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ORIGINAL ARTICLE

Cystinuria in children and young adults: success of monitoring free-cystine urine levels

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Abstract Medical treatment of cystinuria is often disappointing. Patients undergo frequent surgery, which is often followed by early relapse. The aim of our study was to evaluate the efficacy of medical treatment of cystinuria, to prevent formation or to reduce the numbers and dimensions of renal stones. Twenty cystinuric patients were treated with a combined approach, including cystine-binding drugs. Free and bound urine cystine levels were measured every 4 months. Drug dosage was adjusted to maintain free urine cystine level below 100 μmol/mmol creatinine. Eighteen patients completed the study; detection of new stones was reduced from 0.28 per year to 0.03 per year, and, in six patients, the numbers and dimensions of pre-existing renal stones were reduced. Surgery was required in one subject, and no relapse was observed 12 months afterwards. The dosage required to achieve target levels was closely correlated with patient body weight: older children required a lower dose. Medical management of cystinuria is feasible. The treatment must be personalised in children, as the amount of drug required is strictly dependent on body size.

Keywords Cystinuria · Medical treatment · Cystine-binding drugs · Children · Renal stones

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Introduction

Cystinuria is an autosomal recessive disorder characterised by impaired handling of cystine and dibasic amino acids by the renal proximal tubules and intestines [1].

Its main clinical feature is the recurrence of renal stones. A combined medical approach is commonly used to treat this disorder, but its efficacy is still controversial [2] and surgery is frequently required to remove urinary stones. Unfortunately, there is a high incidence of early relapse after surgical intervention [3], most likely due to insufficient preventive therapy.

The aim of our study was to evaluate prospectively, in a paediatric population, the efficacy of a medical approach for long-term treatment of cystinuria, to prevent the formation of new renal stones and to reduce the numbers and dimensions of pre-existing renal stones.

Material and methods

Cystinuria was arbitrarily defined as cystine excretion above 300 μmol/mmol of creatinine in patients with a history of at least one cystine renal stone.

Twenty patients with proven cystinuria, who had had at least one renal stone at the start of the study or in whom a renal stone had previously been removed by surgery or percutaneous lithotripsy, were included in the study.

Two of the 20 patients were excluded from the study in the first 2 months due to allergy to penicillamine and mercaptopropionyl glycine (tiopronin), in one case, and to the development of severe proteinuria in the other. These patients were excluded from all evaluations.

A third patient developed proteinuria 60 months after starting treatment, and mercaptopropionyl glycine was with-

Table 1 Patients' characteristics, urine cystine levels before start of treatment and the number of determinations of free urine cystine achieving targets during follow-up. In the last column, we evaluated the outcome by comparing the numbers and sizes of renal stones at the start of the study and at the end of the follow-up period. More details of the six patients whose clinical situation improved during follow-up are reported in Table 2

Patients	Age at start (years)	Follow-up (months)	Urine cystine level before study (μmol/mmol creatinine)	Free urine cystine target achieved	Outcome
With stones at start	17	54	544	8/9	Worse
	16	86	356	14/17	Stable
	12.1	12	702	1/2	Stable
	8.6	20	601	5/6	Improved
	3.8	14	884	2/4	Improved
	10.7	19	693	9/10	Improved
	24.1	25	320	8/9	Stable
	7.8	86	947	13/16	Stable
	21	34	1420	5/8	Improved
	13.5	46	913	7/11	Improved
	19.8	86	722	9/14	Improved
No stone at start	3.6	70	361	9/12	No stone occurred
	17.1	12	353	3/3	No stone occurred
	13.8	12	402	8/9	No stone occurred
	8.5	60	969	9/14	No stone occurred
	11	68	546	10/11	No stone occurred
	15.9	12	591	3/3	No stone occurred
	1.9	38	780	7/9	No stone occurred

drawn. His data were considered only for the period in which he had been treated.

In all patients spontaneous stone emissions and/or surgical procedures occurring in the 3 years preceding the study were reviewed (stone episodes). Extracorporeal shock wave lithotripsy (ESWL) was not considered as an interventional procedure, due to its low effectiveness in cystine stone removal.

The patients' mean age at the start of study was 12.6 years (range 1.8–24 years). Mean age at diagnosis was 6.3 years (range 0.33–18.42 years). Patients were followed for a mean time of 42 months (median 36 months range 12–86.4 months) (Table 1).

In 11 patients, one or more renal stones was present at the start of the study; six patients had no renal stones at the beginning of the study, as they had previously undergone surgical removal (open surgery in one case and percutaneous lithotripsy in five other cases). One patient, who was also free from renal stones at the start of the study, had had no stone episode during the 3 years preceding the study, although stones had been reported previously.

All patients were treated with a combined approach. Alkali was administered to raise urine pH to between 7 and 8. At the onset of the study, most patients were already receiving alkali treatment (sodium bicarbonate or potassium citrate) and the drug was not changed. For those patients who were not, alkali treatment was started, in all cases, with potassium citrate. All in all, nine patients received sodium bicarbonate and ten potassium citrate.

Urine pH and proteinuria were measured weekly, with dipsticks, and recorded by all patients.

Patients were asked to drink at least 1 l of water per square metre of body surface area.

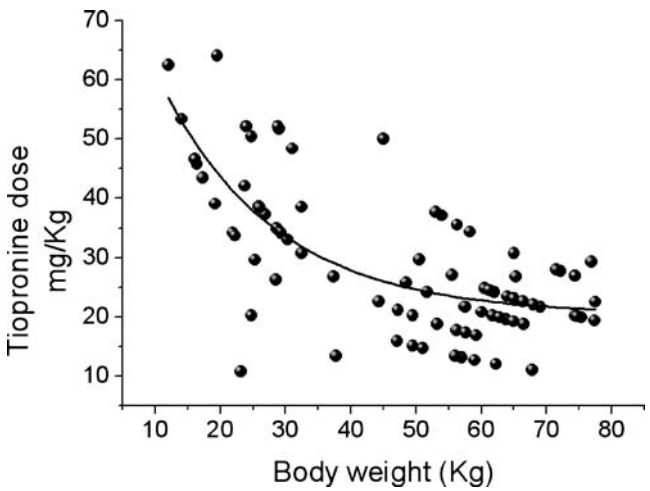

Fig. 1 Alpha-mercaptopropionyl glycine dosage needed to reach the urine free-cystine target level in each patient, according to body weight

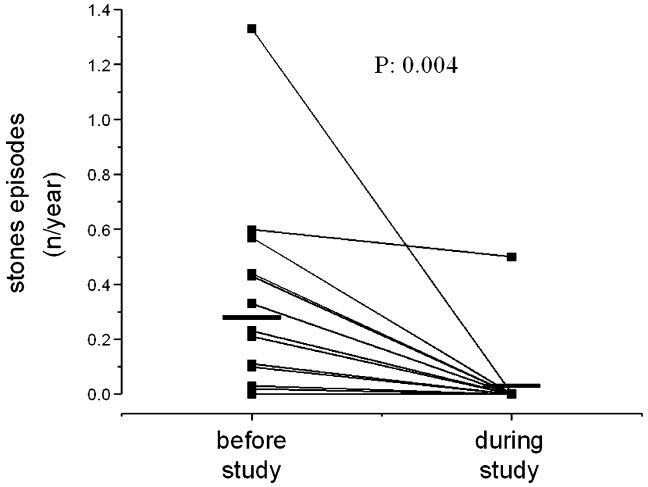

Fig. 2 Number of stone episodes (new stones detected plus surgical interventions). Comparisons between episode frequency in the 3 years preceding the study and in the follow-up period

Cystine-binding drugs were prescribed for all patients: alpha-mercaptopropionyl glycine was our first choice. Two patients had already been switched to D-penicillamine, due to the occurrence of proteinuria following alpha-mercaptopropionyl glycine treatment; proteinuria did not relapse after the switch. Cystine-binding drug dosages were adjusted in order to keep the urine free-cystine level below 100 μmol/mmol creatinine (target level). Normal urine cystine values evaluated in our laboratory in 318 healthy subjects [4] were < 30 μmol/mmol creatinine; therefore, in our patients, urine cystine target level was approximately three-times the normal urine levels of free cystine.

Free and drug-bound urine cystine levels were measured separately in morning urine samples every 4–6 months. The derivatisation and chromatography procedures were performed, with little modifications, as previously reported [4]: briefly, the autosampler collected 10 μl of $NaBH_4$ 4 M (in 333 ml/l of DMSO and 66 mmol/l NaOH), 5 μl of EDTA 2 mmol/l and DTT 2 mmol/l, 5 μl of 1-octanol and 5 μl of HCl 2 mol/l. The mixture was placed in the derivatisation vial containing 10 μl of urine. After incubation for 1 min at room temperature, the autosampler added 25 μl of *N*-ethylmorpholine buffer 2 mol/l, pH 8.0, into the derivatisation vial, and 10 μl of bromobimane 25 mmol/l (in acetonitrile/H_2O 1:1). For free drug determinations, the derivatisation is performed without reducing agent ($NaBH_4$). For cystine and cysteine-drug mixed disulphide determinations, free drug was blocked by the addition of 1 μl of 0.1 mol/l *N*-ethylmaleimide (NEM) before derivatisation. After incubation for 1 min, the autosampler injected 20 μl of this mixture into a 150 mm×4.6 mm Hypersil-ODS HPLC column equilibrated with 30 mmol/l ammonium nitrate and 40 mmol/l ammonium formate buffer, pH 3.6. Calibration curves for each analyte (cysteine, 2-mercaptopropionyl glycine and D-penicillamine) were prepared in 0.1 mol/l HCl containing DTT 100 μmol/l. The amount of free cystine and of the cysteine-drug mixed disulphide was calculated.

Renal stones were monitored by renal ultrasound scan every 4 months to 6 months.

Results

Sixteen patients were treated with alpha-mercaptopropionyl glycine and two with D-penicillamine.

The urine free-cystine target level was steadily maintained in all patients. In each patient the free-cystine level was below target level in 79% of the determinations, and it was less than 50 μmol/mmol of creatinine in most patients in at least 35% of determinations (Table 1).

Mean alpha-mercaptopropionyl glycine dose was 24.65 mg/kg per day (range 13.8–51 mg/kg per day). The dosage required to achieve target level depended on the patient's body weight: older children required a lower dose to achieve target level (Fig. 1). The two patients treated with penicillamine received a mean dose of 17.8 mg/kg per day and 16.3 mg/kg per day, respectively.

Table 2 Details of renal stone evolution in the six patients who had stones at the onset of the study and who improved during follow-up

Renal stones at start of study				Renal stones at last observation			
Right kidney		Left kidney		Right kidney		Left kidney	
Number of stones	Largest stone dimension	Number of stones	Largest stone dimension	Number of stones	Largest stone dimension	Number of stones	Largest stone dimension
4	4 mm	2	12 mm	No stones		2	8 mm
Multiple	4 mm	Multiple	3 mm	Rare	1–2 mm	Rare	1–2 mm
3	2 mm	3	2–3 mm	No stones		No stones	
1	11 mm	Several	1–3 mm	No stones		No stones	
2	3 mm	5	8 mm	No stones		4	6 mm
2	10 mm	1	Staghorn	2	5 mm	1	13 mm

According to the weekly reports, urine pH was above 7 in more than 80% of all determinations in all patients.

Urine osmolality was constantly above 500 mosmol/kg in eight patients, despite the large amount of water they claimed to have drunk.

Stone episodes, as defined above, before the start of the study, were 0.28 per year and fell to 0.03 per year after the free-cystine target level had been reached, with only one patient requiring surgical intervention (percutaneous lithotripsy) to remove an obstructive stone (Fig. 2). In this case, no relapse was observed 12 months after treatment.

In several patients there was a reduction in the numbers and dimensions of pre-existing renal stones, as demonstrated by repeated ultrasound scans (Table 2).

Discussion

Data from the multinational database on cystinuria report an incidence of 0.42 stone episodes for male patients and 0.21 for female patients per annum (spontaneous emissions plus surgical interventions) [5]. These values were obtained despite the fact that many patients in the database were receiving some form of medical treatment.

The large majority of patients with recurrent cystinuria requires some form of surgical treatment [6]. Even though the surgical approach to stone removal is less and less invasive, it is followed by stone relapses in the large majority of patients in the 5 years following treatment [6].

The efficacy of cystine-binding drugs for the treatment of renal cystine stones in adults, without personalisation of the dosage, has been described [7]. In these patients the treatment was effective, and the need for surgery was reduced [7]. However, medical treatment of cystinuria is generally considered very disappointing, due to poor patient compliance or to the occurrence of adverse side effects requiring the withdrawal of treatment [3]. Several methods have been proposed to optimise medical treatment [8], but there is, as yet, no clear indication of which may be the best tool for treatment monitoring.

If a reduction in the likelihood of stone formation is to be achieved, it is essential that the urine free-cystine level be kept above the solubility level. Avoidance of stone formation also depends on two parameters: high urine pH, at least 7–7.5 [1], and low urine osmolality. However, a urine pH of at least 7–7.5 is not easy to be reached and to be steadily maintained. Furthermore, low urine osmolality was not consistently observed in our patients after overnight fasting. Therefore, low urine free-cystine level is the cornerstone of successful medical treatment of cystinuria.

In our series, new renal stones occurred very rarely. This is striking if we compare these results both with the stone incidence recorded in our group of patients in the 3 years preceding the study and with the renal stone incidence observed in a large cohort of previously described patients [5]. In the only patient treated surgically during our study, no stone relapse was observed in the following 12 months, probably due the low urine free-cystine levels maintained after surgery.

One patient developed proteinuria after 6 years of treatment with alpha-mercaptopropionyl glycine, during which period no stone had occurred. This patient was withdrawn from the study. Five months afterwards, a new stone appeared.

This is the first time that medical treatment of cystinuria has been adjusted prospectively on the basis of urine free-cystine levels. In children the treatment had to be individualised, as the dosage varied from patient to patient due to different ages and sizes. In our patients a definite correlation between body weight and required dose was observed: younger children required a much higher dose per kilogramme of body weight than older children. We did not perform a pharmacokinetics study, and, therefore, we are unable to say whether this is due to lower absorption or faster clearance (as occurs for several other drugs) of the treatment employed.

Regular monitoring is therefore needed to personalise treatment, at least in children. It also has other advantages: the attending physician can assess patient compliance, and the patient can judge the effectiveness of the treatment. It may be easier for a patient to accept long-term treatment if the result is immediate, e.g. a “satisfactory” urine free-cystine level instead of a potential future reduction in the likelihood of renal stone recurrence.

Side effects of cystine-binding drugs are not rare and constitute a severe limitation to their prolonged use. In our series adverse events requiring the withdrawal of treatment occurred in three patients (14%). They should be considered second-line treatments in cases where urine dilution and alkalisation alone are not effective.

Cystinuria is a disease of long duration, and our results might show less success over time with regard to patient compliance and to the possible occurrence of adverse side effects leading to withdrawal from medical treatment.

In conclusion, however, our data confirm that conservative medical management of cystinuria is feasible. Treatment must be personalised, at least in children. In order to reduce the risk of early relapse after surgical treatment it is mandatory, in our opinion, to obtain a low urine free-cystine level before performing any invasive procedure to remove stones in patients with cystinuria.

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