MOLECULAR GENETICS AND METABOLISM 64, 237–242 (1998)
ARTICLE NO. GM982725

A Randomized Clinical Trial of Topical Cysteamine Disulfide (Cystamine) versus Free Thiol (Cysteamine) in the Treatment of Corneal Cystine Crystals in Cystinosis

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Received May 7, 1998

In nephropathic cystinosis, corneal cystine crystals cause severe photophobia and corneal erosions. Topical cysteamine dissolves these crystals, but cannot be marketed because it rapidly oxidizes to the disulfide form, cystamine, at room temperature. Since cystamine itself could be used commercially, we compared the efficacy of cystamine and cysteamine with respect to cystine crystal dissolution in a randomized, double-masked clinical trial. One eye each of 14 patients with cystinosis was randomized to either cystamine or cysteamine, 0.5%, with 0.01% benzalkonium chloride; the companion eye was treated with the alternate preparation. Corneal crystals were photographed and a density score was assigned to each slide based on 13 standard slides. After 8–20 months, 6 patients showed significant reduction of the corneal crystal score in only one eye. In each case, the improved eye was the cysteamine-treated eye. Theoretically, cysteamine should dissolve both intracellular and extracellular crystals, whereas cystamine should dissolve only intracellular crystals because it must first be reduced to the free thiol by the cytoplasmic-reducing environment. Hence, the lack of efficacy of the disulfide cystamine suggests that some corneal cystine crystals in cystinosis patients are extracellular, and that another form of stable, topical cysteamine must be developed for cystinosis patients. © 1998 Academic Press

Key Words: cysteamine; cystamine; cystinosis; corneal crystals; cystine.

Nephropathic cystinosis is an autosomal recessive lysosomal storage disorder characterized by renal tubular Fanconi syndrome in the first year of life, growth retardation in children, renal glomerular failure at approximately 10 years of age, hypothyroidism, and a variety of other complications, including photophobia and corneal erosions due to cystine crystal formation within the eye. These signs and symptoms occur because defective lysosomal cystine transport causes nonprotein cystine to accumulate in the lysosomes of many tissues. The responsible gene, CTNS, was mapped to chromosome 17 (1–5) and has been recently cloned (6). It codes for a 367 amino acid protein with six to seven transmembrane regions and a lysosomal membrane targeting motif.

The treatment of choice for cystinosis patients is chronic oral therapy with the free thiol cysteamine. This cystine-depleting agent offers benefits for growth, maintenance of renal glomerular capacity, and preservation of thyroid function (7,8). However, patients treated with oral cysteamine still develop crystals in their corneas (9,10). The crystal deposition, which starts in the periphery, appears at approximately 1 year of age by slit lamp biomicroscopy. With time, the crystals increase in density to involve the entire cornea (11), and patients suffer various degrees of photophobia which may adversely affect quality of life (12). Corneal crystals constitute a critical element in the diagnosis of nephropathic cystinosis.

Fortunately, topical cysteamine depletes the cornea of cystine crystals, reduces the photophobia of

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1096-7192/98 $25.00

cystinosis patients, and appears safe with daily use (13–16). Unfortunately, cysteamine oxidizes to its disulfide, cystamine, at room temperature, making it unstable in the formal, chemical sense. Consequently, cysteamine eye drops must be frozen or kept from oxygen to prevent oxidation. Since this is not practical commercially, the development of cystamine as a topical cystine-depleting agent was considered. Toxicity studies in rabbit eyes, performed at the National Institutes of Health, showed that 2% cystamine was associated with blepharitis and conjunctivitis in rabbit eyes, but that 0.5 and 1.0% cystamine had effects similar to those of placebo (unpublished data).

Based upon the apparent safety of cystamine in rabbit eyes, and its stability in solution, we initiated a randomized, controlled, double-masked clinical trial of topical cystamine versus cysteamine with respect to removal of corneal cystine crystals in cystinosis patients.

METHODS

Patients

Subjects were enrolled in a protocol approved by the National Eye Institute's Institutional Review Board, and written informed consent was obtained from each patient and/or parents. Cystinosis was diagnosed by a leukocyte cystine content above 3.0 nmol half-cystine/mg protein (normal, ≤0.2), in combination with a typical clinical course. All patients were receiving oral cysteamine but had never received topical cysteamine.

Protocol

At the baseline visit, patients underwent an ophthalmologic evaluation focusing on history of photophobia, pain or foreign body sensation, assessment of haziness of the cornea, and observation with slit lamp biomicroscopy for the presence and the degree of crystals in the cornea. Visual acuity was measured with ETDRS charts. For patients younger than school age, picture optotype visual acuity cards were used.

Slit lamp photographs were taken in the same fashion at each visit and slides were graded based on a set of 13 standards graded from "0", which represents clarity at the center, to "3.00", which signifies the greatest recognizable crystal density. The grading system used increments of 0.25 units. This system, which represents a minor modification of that previously described (14), was used by two independent and masked graders (F. I. and E. M. K.). The average of the two scores was used for analysis.

One eye of each patient was randomly assigned to receive either cystamine 0.5% or cysteamine 0.5%, with benzalkonium 0.01%; the companion eye received the other drug. Both preparations were provided by the NIH Pharmaceutical Development Service under Investigational New Drug Exemption No. 45321 (W. A. G.) and were kept frozen before use. The identity was concealed from patients and observers. The patients/parents were instructed and trained in the application of the eyedrops and were evaluated for their performance and retrained if needed at each visit. Patients and/or their parents were instructed to take the different preparations in the specific eyes every hour while awake. The patients were followed at 6–8-month intervals. On each follow-up visit, change in symptoms such as photophobia and foreign body sensation, difference in symptoms between the two eyes, presence of possible side effects, and compliance were repoted. The difference between cysteamine-treated eyes and cystamine-treated eyes in change from baseline to end of follow-up was evaluated by a two-sided 0.05 level paired t test (17). A Data Safety and Monitoring Committee monitored the safety and efficacy of this study.

RESULTS

Baseline Evaluation

Fourteen patients (8 female, 6 male) from 12 families underwent a baseline evaluation for entry into this study (Table 1). All were Caucasian and ranged in age from 3 to 29 years (mean, 15 years). Two patients (14%) chose not to enter the protocol. A 28-year-old woman (No. 9) preferred to be followed locally, and a 16-year-old girl (No. 13) lost interest in the study because of minimal symptoms. Photophobia of various degrees was reported by 11 of the 14 patients; three (Nos. 4, 6, and 13) did not complain of photophobia. No patient reported an episode of acute eye pain. At the baseline visit, gradings of corneal crystals were 2.75 or greater in all patients in both eyes, except for patient No. 8, a 3-year-old girl (Table 1).

Follow-up

Twelve patients (86%) were enrolled in the protocol and had at least one follow-up visit between 11 and 42 months after the baseline visit. One 3-year-old girl (patient No. 4) exited the study due to her parents'

TABLE 1
Summary of Topical Cystamine vs Cysteamine in Cystinosis Patients

Patient	Age (y)/sex	Duration of study (months)	Crystal-density score		Eye treated with cysteamine	Improvement of symptoms[a]	Compliance	
			Baseline OD/OS	End of study OD/OS			Eye drops[b]	Oral cysteamine[c]
1	25/M	20	3.00/3.00	3.00/3.00	OS	+	+++	+
2	11/M	17	3.00/3.00	0.25/2.875	OD	++	+++	+++
3	7/M	16	3.00/2.75	2.50/0.125	OS	++	+++	++++
4	3/F	19	3.00/3.00	2.875/3.00	OD	No symptoms	DCD	++
5	29/M	15	3.00/3.00	1.75/3.00	OD	+	+++	+++
6	5/F	16	3.00/3.00	0.00/3.00	OD	No symptoms	+++	++
7	7/F	16	3.00/3.00	3.00/1.625	OS	++	+++	++
8	3/F	14	2.125/2.375	1.875/2.25	OS	−	++	+
9	28/F	0	2.875/2.875	NA	NA	NA	NA	?
10	25/M	12	3.00/3.00	3.00/2.00	OS	++	++	++
11	18/F	12	3.00/3.00	3.00/3.00	OD	+	+++	++
12	23/M	11	3.00/3.00	3.00/3.00	OD	−	+	+
13	16/F	0	2.75/2.75	NA	NA	NA	NA	+++
14	13/F	8	3.00/3.00	3.00/3.00	OD	−	+++	+

[a] Improvement of symptoms: −, none or questionable; +, moderate, ++, significant; NA, not available.
[b] Eye drops compliance: +, 1 to 4 times per day; ++, 5 to 7; +++, 8 or more; DCD, discontinued.
[c] Oral cysteamine compliance: +, poor; ++, fair; +++, good; ++++, excellent; ?, unknown.

difficulty in administering the eye drops. The period of randomized treatment for the 11 remaining patients ranged from 8 to 20 months (mean, 15 months) and was longer than 12 months in 10 patients.

On follow-up, 6 patients (Nos. 2, 3, 5, 6, 7, and 10) showed significantly different scores between the two eyes. In all 6 patients, the change was observed at the first follow-up visit, i.e., 6 months in 5 patients and 12 months in one patient, who missed the first follow-up. In each case, only one eye showed a score reduction greater than 0.50 unit compared with the baseline visit. In each case the better eye was the one treated with cysteamine (Table 1). The mean reduction in crystal density for cysteamine-treated eyes was 1.02 units compared with 0.07 units for cystamine-treated eyes. The difference between these means, 0.95, was significantly different from zero, by paired t test, with $P = 0.015$. The Data Safety and Monitoring Committee terminated the randomized treatment with cystamine, and all patients were treated with cysteamine eye drops beginning with their next scheduled visit.

Five patients did not show a significant difference in crystal score between the two eyes nor a significant reduction in crystal score in either eye. Three of these patients reported poor complicance. The other two (No. 1, treated for 20 months, and No. 14, treated for 8 months) reported taking the eye drops eight times daily.

Subjective improvement of photophobia and/or discomfort was reported in 5 of the 6 patients who showed a significant reduction in crystal score; patient No. 6 did not complain of photophobia at baseline. For the 5 improved patients, the change in daily life was remarkable. One patient became able to observe sunsets, and others began to play outside during daylight for the first time. Patient No. 3 reported remarkably decreased photophobia, but he still showed persistent squinting in his cystamine-treated right eye on exposure to bright light. After 12 months of cysteamine administration to both eyes, squinting ceased. Most patients did not recognize a difference in photophobia between the two eyes.

The best corrected visual acuity at the end of the randomized treatment showed an improvement of five letters or more compared with the baseline in 3 patients; no patients showed decreased vision. During the study, one 3-year-old was diagnosed with ameotropic amblyopia in the cystamine-treated eye. Two sisters (Nos. 6 and 7) reported an occasional burning sensation, associated with cysteamine eye drops in one girl and with cystamine eye drops in the other. After termination of the study, 1 patient with a history of diabetes mellitus for the past 3 years developed consecutive vitreous hemorrhages in each eye, presumably unrelated to topical medication. There were no other complaints and no adverse reactions were observed on examination.

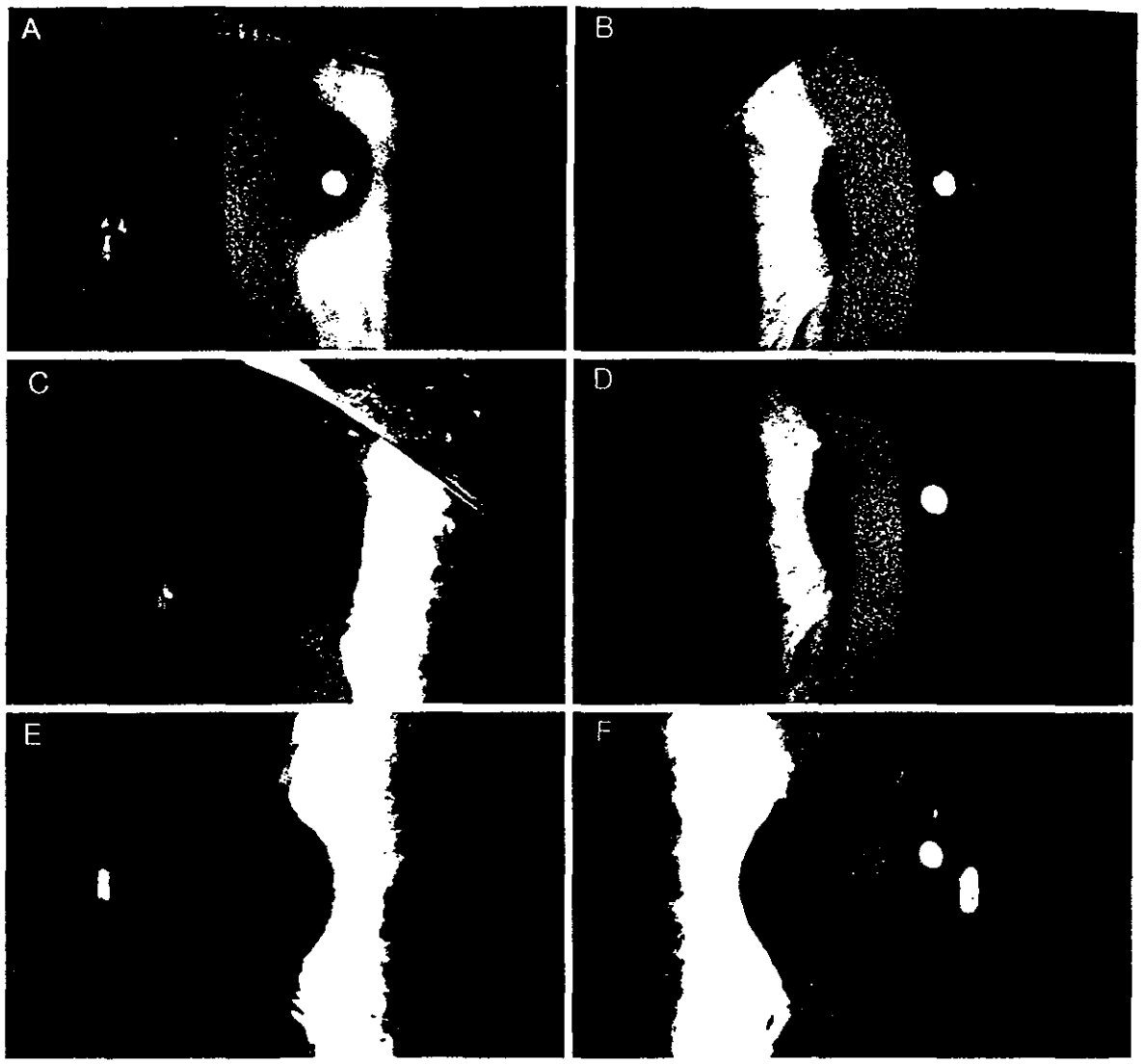

FIG. 1. Slit lamp photographs of corneas in Case 2. The left panels (A, C, E) show the right eye, and the right panels (B, D, F) show the left eye. (A, B) Baseline visit. Diffuse and dense crystals are present in both corneas. (C, D) After 6 months of randomized therapy. The right eye, treated with cysteamine, shows decreased crystals compared to baseline and compared to the cystamine-treated left eye. E, F After 18 additional months of topical cysteamine therapy. Both corneas are almost clear.

Case Report

Patient No. 2, an 11-year-old boy, was noted to be photophobic in the first year of life by his parents. At 2–3 years of age, corneal crystals were observed by an ophthalmologist. His eyes remained very sensitive to light and he occasionally complained of a burning sensation. The boy developed renal failure and underwent a renal allograft procedure at 10 years of age, at which time the diagnosis of nephropathic cystinosis was made.

Upon baseline examination, the corneas had mild haze. Slit lamp biomicroscopy revealed diffuse crystal deposits throughout the entire corneal stroma of both eyes, with crystal scores of 3.00 in each eye (Figs. 1A and B). Punctate fluorescein staining of corneal epithelium was also noted. Crystals were observed on both irides and in both conjunctivas,

especially near the vessels. The lenses were clear. Pigment mottling was noted in the peripheral fundi and some depigmentation and granular pigmentation were observed in the maculae. Visual field, color vision, and dark adaptation, measured by Goldmann perimetry, Farnsworth Panel D-15, and Goldmann-Weekers adaptometer, were normal.

After 6 months of randomized therapy (with cysteamine in the right eye), the patient's eye pain had ceased and his photophobia had lessened, but he could not discern a difference in photophobia between the two eyes. Visual acuity was normal in each eye, with mild hyperopic correction. Corneal haziness was much less in the right than in the left eye. On slit lamp examination, the anterior stroma of the right cornea was almost clear despite the presence of crystals in the deep stroma. In contrast, his left cornea had large condensed crystals throughout the thickness of the stroma, unchanged from the baseline examination. Crystals were not evident in the right conjunctiva, but were abundant in the left conjunctiva. Corneal crystal scores were 1.25 for the right eye and 3.00 for the left eye (Figs. 1C and D). For 17 months, the boy continued the randomized medication without a major change in crystal scores. However, for the first time, the patient could play outside as other children do.

The patient was followed regularly, and 18 months after changing to topical cysteamine in both eyes, slit lamp examination revealed crystals in the peripheral corneas; the central corneas of both eyes were almost clear. In fact, the scores were 0 in the right eye and 0.125 in the left eye (Figs. 1E and F). The patient had no ocular complaints and did not remember his previous photophobia and burning sensation.

DISCUSSION

Although topical cysteamine has proven efficacy in dissolving corneal cystine crystals and reducing the photophobia of cystinosis patients (13–16), it has not been brought to the Food and Drug Administration for New Drug Approval because its ready oxidation to cystamine renders it chemically unstable. Cystamine itself might be suitable for commercial use, but our randomized, double-masked trial has now revealed that it has practically no efficacy compared with cysteamine in dissolving corneal cystine crystals. Although a longer duration of therapy or a higher concentration of cystamine may improve the results, our findings virtually eliminate cystamine as topical therapy for corneal crystals in cystinosis.

Cysteamine ($HS\text{-}CH_2\text{-}CH_2\text{-}NH_3$), or β-mercaptoethylamine, has been shown to deplete cystinotic cells of cystine by first traversing the plasma and lysosomal membranes and then concentrating within the acidic lysosome by virtue of its positively charged amine group. Within the lysosome, the free thiol participates with stored cystine in a disulfide interchange reaction, producing cysteine, which freely leaves the cystinotic lysosome, and the mixed disulfide cysteine-cysteamine. This latter compound resembles lysine and exits the cystinotic lysosome via a lysine transport system which remains functional in cystinotic lysosomes. In this fashion, cysteamine therapy circumvents the defective lysosomal cystine carrier and depletes cells of cystine.

This mechanism of action requires two elements, i.e., an environment which keeps free thiols reduced and an acidic lysosome which concentrates cysteamine. Both of these elements are provided by intact cells. The cytosolic milieu reduces cystamine to cysteamine and makes it an effective cystine-depleting agent. Indeed, previous studies have demonstrated that cystamine depletes cystinotic fibroblast granular fractions of cystine, albeit not as effectively as cysteamine itself (18). It is possible that corneal cells cannot reduce cystamine to cysteamine, and this may account for cystamine's failure to dissolve corneal crystals. However, a more likely explanation is that cystamine remains oxidized as it contacts cystine, because the crystals are outside rather than inside the cells.

This opposes current dogma. Electron microscopy and unstained examination by cross-polarized light and phase contrast microscopy have shown only intracellular crystals, not extracellular crystals, in many ocular tissues including the cornea of one patient (19). Another report showed crystals within corneal stromal cells but not in the extracellular collagenous stroma (20). However, the failure to detect extracellular crystals could be due to fixation methods which favor extracellular crystal dissolution during processing. *In vivo*, intracellular crystals may become extracellular by extrusion from the cell (21) or by cell death, with preservation of residual crystals which were previously intracellular. In any event, the possibility that corneal cystine crystals are found outside of corneal stromal and epithelial cells should be considered in view of the failure of cystamine to dissolve them.

Clinically, we are left with cysteamine eye drops as the treatment of choice for corneal cystine crystals. Cysteamine can be maintained in the free thiol form by freezing or by packaging under argon, which allows for only 9% conversion to cystamine after 4 weeks, compared with 45% in the absence of argon (21). Other chemical means of maintaining cysteamine in the re-

duced state are being pursued, and one formulation is about to be tested for clinical safety and efficacy.

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