (no. 13) who switched treatment from MESNA to tiopronin was not included in this comparison.

As expected, no significant difference in cystine excretion was observed between *Periods 1* and *2* in patients not treated with SH compounds.

The number of routine visits in which renal stone episodes were reported (Fig. 6) was significantly smaller during *Period 2* than during *Period 1* (30 in *Period 1* vs. 11 in *Period 2*; $P < 0.05$). The same was true for the need of active stone removal (11 in *Period 1* vs. 2 in *Period 2*; $P < 0.01$).

## Discussion

From the results obtained, the division of urine sampling into day and night portions undoubtedly revealed supersaturation episodes that would have evaded detection if only standard 24-h urine samples had been analyzed. Almost 47% of the supersaturation episodes in the whole group of patients and 52% in the patients treated with SH compounds would have escaped detection with 24-h analysis. To our surprise, there was no difference in the total number of supersaturation episodes between day and night samples. Nighttime is usually recognized as the main period of risk [28], but our result suggests that the daytime period also requires careful attention. It seems reasonable that the supersaturation episodes during the day were explained by a dietary load with methionine and cystine, which resulted in an increased excretion of cystine with high cystine concentrations in spite of the usually higher fluid intake during the day.

Cystine stone formation is precipitated by cystine from supersaturated urine, and the primary objective in stone-preventing therapy is to maintain the concentration of free cystine below this level of supersaturation. One of the critical factors in management of stone-forming cystinuria is, therefore, to monitor the level of free cystine in urine. The traditional way to monitor the cystine concentration has been to analyze 24-h urine samples. Several investigators have shown that urinary cystine concentrations are at the highest level during the night [1, 12, 34]. Our group showed that there were diurnal variations in urinary cystine saturation with the highest levels during the night (00.00–06.00 hours) and lowest during the afternoon (12.00–18.00 hours). The peak concentrations of cystine in these 6-h urine portions were about 90% higher than the corresponding 24-h urine levels [28].

The importance of increased control of diurnal variations in free, urinary cystine was emphasized by our finding that while stone formation in 2/3 of the patients was arrested with treatment guided by 24-h urine analysis, 1/3 of the patients continued to form stones in spite of urinary cystine concentrations below the risk level of 1,200 μmol/l. These results indicated that there was a risk of stone formation even when the average cystine concentration was as low as 500 μmol/l [27]. One explanation for this might to be that the periods with the highest urinary cystine concentration entail a risk of cystine crystallization, even though the 24-h urine cystine was below the risk level.

Thus, we obviously needed a more sensitive way of monitoring cystine concentrations than provided by the 24-h urine samples. Conducting 6-h urine samples in

**Table 2** Concentrations of free cystine, urine volumes and free cystine excretion during *Periods 1 and 2* and observed periods of high cystine supersaturation during *Period 2*

| Patient no | Urinary free cystine concentration (μmol/l) *Period 1* [a,b] | Urinary free cystine concentration (μmol/l) *Period 2* [a,b] | Urinary volumes (ml) *Period 1* [a,b] | Urinary volumes (ml) *Period 2* [a,b] | Urinary free cystine excretion (μmol/24 h) *Period 1* [a,b] | Urinary free cystine excretion (μmol/24 h) *Period 2* [a,b] | Super-saturation episodes day sample [c,d] | Super-saturation episodes night sample [c,d] | Super-saturation episodes 24-h sample [c,d] | Super-saturation episodes not detected in 24-h samples [c,d] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 741 (6) | 844 (7) | 2,190 (6) | 2,119 (7) | 1,573 (6) | 1,868 (6) | 3 | 0 | 1 | 2 |
| 2 | 962 (7) | 878 (7) | 1,350 (7) | 1,591 (7) | 1,307 (7) | 1,186 (7) | 1 | 4 | 3 | 1 |
| 3 | 912 (6) | 750 (6) | 1,333 (7) | 1,726 (6) | 1,133 (6) | 973 (6) | 0 | 0 | 0 | 0 |
| 4 | 686 (7) | 613 (7) | 2,022 (7) | 2,397 (7) | 1,388 (7) | 1,257 (7) | 0 | 0 | 0 | 0 |
| 5 | 845 (7) | 689 (7) | 1,609 (7) | 1,768 (7) | 1,334 (7) | 1,114 (7) | 1 | 0 | 0 | 1 |
| 6 | 780 (7) | 631 (7) | 1,255 (7) | 1,841 (7) | 988 (7) | 780 (7) | 1 | 1 | 1 | 1 |
| 7 | 481 (6) | 554 (6) | 4,759 (7) | 4,297 (6) | 2,315 (6) | 2,704 (6) | 0 | 0 | 0 | 0 |
| 8 | 691 (7) | 610 (7) | 3,279 (7) | 3,334 (7) | 2,260 (7) | 2,044 (7) | 1 | 0 | 1 | 0 |
| 9 | 903 (7) | 567 (7) | 2,423 (7) | 2,558 (7) | 2,132 (7) | 1,368 (7) | 0 | 0 | 0 | 0 |
| 10 | 652 (6) | 623 (7) | 2,512 (7) | 2,776 (7) | 1,640 (6) | 1,524 (7) | 1 | 0 | 1 | 0 |
| 11 | 551 (7) | 457 (6) | 2,113 (7) | 2,758 (6) | 1,171 (7) | 1,001 (6) | 0 | 0 | 0 | 0 |
| 12 | 883 (6) | 738 (6) | 2,206 (6) | 2,130 (5) | 1,935 (7) | 1,547 (6) | 0 | 1 | 0 | 1 |
| 13 | 815 (7) | 439 (7) | 1,515 (7) | 2,544 (7) | 1,171 (7) | 679 (7) | 1 | 0 | 1 | 0 |
| 14 | 808 (7) | 720 (7) | 2,123 (7) | 2,618 (7) | 1,688 (7) | 1,534 (7) | 0 | 2 | 0 | 2 |
| 15 | 619 (7) | 588 (7) | 1,687 (7) | 1,581 (7) | 962 (7) | 984 (7) | 2 | 0 | 0 | 2 |
| 16 | 686 (7) | 414 (6) | 1,628 (6) | 1,794 (6) | 1,110 (6) | 665 (5) | 0 | 0 | 0 | 0 |
| 17 | 1,185 (7) | 1,048 (7) | 2,146 (7) | 2,469 (7) | 2,494 (7) | 2,511 (7) | 3 | 0 | 1 | 2 |
| 18 | 658 (7) | 675 (6) | 2,138 (6) | 1,871 (6) | 1,431 (6) | 1,288 (6) | 1 | 0 | 1 | 0 |
| 19 | 508 (7) | 280 (6) | 3,645 (7) | 3,513 (6) | 1,840 (7) | 977 (6) | 0 | 0 | 0 | 0 |
| 20 | 780 (7) | 547 (7) | 3,146 (7) | 3,534 (7) | 2,454 (7) | 1,933 (7) | 0 | 1 | 0 | 1 |
| 21 | 2,004 (7) | 1,779 (7) | 1,831 (7) | 2,239 (7) | 3,669 (7) | 3,983 (7) | 7 | 6 | 7 | 0 |
| 22 | 876 (7) | 928 (7) | 2,986 (7) | 3,350 (7) | 2,616 (7) | 3,109 (7) | 2 | 1 | 1 | 1 |
| 23 | 1,117 (5) | 556 (5) | 2,226 (5) | 3,324 (4) | 2,486 (5) | 1,848 (4) | 1 | 1 | 1 | 0 |
| 24 | 1,412 (7) | 1,141 (7) | 2,298 (6) | 2,871 (7) | 3,245 (6) | 3,276 (7) | 1 | 4 | 3 | 1 |
| 25 | 1,548 (7) | 893 (6) | 1,518 (4) | 1,992 (6) | 2,350 (4) | 1,79 (6) | 1 | 5 | 1 | 4 |
| 26 | 880 (6) | 911 (6) | 2,841 (7) | 2,879 (7) | 2,500 (6) | 2,623 (6) | 1 | 2 | 1 | 2 |
| Median | 812 | 653 | 2,142 | 2,520 | 1,764 | 1,529 | | | | |
| Total [e] | | | | | | | 28 | 28 | 24 | 21 |
| 10th percentile | 558 | 441 | 1,366 | 1,730 | 1,112 | 799 | | | | |
| 90th percentile | 1,389 | 1,036 | 3,265 | 3,497 | 2,604 | 3,068 | | | | |

[a] Mean value of the period
[b] Number of observations (in parentheses)
[c] Defined as urinary cystine concentration above 1,200 μmol/l
[d] Measurements during *Period 2*
[e] On 11 occasions, cystine concentrations were above 1,200 μmol/l in all samples. This explains the difference when comparing the total registered supersaturation episodes in day and night collections (i.e. 56) with the total sum of missed and detected supersaturation episodes in the 24-h samples (i.e., 45)

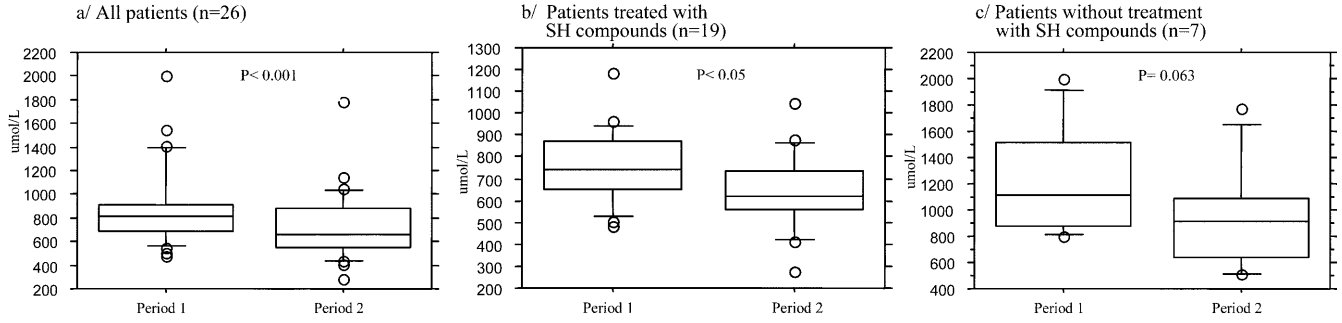



**Fig. 2a–c** Box plot of urinary free cystine concentration (μmol/l) in 26 patients with and without treatment with SH compounds (tiopronin, D-penicillamine and MESNA). *Period 1*: 24-h urine samples. *Period 2*: Urine samples divided into day and night collections. *Horizontal lines* represent 10th–25th–50th–75th and 90th percentiles, respectively. *Circles* represent values below the 10th and above the 90th percentile

clinical practice would, however, probably prove difficult because of low patient compliance. The use of day and night portions therefore was considered a realistic compromise.

The basic method for lowering the concentration of free cystine in urine is to maintain a high fluid intake. In case this measure is insufficient, treatment with SH compounds such as D-penicillamine and tiopronin should be considered [5, 6, 8, 22, 29, 33, 36, 37]. In an experimental study, Dent and Senior determined the solubility of cystine in human urine to be about 1,200 μmol/l [12]. Later Pak and Fuller observed inter-individual differences in urinary cystine solubility attributable to differences in urinary concentrations of solutes and macromolecules [32]. We used a urinary cystine concentration of 1,200 μmol/l as the level indicating a high cystine supersaturation. A rough discriminating level is probably sufficient in clinical practice because it is difficult to get exact information on the pH level.

The patients in the present study showed significant differences in mean urinary cystine concentrations between the two periods in the group as a whole, as well as in the patients treated with SH compounds. A similar tendency, although statistically not significant, was recorded in the small group of patients receiving basic treatment. It also should be noted that these individuals did not tolerate treatment with SH-compounds and that a high fluid intake had been emphasised for many years as the only method to lower the concentration of free cystine in the urine. This made it difficult to further increase fluid intake, even in the light of high cystine concentrations. The division into separate day and night samples, however, made it possible to instruct the patients to increase their fluid intake during the period of particular risk. Investigations of the pharmacokinetics of tiopronin have shown that its effect on lowering the excretion of free cystine lasts 6–12 h [3, 4]. This observation suggests that the drug should be given at least twice daily. Similar conclusions can be drawn from the results reported by other authors [1, 18, 25]. Unfortunately, a regimen with three or four doses per day tends to decrease patient compliance. We have, therefore, given tiopronin in the morning and at bedtime with the dose adjusted to the cystine concentration measured during the actual period.

We believe that the lower cystine concentration recorded during *Period 2* was the result of an increased awareness of the diurnal variations in cystine concentration, and the following individualized instructions for fluid intake and dosage of SH compounds. The tiopronin dose, accordingly, was significantly higher at the end of *Period 2* than at the end of *Period 1*. The higher tiopronin dose was accompanied by a significant decrease in the excretion of free cystine in the urine in the patients treated with tiopronin or D-penicillamine during *Period 2* (Fig. 4). The dose in the patients treated with D-penicillamine was not significantly increased, and the median dose remained unchanged between the end of *Periods 1* and *2* (Table 3).

Since no change in the diet was prescribed, no significant difference in the excretion of free cystine was

**Fig. 3a–c** Box plot of urinary volumes in 26 patients with and without treatment with SH compounds (tiopronin, D-penicillamine and MESNA). *Period 1*: 24-h urine samples. *Period 2*: Urine samples divided into day and night collections

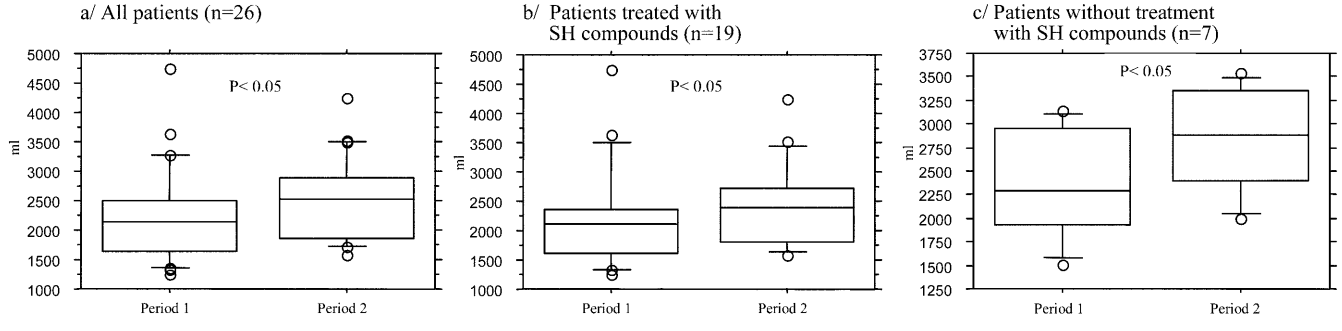


**Table 3** Doses of sulfhydryl compounds in 19 patients (tiopronin, D-penicillamine and MESNA)

| Pat no | Sulfhydryl dose Study start (mg) | Sulfhydryl dose End of *Period 1* (mg) | Sulfhydryl dose End of *Period 2* (mg) |
|---|---|---|---|
| 1 | 2,750[a] | 2,750[a] | 3,000[a] |
| 2 | 2,500[a] | 2,500[a] | 3,000[a] |
| 3 | 2,500[a] | 2,500[a] | 3,250[a] |
| 4 | 2,000[a] | 2,000[a] | 2,250[a] |
| 5 | 2,250[a] | 2,250[a] | 2,250[a] |
| 6 | 3,000[a] | 3,000[a] | 3,000[a] |
| 7 | 1,000[a] | 1,000[a] | 1,500[a] |
| 8 | 500[a] | 500[a] | 750[a] |
| 9 | 1,250[a] | 1,250[a] | 2,250[a] |
| 10 | 2,500[a] | 2,500[a] | 2,500[a] |
| 11 | 1,500[a] | 1,500[a] | 1,750[a] |
| 12 | 1,500[a] | 1,500[a] | 1,750[a] |
| 13 | 800[c] | 1,250[a] | 2,000[a] |
| 14 | 1,200[c] | 1,200[c] | 1,200[c] |
| 15 | 1,000[b] | 1,250[b] | 1,500[b] |
| 16 | 1,000[b] | 1,000[b] | 1,000[b] |
| 17 | 1,750[b] | 1,750[b] | 2,500[b] |
| 18 | 750[b] | 750[b] | 750[b] |
| 19 | 1,000[b] | 1,250[b] | 1,250[b] |
| Tiopronin | | | |
| Median | 2,125 | 2,125 | 2,250 |
| 10th percentile | 850 | 850 | 1,275 |
| 90th percentile | 2,825 | 2,825 | 3,075 |
| D-penicillamine | | | |
| Median | 1,000 | 1,250 | 1,250 |
| 10th percentile | 750 | 750 | 750 |
| 90th percentile | 1,750 | 1,750 | 2,500 |

[a] Tiopronin
[b] D-penicillamine
[c] MESNA

observed in the patients treated with hydration and urine alkalinization only.

The reported frequency of renal stone episodes and the need for active stone removal was lower during *Period 2*. It seems reasonable to believe that the higher detection rate of episodes with high cystine supersaturation and the lower mean concentration of urinary cystine contributed to this effect.

We conclude that separate day and night urine sampling for analysis of cystine reveals supersaturation episodes that will not be detected with analysis of cystine in 24-h urine collections. This strategy thus increased our possibilities to optimize and individualize the treatment by better coping with the diurnal variations in cystine concentration and made it possible to reach a new level of patient-oriented approach in the treatment of stone-forming cystinuria.

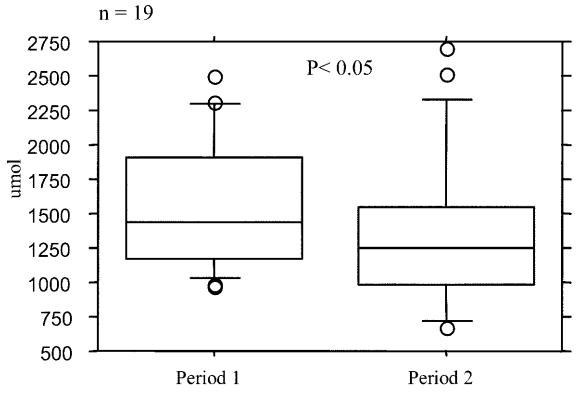



**Fig. 4** Box plot of urinary cystine excretion (μmol/l) in 19 patients treated with SH compounds (tiopronin, D-penicillamine and MESNA). *Period 1*: 24-h urine samples. *Period 2*: Urine samples divided into day and night collections

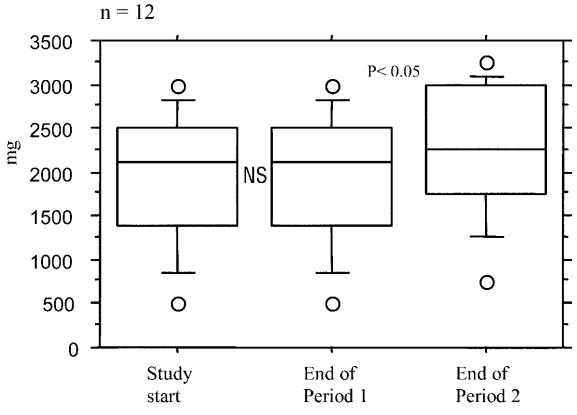
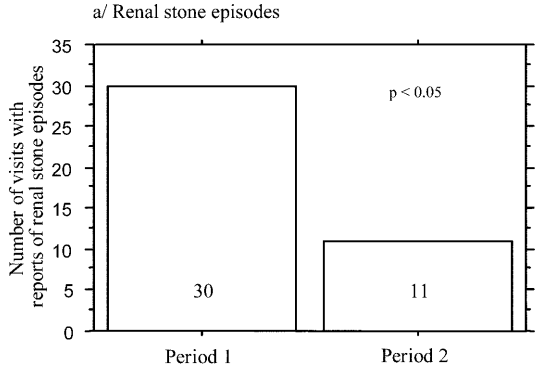
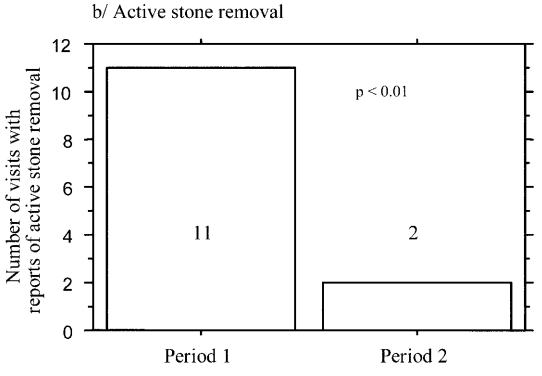



**Fig. 5** Box plot of tiopronin dose at study start and at the end of *Periods 1* and *2*. *Period 1*: 24-h urine sample. *Period 2*: Urine samples divided into day and night collections. *N.S.* Not statistically significant

**Fig. 6a, b** Renal stone episodes and active stone removal between *Periods 1* and *2* calculated with Mantel-Haenszel test



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## References


1. Backer ET, van den Ende A, Geus WP (1987) Determination of urinary cystine for monitoring anticystinuric therapy. Clin Chim Acta 165:351
2. Bostrom H, Hambraeus L (1964) Cystinuria in Sweden. Acta Med Scand Suppl:411
3. Carlsson MS, Denneberg T, Emanuelsson B-M, Kågedal B, Lindgren S (1993) Pharmacokinetics of oral tiopronin. Eur J Clin Pharmacol 45:79
4. Carlsson MS, Denneberg T, Emanuelsson B-M, Kågedal B, Lindgren S (1994) Steady state pharmacokinetics of 2-mercaptopropionylglycine (tiopronin) in healthy volunteers and patients with cystinuria. Drug Invest 7:41
5. Chow GK, Streem SB (1996) Medical treatment of cystinuria: Results of contemporary clinical practise. J Urol 156:1576
6. Crawhall JC (1987) Cystinuria – an experience in management over 18 years. Miner Electrolyte Metab 13:286
7. Crawhall JC, Scowen EF, Watts RWE (1963) Effects of penicillamine on cystinuria. Br Med J 1:588
8. Dahlberg PJ, van den Berg CJ, Kurtz SB, Wilson DM, Smith LH (1977) Clinical features and management of cystinuria. Mayo Clin Proc 52:533
9. Denneberg T, Jeppsson J-O (1992) The management of cystinuric patients with sodium 2-mercaptoethane sulfonate (MESNA), XXIX:th Congr EDTA-ERA; 1992 June 28–July 1; Paris, p 34 (abstract)
10. Denneberg T, Jeppsson J-O, Stenberg P (1983) Alternative treatment of cystinuria with alpha-mercaptopropionylglycine, Thiola®. Proc Eur Dial Transplant Assoc 20:427
11. Denneberg T, Fjellstedt E, Lindell Å, Tiselius H-G (1995) Fractionation of urinary cystine – a new strategy in treatment of cystinuria. Paper read at the Swedish Association of Urology, Proceedings of the Annual Meeting. Published in: Scand J Urol Nephrol 29 [Suppl 174]: 49 (Abstract)
12. Dent CE, Senior B (1955) Studies on the treatment of cystinuria. Br J Urol 27:317
13. Dent CE, Friedman M, Green H, Watson LCA (1965) Treatment of cystinuria. BMJ 1:403
14. DiSilverio F, Gallucci M, Ricciuti GP, Alpi G, Fini D, Cassanelli A, Molinari C (1987) Mercaptoethane sulphonate in cystinic calculosis therapy. Contrib Nephrol 58:193
15. Granerus G, Aurell M (1981) Reference values for 51Cr-EDTA clearance as a measure of glomerular filtration rate. Scand J Clin Lab Invest 41:611
16. Granerus G, Moonen M, Ekberg S (1990) A comparison between 99mTc-MAG3 and 99mTc-DTPA with special reference to the measurement of relative and absolute renal function. In: Schmidt HAE, van der Schoot JB (eds) Nuclear Medicine: The state of the art of nuclear medicine in Europe. Schattauer, Stuttgart, p 284
17. Halperin EC, Thier SO, Rosenberg LE (1981) The use of D-penicillamine in cystinuria: efficacy and untoward reactions. Yale J Biol Med 54:439
18. Harbar JA, Cusworth DC, Lawes LC, Wrong OM (1986) Comparison of 2-mercaptopropionylglycine and D-Penicillamine in the treatment of cystinuria. J Urol 136:146
19. Hautman RE (1981) Cystine-stone therapy with alpha-mercaptoporpionylglycine – Ten years' experience with forty-two patients. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis: clinical basis and research. Plenum, New York, p 139
20. Jaffe IA (1986) Adverse effects profile of sulfhydryl compounds in man. Am J Med 80:471
21. Jeppsson J-O, Karlsson IM (1972) Ion-exchange chromatography of physiological sulphur amino acids on a highly cross-linked resin. J Chromatogr 72:93
22. Joly D, Pieux P, Méjean A, Gagnadoux M-F, Daudon M, Jungers P (1999) Treatment of cystinuria. Pediatr Nephrol 13:945
23. Kallistratos G, Timmerman A (1968) Uber die Wirkung von Thiola. Naturwissenschaften 55:648
24. King JS (1968) Treatment of cystinuria with alpha-mercaptopropionylglycine. A preliminary report with some notes on column chromatography of mercaptans. Proc Soc Exp Biol Med 129:927
25. Koide T, Kinoshita K, Takemoto M, Yachiku S, Sonoda T (1982) Conservative treatment of cystine calculi: effect of oral alpha-mercaptopropionylglycine on cystine stone dissolution and on prevention of stone recurrence. J Urol 128:513
26. Linari F, Marangella M, Fruttero B, Bruno M (1981) The natural history of cystinuria: a 15-year follow-up in 106 patients. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis: clinical basis and research. Plenum, New York, p 145
27. Lindell Å, Denneberg T, Hellgren E, Jeppsson J-O, Tiselius H-G (1995) Clinical course and cystine stone formation during tiopronin treatment. Urol Res 23:111
28. Lindell Å, Denneberg T, Jeppsson J-O, Tiselius H-G (1995) Measurements of diurnal variations in urinary cystine saturation. Urol Res 23:215
29. Lotz M, Potts JT, Holland JM, Kiser WS, Bartter FC (1966) D-Penicillamine therapy in cystinuria. J Urol 95:257
30. Miano L, Gallucci M, Petta S (1979) Results of medical treatment of cystine lithiasis. Eur Urol 5:265
31. Morner CT (1932) Kan nagot rationellt goras for minskande av stenbildningsrisken hos cystinurici. Uppsala Läkför Förh 37:295
32. Pak CYC, Fuller C (1983) Assessment of cystine solubility in urine and of heterogenous nucleation. J Urol 129:1066
33. Pak CYC, Fuller C, Sakhaee K, Zerwekh JE, Adams BV (1986) Management of cystine nephrolithiasis with alpha-mercaptopropionylglycine. J Urol 136:1003
34. Purkiss P, Watts RWE (1977) Low-dose D-Penicillamine in cystinuria. Proc R Soc Med 70:27
35. Remien A, Kallistratos G, Burchhart P (1975) Treatment of cystinuria with Thiola (alpha-mercaptopropionylglycine). Eur Urol 1:227
36. Sakhaee K, Sutton AL (1996) Pathogenesis and medical management of cystinuria. In: Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger GM (eds) Kidney stones: medical and surgical management. Lippincott-Raven, Philadelphia, p 1007
37. Stephens AD (1989) Cystinuria and its treatment: 25 years' experience at St. Bartholomew's Hospital. J Inherit Metab Dis 12:197
38. Wollaston WH (1810) On cystic oxide: A new species of urinary calculus. Trans R Soc London 100:223