Steady-state pharmacokinetics and pharmacodynamics of cysteamine bitartrate in paediatric nephropathic cystinosis patients

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Aims Cysteamine is used to reduce tissue cystine content in patients suffering from nephropathic cystinosis. The objectives of the current study were to investigate pharmacokinetics and pharmacodynamics of cysteamine bitartrate in children and young adults with nephropathic cystinosis.

Methods Cysteamine bitartrate was administered to 11 cystinosis patients at their regular dose level in a single-dose, open-label, steady-state study. Blood samples were collected and analysed for plasma cysteamine and white blood cell cystine content and pharmacokinetic and pharmacodynamic parameters estimated by NONMEM analysis using a linked pharmacokinetic–pharmacodynamic model.

Results Cysteamine was rapidly cleared from the plasma (mean $CL/F = 32.3 \text{ ml min}^{-1} \text{ kg}^{-1}$, range = 17.3–52.2), appeared to be extensively distributed (mean $V_{ss}/F = 15.1 \text{ l}$, range 2.7–32.3) and exhibited a mean T_{max} of 1.4 h. White blood cell cystine content post-dosing was significantly decreased compared with pre- and post-dose values (average decrement approximately 47%). A counter-clockwise hysteresis was noted in all patients, suggestive of a lag time (mean $T_{lag} = 0.44 \text{ h}$, range 0.22–0.92) between drug concentration and effect.

Conclusions The results of this study establish that cysteamine is rapidly cleared from the plasma but that an every 6 h dosing interval adequately maintains white blood cell cystine content below the target of 1 nmol cystine per mg protein.

Keywords: cystinosis, pharmacodynamics, pharmacokinetics

Introduction

Nephropathic cystinosis is a rare autosomal recessive disorder. Cystinosis results from accumulation of the disulphide amino acid, cystine, within the lysosomes of various cells of the body [1]. Lysosomal cystine accumulation in cystinosis results from the defective transport of cystine across the lysosomal membrane into the cytoplasm. This accumulation of cystine is usually 10–1000 times that of normal, leading to crystal formation, cellular damage, and resulting in multiple organ damage to kidney, pancreas, thyroid gland, and other organs [2]. During the first decade of life, this damage is most prevalent in the kidneys, resulting in renal failure. Before the advent of renal transplantation, it was rare for infantile nephropathic cystinosis patients to live past 10 years

of age [2]. Fibroblasts and leucocytes isolated from patients with cystinosis exhibit cystine levels up to 100 times the level found in normal individuals, serving as a readily measurable tissue for cystine concentrations [1]. The goal of treatment is to maintain white blood cell (WBC) cystine levels below 1 nmol cystine mg^{-1} protein.

The incidence of nephropathic cystinosis is approximately 1 in 100 000–200 000 live births. There are approximately 600 known cases in North America and 500 known cases in Europe. In the USA, approximately one-third of these patients have had renal transplants, and the oldest patients are now about 40 years old [3]. Infantile/nephropathic cystinosis usually appears several months after birth and is characterized by renal Fanconi syndrome, cystine crystals in the cornea and failure to thrive [3]. This is the most frequent type of cystinosis diagnosed, making up approximately 95% of diagnosed cases.

Limited information is available on the pharmacokinetics and pharmacodynamics of cysteamine, with most studies involving only healthy adult subjects [4–6]. One study assessed the effects of cysteamine HCl or phospho-

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cysteamine and at substantially higher doses than those of the cysteamine bitartrate formulation used in the current study [7]. The objectives of the current study were to evaluate the pharmacokinetics of cysteamine bitartrate under clinical treatment conditions in paediatric patients and to investigate the pharmacokinetic and pharmacodynamic relationship for cysteamine bitartrate in these same subjects.

Methods

Eleven patients (four females and seven males) aged 3–15 years and whose weight ranged from 14.3 to 60.2 kg participated in the study. If the subject was <18 years of age, their parent or guardian signed an Informed Consent Form. Children >7 years of age signed an Informed Assent Form in addition to the Informed Consent Form the parent or guardian signed. All protocols were approved by the University of California, San Diego Institutional Review Board.

Study design

This was a single-dose, open-label study in cystinotic patients, without renal transplant, at steady state on cysteamine bitartrate. The assumption of steady state is based upon compliance of the patient with the absolute knowledge of the last two doses taken and the dosing interval between those doses. Patients with nephropathic cystinosis weighing at least 10 kg were enrolled in this study. All prospective patients had documented evidence of nephropathic cystinosis and the use of cysteamine for at least 12 months.

The patients received their regular dose of medication at approximately 08.00 h with 100 ml of ambient temperature water. Patients were served a standardized low-fat/low-protein breakfast 30 min before dosing, and a standardized lunch 4 h after dosing.

Blood samples were collected in heparinized tubes before dose and at the following times after dosing: 0.5, 1, 1.5, 2, 3 and 6 h post dose. These samples were used for plasma cysteamine concentration assays and for WBC cystine concentration assays.

Cysteamine and cystine assays

Cysteamine plasma concentrations were determined by PPD/Pharmaco (Richmond, VA, USA). The assay method used high-performance liquid chromatography (HPLC) with ultraviolet detection. Following the addition of internal standard (2-dimethylaminoethanethiol) to each plasma sample, proteins were precipitated by the addition of 8 M urea. Samples were then treated with sodium borohydride to reduce all disulphide linkages and

the resulting mixture acidified with phosphoric acid to quench the residual borohydride. The solution was then adjusted to a pH of approximately 7.8 by the addition of 1.0 M KOH and 0.1 N phosphate buffers. Derivatization of cysteamine and internal standard was performed with Ellman's Reagent (5,5 dithio-bis(2-nitrobenzoic acid)). The derivatized compounds were then purified using a benzene sulphonate ion exchange cartridge, the eluent evaporated to dryness and then reconstituted in mobile phase. The HPLC conditions consisted of a mobile phase of acetonitrile : potassium phosphate buffer (10 : 90) flowing at 1.8 ml min⁻¹ through a Supelco LC-8 column (5 µm, 4.6 × 250 mm). Analytes were quantified using ultraviolet detection at a wavelength of 330 nm. The method has been validated for cysteamine over the concentration range of 1.3–130 µM with a lower limit of quantification (LLOQ) equal to the lowest calibration level of 1.3 µM. The between-day precision of the assay was ≤9.9%, and the between-day accuracy varied within –3.5% and 16.2% of the nominal concentration for cysteamine. The chromatograms were free of interference from any of the concomitant medications.

WBC cystine content was assayed at the Cystine Determination Laboratory at the University of California (San Diego, CA, USA) according to the methods of Smith *et al.* [8]. After the plasma was removed for plasma cysteamine determination, normal saline was added to the spun blood to bring it back to its original volume. The samples were then mixed with an equal volume of ACD-dextran, capped, inverted, then allowed to stand on ice for 30 min. The supernatant was then transferred to a clean tube and centrifuged at 450 g for 10 min at 5 °C. The resulting supernatant was then discarded. To the pellet, 0.8 ml of 0.9% NaCl and 2.4 ml of distilled water were added. The mixture was vortexed continuously at moderate speed for 90 s and then 0.8 ml of 3.6% NaCl added and the mixture centrifuged at 450 g for 3 min at 5 °C. The supernate was then discarded and the step repeated, again discarding the resulting supernatant. To the pellet, 3.0 ml of 0.9% NaCl was added, the pellet resuspended by gentle vortexing and the mixture centrifuged again at 450 g for 3 min at 5 °C. Following discarding of the supernatant, 0.3 ml of distilled water was added to the pellet and the white cells lysed by sonication. The lysed cell solution was then transferred to a clean tube containing 0.1 ml 12% sulfosalicylic acid (SSA) and vortexed. The samples were then frozen at –20 °C until assay for leucocyte cystine content. The samples were stable under these conditions for up to 2 weeks.

For analysis of cystine content, the samples were thawed at room temperature and centrifuged at 450 g for 10 min at 4 °C. The supernatant was then transferred to a clean tube and the pellet saved for assay of protein content. To the tube containing supernatant, 50 µl of 1 M

sodium acetate pH 5.0 were added, followed by 50 µl of 1.66 M sodium hydroxide and the samples vortexed. One microlitre of the sample was placed on pH paper to ensure the pH was at 5.0 (if adjustment was needed, 1.66 N NaOH or 12% SSA were added to achieve a pH = 5.0 and the amount of acid or base added was recorded). The samples were then diluted to 0.5 µl with distilled water and vortexed. Sample (50 µl) was then added to 60 µl of Cystine Binding Protein cocktail, mixed and the samples incubated at room temperature for 10 min. Solution (100 µl) was then filtered through 24 mm diameter (0.45 µm pore size) Protran nitrocellulose filters (Protran filters; Schleicher & Schuell, Keene, NH, USA) at 10 in of Hg vacuum pressure. The filter was then rapidly rinsed with 0.6 ml of 0.01 M sodium acetate buffer and dried under a heat lamp for 10 min. Each filter was then placed in a scintillation vial and 6 ml of scintillation counting fluid (EcoLune®; ICN, Costa Mesa, CA, USA) added. The ^{14}C -cystine-protein complex bound to the filter was measured by liquid scintillation counting. Protein was measured by the method of Lowry *et al.* [9].

Data analysis

Pharmacokinetic and pharmacodynamic parameters for cysteamine and cystine were calculated sequentially with compartmental techniques using the nonlinear regression program NONMEM [10]. The pharmacokinetics of a two-compartment model with first-order absorption and a lag time were first determined in each individual using a proportional residual error model. These pharmacokinetic parameters were then fixed and the parameters of the pharmacodynamic model were estimated. A pharmacodynamic link model was used to account for the equilibration delay between concentration and effect. Ke_0 is a first-order rate constant that controls the disappearance of drug from a hypothetical effect compartment. The drug concentration in the effect compartment was related to the observed effect using a fractional inhibitory E_{max} model [11]. With this model, the effect is constrained from negative, and will approach zero as the concentration increases.

$$E = \text{BL} \cdot \left(1 - \frac{\text{Ce}}{\text{EC}_{50} + \text{Ce}} \right)$$

where E is the pharmacodynamic effect, BL is the baseline effect at zero concentration, Ce is the concentration in the hypothetical effect compartment, and EC_{50} is the concentration at the effect site that results in 50% of maximal response.

Repeated measures analysis of variance (general linear model) followed by multiple comparison of means (with Bonferroni correction) was used to assess statistical sig-

nificance at $P < 0.05$. Statistical analysis was performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographic characteristics of the patients as well as concomitant medications are presented in Table 1. The mean plasma concentration *vs.* time profiles for both cysteamine and WBC cystine content are illustrated graphically in Figure 1A,B. The mean $\pm$ SD C_{max} for cysteamine was $36.3 \pm 11.7 \mu\text{M}$ (range 16.9–53.2 µM) and the mean T_{max} was 1.4 h (range 1.0–2.0 h). A two-compartment model with first-order absorption and a lag time was adequate to describe the pharmacokinetic data in all subjects. Cysteamine compartmental pharmacokinetic parameter estimates for each subject are presented in Table 2.

When WBC cystine content was compared to both the pre-dose and the 6-h post-dose time points, there was a statistically significant decrease ($P < 0.05$) at the 1, 2, and 3-h post-dose time values. WBC cystine content returned to baseline levels by 6 h post dose (the time of the next scheduled dose). The mean $\pm$ SD maximum decrement in WBC cystine content was $0.46 \pm 0.23 \text{ nmol cystine mg}^{-1} \text{ protein}$ (range 0.07–0.81) and the mean $\pm$ SD time to maximum effect was $1.8 \pm 0.8 \text{ h}$. Pharmacodynamic estimates of the effect on WBC cystine content using the model presented in equation 1 are listed in Table 3. Data were not sufficient to fit a sigmoid E_{max} model to the pharmacodynamic data.

Examination of Figure 1 suggests that the peak cysteamine plasma concentration occurred before the maximum pharmacodynamic response (decrease in WBC cystine), suggesting a lag in effect. The mean plasma cysteamine concentration *vs.* absolute decrement in WBC cystine content is depicted in Figure 2 in a temporal fashion and clearly shows a counter-clockwise hysteresis, confirming a lag in time to maximum effect.

Discussion

Cystinosis is a rare disease that has unfortunately not been the subject of extensive research, probably due to the relatively small number of patients who suffer from the disease (approximately 1400 patients world-wide, 1100 in North America and Europe). If left untreated, cystinosis progresses to renal failure and eventually death. Cysteamine has been demonstrated to be an effective agent in the reduction of intracellular cystine, the elevation of which has been shown to be the primary cause of cellular damage in cystinosis patients. The purpose of the current study was to assess pharmacokinetic and pharmacodynamic parameters associated with cysteamine bitartrate therapy in patients with cystinosis. Our results

Table 1 Demographic characteristics of study patients and dose administered.

Patient	Sex	Race	Age (years)	Weight (kg)	Height (cm)	Dose (mg) ^a	Concomitant medications (per day)
1	F	Caucasian	12	43.8	144.25	500	Potassium citrate, sodium citrate and citric acid; levocarnitine; phosphorus, potassium and sodium; indomethacin; omeprazole; calcitriol; somatropin; cysteamine eye drops
2	M	Caucasian	11	39.0	143.50	350	Sodium bicarbonate; potassium citrate; calcitriol
3	M	Caucasian/Hispanic	12	57.2	155.25	550	Phosphorus and potassium; potassium chloride; cysteamine eye drops
4	F	Caucasian	9	22.5	115.50	250	Potassium citrate and citric acid; potassium chloride; levothyroxine; sodium chloride; potassium, phosphorus and sodium; cysteamine eye drops; somatropin
5	F	Caucasian	5	18.4	106.80	250	Sodium bicarbonate; potassium acid phosphate and sodium acid phosphate; potassium chloride; calcitriol; iron; fluoride; cetirizine
6	M	Caucasian	3	14.3	93.00	225	Levocarnitine; potassium chloride; dihydrotachysterol; phosphorus and potassium; calcium carbonate; kanana
7	M	Caucasian	10	29.1	138.50	450	Indomethacin; Joule's solution; potassium citrate
8	F	Caucasian	15	47.0	147.00	550	Potassium bicarbonate and potassium citrate; phosphorus, potassium and sodium; levocarnitine; calcitriol; potassium citrate and citric acid; cysteamine eye drops
9	M	Caucasian	14	60.2	162.00	500	Potassium acid phosphate and sodium acid phosphate; potassium citrate, sodium citrate and citric acid; levocarnitine; calcitriol; cysteamine eye drops
10	M	Caucasian	11	38.7	141.25	400	Potassium citrate and citric acid; levocarnitine; calcitriol; phosphorus and potassium, cysteamine eye drops
11	M	Caucasian	9	31.3	138.50	400	Calcitriol; indomethacin; mineral oil; phosphorus, potassium and sodium; potassium citrate, sodium citrate and citric acid; levocarnitine; iron; cysteamine eye drops; multivitamin/fluoride tablet.

^aDaily dosing of cysteamine was at same level (mg) given four times daily.

Table 2 Cysteamine compartmental pharmacokinetic parameter estimates.

	$CL/F, \text{ ml min}^{-1} \text{ kg}^{-1}$	$V_c, \text{ l kg}^{-1}$	$Q, \text{ ml min}^{-1} \text{ kg}^{-1}$	$V_{ss}/F, \text{ l kg}^{-1}$	$K_a, \text{ h}^{-1}$	$Alag, \text{ h}$
Mean	32.3	2.0	29.8	15.1	1.7	0.44
Geometric mean	30.6	1.6	24.6	11.0	1.6	0.41
Median	29.1	1.7	22.5	9.9	1.7	0.36
Minimum	17.3	0.1	8.7	2.7	0.7	0.22
Maximum	52.2	3.6	83.1	32.3	2.5	0.92

CL/F, Apparent clearance; V_c , volume of the central compartment; Q , intercompartmental clearance; V_{ss}/F , apparent steady-state volume of distribution; K_a , absorption rate constant; $Alag$, absorption lag time.

indicate that cysteamine bitartrate therapy produces clinically significant reductions in WBC cystine content, roughly inversely parallel to plasma cysteamine concentrations with a slight lag time.

No studies of the pharmacokinetics and pharmacodynamics of cysteamine bitartrate in children with cystinosis have been published. However, Smolin *et al.* [7], using two different forms of the drug (HCl salt and phospho-

cysteamine) did report the pharmacokinetics and pharmacodynamics of these formulations in patients with the disease. It is of note that the previous study [7] used doses of cysteamine (on a molar basis) that were four-fold higher than those used by us and the patient's dosing of cysteamine was discontinued for 1 or 7 days prior to study, whereas the current study was conducted under steady-state conditions. Cysteamine bitartrate exhibited a

Table 3 White blood cell cystine pharmacodynamic parameter estimates.

	<i>EC</i> 50, μM	<i>Ke</i> 0, h^{-1}	<i>BL</i> cystine, nmol cystine mg^{-1} protein
Mean	15.3	2.2	0.91
Median	6.5	1.4	0.79
Geometric mean	5.6	1.3	0.76
Minimum	0.6	0.2	0.13
Maximum	61.1	8.9	1.9

*EC*50, Concentration producing 50% of maximal effect; *Ke*0, elimination rate constant from the theoretical effect compartment; *BL*, baseline (predose) cystine content.

Figure 1 (A) Mean ($\pm$ SD) cysteamine plasma concentrations over the 6-h dosing interval. (B) Mean ($\pm$ SD) white blood cell cystine concentrations during cysteamine therapy over the dosing interval. #Statistically different from 0 and 6-h values ($P < 0.05$).

$T_{\max}$ of 1.4 h, indicative of a moderate rate of absorption and substantially longer than the 0.75-h $T_{\max}$ reported after administration of either cysteamine HCl or phosphocysteamine [7]. Using doses of cysteamine bitartrate one-quarter ($0.05 \text{ mmol kg}^{-1}$) those of cysteamine HCl or phosphocysteamine (0.2 mmol kg^{-1}), the $C_{\max}$ achieved

Figure 2 Temporal representation of white blood cell cystine concentration *vs.* plasma cysteamine concentration. Manifestation of counter-clockwise hysteresis loop is suggestive of lag in concentration-effect relationship.

in the current study ($36 \mu\text{M}$) was approximately three-quarters those reported previously (approximately $50 \mu\text{M}$) [7]. Thus, it appears that cysteamine bitartrate formulation results in a slower time to peak concentrations and a slightly lower peak concentration. However, the observed maximum decrement in WBC cystine concentration was roughly comparable (48% in the current study *vs.* approximately 60% with the other formulations [7]), suggesting comparable efficacy. Though not directly comparable due to the differences in study design (steady state *vs.* single dose), cysteamine bitartrate appears to produce a more constant cysteamine concentration compared with the HCl or phosphocysteamine formulations, which produced a rapid increase to a high peak concentration followed by a rapid decrement.

One of the therapeutic goals in the treatment of cystinosis is to maintain WBC cystine content $<1 \text{ nmol cystine mg}^{-1}$ protein. In the present study, administration of cysteamine [approximately $0.05 \text{ mmol (10 mg) kg}^{-1}$ every 6 h] on average maintained cystine content below this level, even predose (Figure 1A,B), suggesting that every 6 h cysteamine administration results in achievement of WBC cystine content within the desired therapeutic range. However, it is of note that in the present study the maximum decrement in cystine content was not achieved until 1.8 h on average, compared with an average $T_{\max}$ for cysteamine of 1.4 h. Individual patient differences were even greater than implied by the mean values. Because of this apparent discrepancy between cysteamine concentrations and time to effect, we evaluated whether a lag occurred and if a hysteresis relationship might be present. As depicted in Figure 2, a very pronounced counter-clockwise hysteresis relationship can be noted. This finding suggests that a lag time was involved before the drug was distributed to the active site or an

active metabolite was involved in the pharmacodynamic response process. Based on the known metabolic fate of cysteamine (no known active metabolites) and the extensive distribution observed, it is presumed that time of distribution to the effect site is the more likely explanation. With respect to the mechanism of action of cysteamine, it must enter the lysosomal compartment of cells through a specific transporter. The cysteamine can then react with cystine (cystine is a cysteine disulphide) to form a mixed disulphide, cysteamine–cysteine. The mixed disulphide exits the lysosomes through an intact lysine transporter [2, 12]. This mixed disulphide is then reduced to cysteamine and cysteine by glutathione in the cytoplasm, resulting in reduction of cystine in the lysosomes. Subsequently, the released cysteamine can cycle back into the lysosomes and remove another 1 mole of half-cystine per mole of cysteamine. Though the rate at which cysteamine is transported into the lysosomes and subsequently the mixed disulphide is transported out of the lysosomes is unknown, this transport requirement combined with the recycling of cysteamine could explain the observed counter-clockwise hysteresis phenomenon. The median Ke_0 determined in the pharmacodynamic modelling corresponds to an equilibration half-time of 0.48 h. Unfortunately, this equilibrium is relatively rapid and the effect associated with a given concentration will not be maintained for a long period of time as the concentration falls off.

Cysteamine bitartrate appeared to be well tolerated with no adverse effects reported. It should be noted that all patients were on chronic therapy with cysteamine bitartrate prior to the study and thus may have become tolerant to any adverse effects. The most commonly reported adverse effects of cysteamine bitartrate therapy are nausea/vomiting, anorexia, diarrhoea, drowsiness, rash, and halitosis [13]. These effects are most frequent at initiation of therapy and may diminish with time. In a study of the relative bioavailability of various cysteamine formulations, Tenneze *et al.* reported vomiting occurring in almost half the subjects, though the effect diminished with time [5].

In summary, cysteamine bitartrate administration results in suppression of WBC cystine content to target levels sought during the treatment of nephropathic cystinosis. Plasma cysteamine concentrations are correlated with reductions in WBC cystine content, though a lag exists between drug concentration and effect. Thus, there appears to be a predictable pharmacokinetic–

pharmacodynamic relationship when cysteamine is used in the treatment of nephropathic cystinosis.

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