UNDERSTANDING INTESTINAL CYSTEAMINE BITARTRATE ABSORPTION

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Objectives To test the hypothesis that a controlled-release preparation of cysteamine, with fewer daily administrations, would improve the quality of life for patients with cystinosis.

Study design A specifically designed nasoenteric tube was used to administer cysteamine directly into the stomach, small intestine (SI) and colon and serial plasma cysteamine, serum gastrin and leukocyte cystine levels were measured.

Results Eight control subjects (mean age 23.2 years) and 6 subjects with cystinosis (mean age 15.2 years) were studied. Cysteamine absorption (maximum concentration and area under the curve of the concentration-time gradient) was greater from the SI than stomach or cecum ($P < .01$). Leukocyte cystine depletion was greater after delivery of cysteamine into the SI than stomach or cecum; this effect was associated with the plasma cysteamine maximum concentration and area under the curve ($P < .001$ and $< .02$, respectively). Gastrin levels were not affected by site of drug delivery and were elevated only in patients with cystinosis with gastrointestinal symptoms.

Conclusions The absorption of cysteamine and the effect of this agent on leukocyte cystine depletion are more profound after SI administration. Enteric-coated cysteamine, targeted for SI release, may require fewer daily dosages. Not all patients with cystinosis require acid-suppression therapy. *(J Pediatr 2006;148:764-9)*

Cystinosis is a rare, autosomal recessive disease caused by intra-lysosomal accumulation of the amino acid cystine within various tissues, including the spleen, liver, lymph nodes, kidney, bone marrow, and eyes.[1] Nephropathic cystinosis is associated with progressive kidney failure that necessitates kidney transplantation.[1,2] To date, the only specific treatment for nephropathic cystinosis is the sulfhydryl agent, cysteamine; this drug has been shown to lower intracellular cystine levels, thereby reducing the rate of progression of kidney failure in children.[1-3]

Cysteamine, through a mechanism of increased gastrin and gastric acid production, is known to be ulcerogenic in laboratory animals.[4-6] When administered orally to children with cystinosis, cysteamine has also been shown to cause a 3-fold increase in gastric acid production and a 50% rise of serum gastrin levels above baseline.[7,8] As a consequence, some patients with cystinosis on cysteamine therapy frequently suffer gastrointestinal (GI) symptoms and are often unable to take cysteamine regularly or at full dose.[8-10] To achieve sustained reduction of leukocyte cystine levels, patients are normally required to take oral cysteamine every 6 hours, which invariably means having to awaken from sleep. When a single dose of cysteamine was administered intravenously the leukocyte cystine level remained suppressed for more than 24 hours, possibly because plasma cysteamine concentrations were higher and achieved more rapidly than when the drug is administered orally.[11] Clearly, regular intravenous administration of cysteamine would not be practical; however, if the drug could be administered in a way that would result in higher plasma, and thus intracellular, concentration, it might be possible to decrease the number of daily doses and therefore improve the quality of life for patients.

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METHODS

This study was approved by the University of California at San Diego, Human Research Protection Program, and informed consent was obtained for each participant. Study subjects were recruited nationwide and admitted to the University of California at San Diego General Clinical Research Center.

AOC	Area over the curve	HSD	Honestly significant difference test
C_{max}	Maximum concentration	REML	Restricted maximum likelihood
GI	Gastrointestinal	SI	Small intestine

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Table I. Cystinosis patient data

Patient	Age (yrs)	Sex	Weight (kg)	Cysteamine dose (mg)*	Serum creatinine (mg/dL)
1	16	Male	61.5	500	1.0
2	14	Male	39.4	406	1.2
3	13	Female	39.1	406	1.5
4	19	Female	38.1	406	1.4
5	13	Female	50.1	500	1.0
6	16	Male	58.7	500	3.1

*Dose of cysteamine base delivered into varying intestinal sites.

Subjects

Children with cystinosis, ≥12 years old, and taking regular cysteamine bitartrate (Cystagon; Mylan, Morgantown, WV) were recruited to the sttudy (Table I). Adult control patients were recruited locally. Patients with cystinosis had a mean leukocyte cystine level of less than 2.0 nmol half-cystine/mg protein over the past year. Cysteamine therapy was discontinued 2 days before admission, and acid suppressants, antibiotics, nonsteroidal antiinflammatory drugs, pro-kinetic agents, and antihistamines were discontinued 2 weeks before admission. None of the patients had undergone kidney transplantation. Baseline chemistry, *Helicobacter pylori* serologic study, complete blood count, and urinalysis were performed.

Cysteamine bitartrate delivery

Cysteamine was infused through a silicone rubber nasoenteric tube (Dentsleeve Pty Ltd, Australia), 3 mm in diameter and 4.5 meters long. The tube, specifically made for this study, had a tungsten-weighted tip, and immediately proximal to this was an inflatable balloon (5-mL capacity). Immediately proximal to the balloon was an infusion port (1 mm diameter) through which the drug was delivered. After an overnight fast (except for water), the dose of cysteamine bitartrate (10 mg/kg/dose of base, maximum of 500 mg) was dissolved in 10 mL of water and infused over 1 to 2 minutes. On day 1 of the study, the nasoenteric tube was inserted into the stomach. By day 3 of the study the tube had passed into the proximal small intestine (SI) just distal to the ligament of Treitz (confirmed fluoroscopically). The balloon was then inflated, and peristalsis propelled the tube distally. Tube position within the cecum was confirmed fluoroscopically on day 5 (day 7 in 4 patients because of slow transit). If the tube had migrated too far, it was retracted into the desired location.

Serum gastrin, cysteamine and leukocyte cystine measurements

After an overnight fast (except for water) blood samples were taken at baseline and at varying intervals after intraluminal delivery of cysteamine. Serum gastrin levels were then measured at 30, 60, 90, and 120 minutes and 3 and 4 hours; cysteamine levels were measured at 0, 5, 10, 20, 30, 45, 60, 75, 90, 105, 120, and 150 minutes and 3, 4, 6, 8, 10, 12, and 16 hours; leukocyte cystine levels were measured at 1, 2, 3, 4, 6, and 12 hours in patients with cystinosis only. Gastrin was measured in picograms/mL with the Diagnostic Products Corporation (Los Angeles, Calif) gastrin^{125} radioimmunoassay-assay kit.[12] Leukocyte cystine levels were measured in nmol half-cystine per mg protein by the Cystine Determination Lab (La Jolla, Calif).[13]

To measure plasma cysteamine, 100-μL plasma samples were collected in heparinized Vacutainers and spun in a centrifuge within 1 hour, and plasma was stored at −18° C. With a previously described method,[10] the concentration of cysteamine was measured by use of tandem mass spectroscopy (API 2000 LC/MS/MS; Applied Biosystems, Foster City, Calif). Cysteamine concentrations were calculated with a calibration curve that was prepared by spiking plasma with buffered cysteamine solutions, and quality control samples were analyzed with each batch.[10,14]

Statistical analysis

Mixed model restricted maximum likelihood (REML) repeated measures analysis of variance with subjects as a random effect was performed on the absolute leukocyte cystine levels, on the leukocyte cystine level changes from baseline, and on what we term "area over the curve" (AOC) for leukocyte cystine level changes from baseline after cysteamine administration for the subjects with cystinosis. AOC is computationally analogous to area under the curve, but it is applied when values are predominantly decreasing below baseline values. Large AOC values reflect large decreases, and a negative AOC reflects a net increase in value. Main effects for site of delivery, time after delivery, and the interaction between site and time were tested, except just the site effect was tested for AOCs. In the absence of significant interaction when a main effect was detected, Tukey's honestly significant difference test (HSD) was applied to identify where differences occurred within a 5% family wise error rate. The Tukey HSD procedure controls for overall significance level when performing all pairwise comparisons. An additional analysis was performed with plasma cysteamine C_{max} added to the AOC model.

REML repeated measures analyses of variance with subjects as a random effect were also performed as described above on AUC and the C_{max} over time for plasma cysteamine levels separately for the subjects with cystinosis and control subjects and with both subject groups combined. Differences between means for the 3 sites were tested, plus group and group × site interaction effects for the combined groups. If a site effect was detected, Tukey's HSD was applied to determine which sites differed from each other.

REML repeated measures analyses of variance were also performed as described above on gastrin levels. The analyses were performed on 2 versions of datasets: the full dataset and all data after omitting observations collected at 30 minutes (1 subject was missing a blood sample taken at 30 minutes after small intestinal cysteamine delivery). A 5%

significance level was used without adjustment for all statistical testing.

RESULTS

Six patients with cystinosis, (3 male, 3 female) with a mean age of 15.2 years (range 13-19 years) were recruited into the study (Table I). Eight healthy adult control patients (6 male, 2 female) with a mean age of 23.2 years (range 19-28 years) were enrolled. None of the children with cystinosis had undergone kidney transplantation. All control subjects received 500 mg cysteamine base, whereas the mean dose for subjects with cystinosis was 453 mg (range 406-500 mg). All subjects had normal liver function test results. In all subjects the nasoenteric tube passed successfully from the stomach into the upper SI; however, it did not progress any further in 2 subjects with cystinosis. In 2 of the control subjects the tube only reached the mid-ileum but did, however, progress to the cecum in 8 subjects (4 control subjects, 4 with cystinosis). There were no reported adverse effects with the insertion or removal of the nasoenteric tube (Figure 1).

Symptoms

Only 2 patients (1 male, 1 female) with cystinosis reported regular GI symptoms before the study, and these had responded to acid-suppression therapy. The male subject had severe retching and emesis about 15 minutes after receiving intragastric cysteamine but did not have any symptoms when the drug was infused into the proximal small intestine. The female child with cystinosis had mild transient nausea after SI drug delivery only. No other symptoms were reported after any other cysteamine delivery in the children with cystinosis. There were no associated adverse events with tube placement or removal.

Plasma cysteamine

Among the subjects with cystinosis as measured by analysis of variance, the mean plasma cysteamine C_{max} and AUCs (of the concentration-time gradient) differed by site of cysteamine delivery (both $P < .03$). Site (†) refers to either patients with cystinosis or control subjects. For the plasma cysteamine AUCs, the means differed between the duodenal and both gastric and cecal sites of delivery (Tukey HSD global $P < .05$). Among control subjects, the mean AUC did not differ among delivery sites ($P > .4$), but mean C_{max} did ($P < .05$). For both cystinosis and control groups the mean C_{max} values differed only between the duodenum and cecum; mean C_{max} values after duodenal versus gastric or gastric versus cecal delivery were not statistically different (Tables II and III).

When data from the control subjects were combined with cystinosis subject data, there was both a group effect ($P < .05$) and a site effect ($P < .01$) for AUCs, with a significant difference between mean AUC levels for the duodenum versus both the stomach and cecum. C_{max} values differed among sites ($P < .01$) but not between groups ($P > .4$). Group (*) refers to site of intestinal delivery. C_{max} differed between duodenum versus both stomach and cecum (Figure 2).

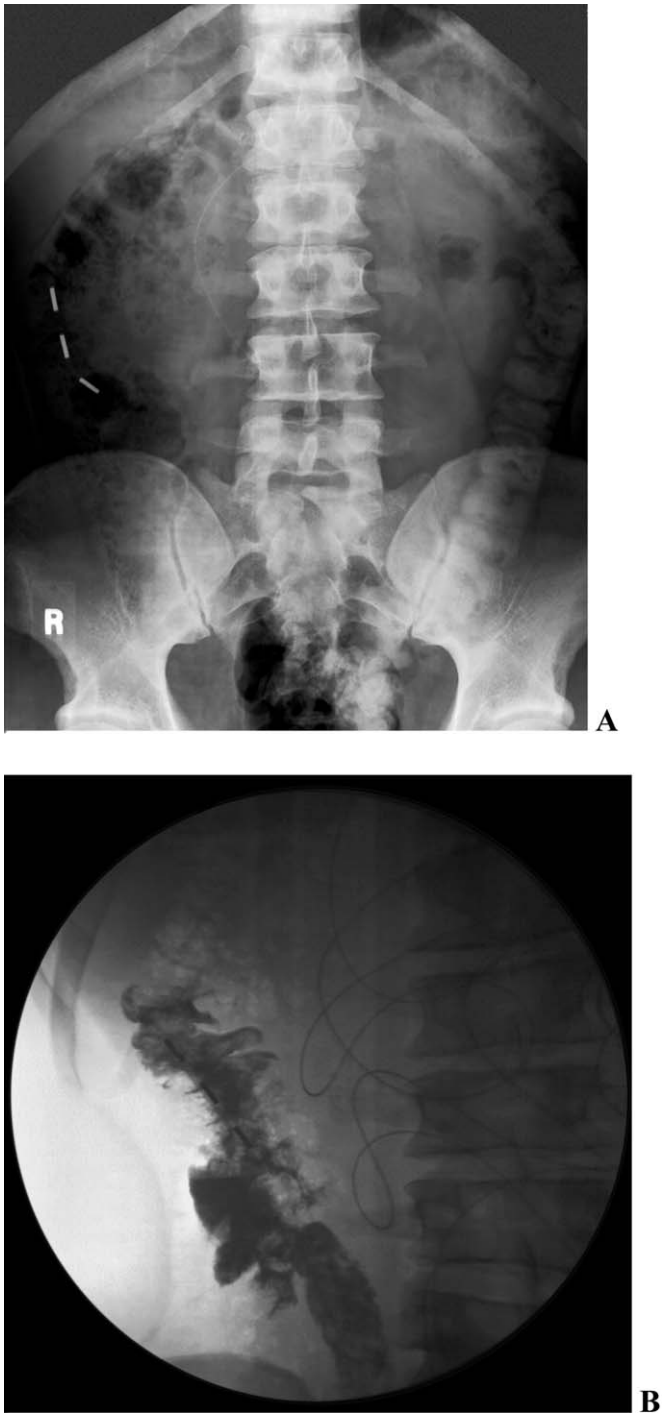

Figure 1. Enterocolonic tube. **A**, Abdominal X-ray film shows radiopaque weighted tip of tube entering ascending colon. **B**, Contrast infused. Tube has passed through small intestine and position of tip is confirmed.

Leukocyte cystine

There were significant differences among the 3 sites of delivery for cystine levels ($P < .04$), changes from baseline

Table II. Mean plasma cysteamine C_{max} levels (μmol/L) and area under curve (AUC) measurements in cystinosis subjects, controls, and combined cystinosis and control subjects, after delivery of cysteamine into the stomach, small intestine, and cecum

	C_{max} Cystinosis	AUC Cystinosis	C_{max} Control	AUC Control	C_{max} Combined	AUC Combined
Stomach	35.5 (20.5)	3006 (1112)	39.5 (16.4)	3613 (1384)	37.8 (17.6)	3353 (1267)
Small Intestine	55.8 (13.0)	4299 (1056)	51.1 (20.7)	3988 (1659)	53.2 (17.4)	4047 (1376)
Cecum	21.9 (13.1)	3002 (909)	23.1 (15.3)	2804 (1323)	22.5 (13.2)	2903 (1056)

The standard deviations are in parenthesis.

Table III. Comparisons of mean plasma cysteamine C_{max} (μmol/L) and AUC measurements for combined cystinosis subjects and control subjects among delivery sites

	AUC	C_{max}
P value*	<0.01	<0.01
Stomach vs SI	+	+
Stomach vs Cecum	−	−
SI vs Cecum	+	+

+Significant difference using Tukey's HSD test (α = 0.05)
−No significant difference.
*ANOVA test for equality of three delivery sites

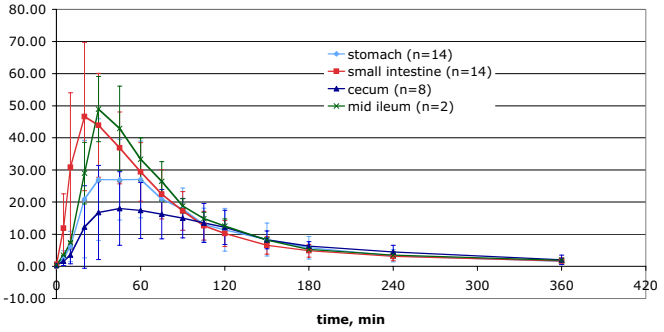

Figure 2. Mean plasma cysteamine levels taken from patients with cystinosis and control subjects after delivery of drug into various intestinal sites. *Error bars* are standard error of mean. In 2 patients (control subjects), most distal point of delivery was mid-ileal region. Available in color at www.jpeds.com.

values ($P < .0001$), and AOCs for changes from baseline ($P < .02$). A Tukey HSD test, which controls for multiple comparisons, showed that mean leukocyte cystine levels differed between the cecum and stomach sites, but that cecum versus duodenum and stomach versus duodenum produced similar mean values. When the absolute cystine levels or AOCs for changes from baseline levels were evaluated, the significant differences in sites were found between the duodenum and both the stomach and cecum, but not between stomach and cecum (Tukey HSD global $P < .05$) (Figure 3). Plasma cysteamine C_{max} and AUC contributed a statistically

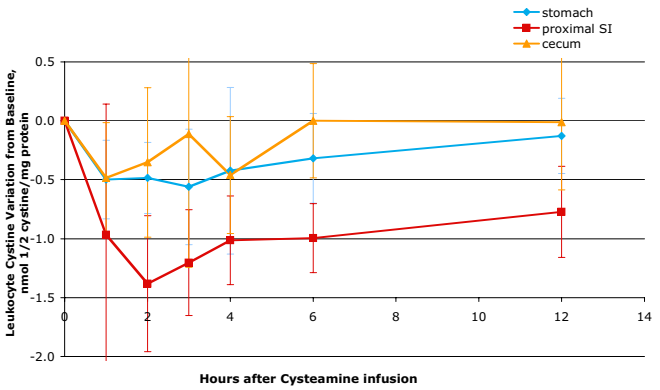

Figure 3. Shows mean change in leukocyte cystine levels, compared with baseline levels, over 12-hour period after delivery of cysteamine into varying intestinal sites. Negative levels signify increased leukocyte cystine depletion compared with baseline. Available in color at www.jpeds.com.

significant effect on AOC ($P < .001$ and $< .02$, respectively), even after controlling for delivery site (Figure 4).

Blood gastrin

For the full gastrin dataset, there was a significant difference among the means for the different delivery sites ($P < .01$), with the cecum resulting in a lower mean from that of the stomach and small intestine. Both group* and site† significant effects were detected after omitting observations from 30 minutes after delivery ($P < .05$ and $P < .01$, respectively). The 30-minute observations were omitted because of a missing data set. For these observations, mean levels of gastrin after delivery in the cecum were different from those from both the duodenum and stomach, although the latter did not differ from each other. The 1 boy (14 years) who had severe GI symptoms after intragastric, but not enteric or cecal, cysteamine delivery had a rise in baseline gastrin from 70 pg/mL to 121 pg/mL at 30 minutes after gastric cysteamine. Within the control group, more than half of the baseline and post-cysteamine gastrin levels remained undetectable (<25 pg/mL), and none of the control subjects had a significant rise in gastrin after cysteamine delivery into any site.

DISCUSSION

Patients with cystinosis are required to ingest oral cysteamine (Cystagon) every 6 hours, day and night. When taken

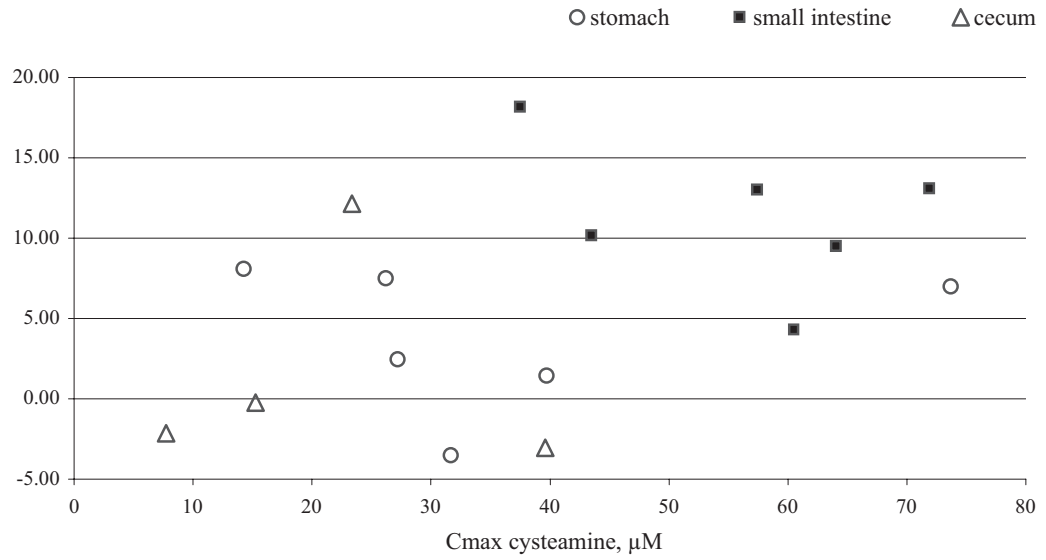

Figure 4. Scatterplot of plasma cysteamine C_{max} vs AOC of WBC Cystine changes from Baseline. Positive value means decrease from baseline. Negative value means increase from baseline. AOC change from baseline was affected by C_{max} for cysteamine ($P < .001$).

regularly, cysteamine can deplete intracellular cystine by up to 90% (as measured in circulating white blood cells), and this has been shown to reduce the rate of progression to kidney failure/transplantation and also to obviate the need for thyroid replacement therapy.[1–3,15,16] Unfortunately, because of the strict treatment regimen and the associated symptoms, nonadherence with cysteamine therapy remains a problem, particularly among adolescent and young adult patients.[17] Certainly, by reducing the frequency of required cysteamine dosing adherence can be improved. Our present study shows a strong statistical association between the maximum plasma concentration (C_{max}) of cysteamine and AOC measurements for leukocyte cystine ($P < .001$). A higher C_{max} is achieved after delivery of cysteamine into the small intestine than when infused into the stomach or colon; this may be due to improved absorption rate from the SI, greater surface area of the SI, or less cysteamine undergoing hepatic first pass elimination when absorbed rapidly through the small intestine. When data were combined for patients with cystinosis and control subjects, there was a statistical difference between duodenal versus both gastric and colonic delivery for plasma cysteamine C_{max} and AUC levels (both $P < .05$). The lack of similar statistical significance for the cystinosis group alone may simply reflect the small number of patients studied. Changes from baseline leukocyte cystine levels were statistically significant for absolute cystine levels and for AOC when cysteamine was infused into the duodenum compared with both stomach and colon. As shown in Figure 3, the leukocyte cystine levels remained below pre-delivery levels for up to 12 hours after a single dose of cysteamine into the small intestine. This would suggest that effective absorption of cysteamine through the SI, by causing a higher C_{max} and AUC on the cysteamine concentration-time gradient, could lead to prolonged depletion of leukocyte cystine and possibly less frequent daily dosing. Another explanation would be that by achieving a high enough plasma cysteamine concentration, more drug reaches the lysosome (where cystine accumulates). In the lysosome the cysteamine reacts with cystine forming the mixed disulfide of cysteamine and cysteine.[1] The mixed disulfide exits the lysosome presumably via the lysine carrier. In the cytosol the mixed disulfide can be reduced by its reaction with glutathione. The cysteine released can be used for protein or glutathione synthesis. We hypothesize that the cysteamine released from the mixed disulfide reenters the lysosome where it can react with another cystine molecule.[1] Thus 1 molecule of cysteamine may release many molecules of cystine from the lysosome. Our study findings complement those of Thoene et al[11] in their study of leukocyte cystine response to intravenous cysteamine administered in a similar dose to that taken orally in a single patient with cystinosis. This study showed a dramatic decrease in leukocyte cystine within an hour of cysteamine delivery. In retrospect, the remarkable finding from this study was that the leukocyte cystine levels remained at the 1-hour level for 24 hours, and even at 48 hours after delivery the levels had not returned to the pre-cysteamine level.

Cysteamine is a potent gastric acid–secretagogue that has been used in laboratory animals to induce duodenal ulceration[4–6]; studies in humans and animals have shown that cysteamine-induced gastric acid hypersecretion is most likely mediated through hypergastrinemia.[7,8,10,18] In previous studies performed in children with cystinosis who suffered regular upper gastrointestinal symptoms, a single oral dose of cysteamine (11-23 mg/kg) was shown to cause hypergastrinemia and a 2- to 3-fold rise in gastric acid–hypersecretion.[8] Symptoms suffered by these individuals included abdominal pain, heartburn, nausea, vomiting, and anorexia. Interestingly, only 2 of 6 subjects with cystinosis (who were known to suffer regular cysteamine-induced GI symptoms) had increased gastrin levels and symptoms, including nausea, retching, and

discomfort after intragastric cysteamine. Gastrin levels were only available after small intestinal administration in 1 of the 2 children and the levels remained the same as baseline. Neither child had symptoms after enteric cysteamine delivery. None of the other patients with cystinosis or control subjects had an increase in gastrin levels with cysteamine infused into any site. This would suggest that cysteamine-induced hypergastrinemia may arise as a local effect on the gastric antral-predominant G-cells only in susceptible individuals. In addition, plasma gastrin levels usually peaks after intragastric delivery within 30 minutes, whereas the plasma cysteamine levels peaked later.[8,10] In 2 previous studies, children with cystinosis were shown to have a significant rise in plasma gastrin levels after receiving intragastric cysteamine[8,10]; as part of these study's entry criteria all subjects did, however, suffer with regular GI symptoms. Data from this study would suggest that cysteamine does not cause hypergastrinemia, and therefore acid-hypersecretion, in all patients with cystinosis. Thus acid suppression therapy would not be recommended in patients with cystinosis without upper GI symptoms.

Our data suggest that direct administration of cysteamine into the jejunum may result in prolonged leukocyte cystine depletion; however, we have not yet determined the actual dose and frequency of administration of the drug when it is given in this fashion. In a previous study, a child who had a gastrojejunal feeding tube for oral feeding aversion and severe UGI symptoms, responded to intrajejunal cysteamine with a 3-fold rise in serum gastrin as compared with drug administration into the stomach. The leukocyte cystine response was not measured in this child. Therefore patients with jejunal feeding tubes will have to be further evaluated.

This study provides data that may be used to improve the quality of life for patients with cystinosis. The present formulation of Cystagon comprises cysteamine in a capsule that will dissolve rapidly on contact with water, most likely within the stomach. In our next study we hope to test an enteric-released preparation of cysteamine to determine whether it can cause sustained depletion of leukocyte cystine, therefore requiring fewer daily drug dosages. In addition to improving quality of life, it may also result in fewer problems with compliance and possibly even diminish gastrointestinal symptoms.

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