doi: 10.1111/fcp.12009

ORIGINAL ARTICLE

Pharmacokinetics of cysteamine bitartrate following intraduodenal delivery

Ranjan Dohil[a,b]*, Betty L. Cabrera[a,b], Jon A. Gangoiti[a,b], Bruce A. Barshop[a,b], Patrice Rioux[c]

[a]*Department of Pediatric, University of California, San Diego, CA, USA*
[b]*Rady Children's Hospital, San Diego, CA, USA*
[c]*Raptor Pharmaceutical Corp., Novato, CA, USA*

Keywords
bioavailability, biodistribution, cerebrospinal fluid and tissue cystine levels, cystinosis, intraduodenal, tissue

Received 26 April 2012; revised 28 August 2012; accepted 24 September 2012

*Correspondence and reprints: rdohil@ucsd.edu

ABSTRACT

Cysteamine is approved for the treatment of cystinosis and is being evaluated for Huntington's disease and non-alcoholic fatty liver disease. Little is known about the bioavailability and biodistribution of the drug. The aim was to determine plasma, cerebrospinal fluid (CSF), and tissue (liver, kidney, muscle) cysteamine levels following intraduodenal delivery of the drug in rats pretreated and naïve to cysteamine and to estimate the hepatic first-pass effect on cysteamine. Healthy male rats ($n = 66$) underwent intraduodenal and portal (PV) or jugular (JVC) venous catheterization. Half were pretreated with cysteamine, and half were naïve. Following intraduodenal cysteamine (20 mg/kg), serial blood samples were collected from the PV or the JVC. Animals were sacrificed at specific time points, and CSF and tissue were collected. Cysteamine levels were determined in plasma, CSF, and tissue. The C_{max} was achieved in 5–10 min from PV and 5–22.5 min from JVC. The PV-C_{max} ($P = 0.08$), PV-AUC_{0-t} ($P = 0.16$), JVC-C_{max} ($P = 0.02$) and JVC-AUC_{0-t} ($P = 0.03$) were higher in naive than in pretreated animals. Plasma cysteamine levels returned to baseline in ≤ 120 min. The hepatic first-pass effect was estimated at 40%. Peak tissue and CSF cysteamine levels occurred ≤ 22.5 min, but returned to baseline levels ≤ 180 min. There was no difference in CSF and tissue cysteamine levels between naïve and pretreated groups, although cysteamine was more rapidly cleared in the pretreated group. Cysteamine is rapidly absorbed from the small intestine, undergoes significant hepatic first-pass metabolism, crosses the blood brain barrier, and is almost undetectable in plasma, CSF, and body tissues 2 h after ingestion. Sustained-release cysteamine may provide prolonged tissue exposure.

INTRODUCTION

Cysteamine bitartrate is an aminothiol agent approved for the treatment of the lysosomal storage disorder cystinosis [1,2]. Cysteamine (Cystagon®, Mylan, Morgantown, WV, USA) enters the lysosome and forms a mixed disulfide (MDS) with cystine, which then leaves the lysosome. Sustained suppression of tissue cystine will result in the reduced rate of progression to renal failure, but this can only be achieved by giving cysteamine every 6 h [3,4]. Tissue levels of cystine are monitored using the surrogate marker white blood cell (WBC) cystine, and levels of <1 nmol 1/2 cystine per mg protein are considered optimal. Cysteamine and its oxidized form, cystamine, have antioxidant and transglutaminase reducing activity and are being evaluated as potential therapies for Huntington's disease (HD) and non-alcoholic fatty liver disease (NAFLD) as well as other disorders associated with severe oxidative stress and abnormal fibrosis [5–14].

Although cysteamine bitartrate has been commercially available since 1994, little is known about its bioavailability and biodistribution. Previous studies in humans showed that direct delivery of cysteamine into the small intestine (SI) resulted in better drug absorption, prolonged suppression of WBC cystine levels, and possibly fewer gastrointestinal (GI) symptoms [15–17]. These data were important in the development of cysteamine bitartrate delayed release (DR cysteamine), which recently was shown in clinical studies to be effective in treating cystinosis using fewer daily doses [18]. For the purposes of our study, it was important to evaluate the biodistribution of cysteamine following SI drug delivery. This was more likely to mimic DR cysteamine and also to reduce the effects of cysteamine on delaying gastric emptying and therefore the rate at which orally administered drug would be delivered to the SI [19].

In our study, we administered cysteamine to rats directly into the SI through an indwelling duodenal catheter and measured cysteamine levels in plasma (from the portal and jugular veins), in cerebrospinal fluid (CSF), and from liver, kidney, and muscle tissue. We also compared the plasma and tissue cysteamine concentrations in rats that were pretreated with those that were naïve to the drug to determine if cysteamine pretreatment impacts absorption or the rate of clearance of the drug.

METHODS

All animal procedures were reviewed by the Institutional Animal Care and Use Committee and performed by Absorption Systems, Inc. (San Diego, CA, USA). Healthy male Sprague–Dawley rats (Charles Rivers Laboratories, Wilmington, MA, USA) underwent intraduodenal and venous catheterization under anesthesia 1 week before the study commenced. Animals were housed individually and allowed free access to water and food during the acclimatization period.

Rats were randomly assigned into one of two groups. The pretreated group (n = 33) received intraduodenal cysteamine bitartrate (20 mg/kg) twice daily for 48 h with the last dose given 12 h before the study test dose was administered. The naïve group (n = 33) were not pretreated with cysteamine. Food was not given for 12 h before study samples were taken but water was permitted. The dose of cysteamine used in this study was based upon previous work in human adults, which showed that the mean plasma drug concentration was satisfactory (51 μM) following intraduodenal delivery of 6 mg/kg body weight of cysteamine [20]. We used a dose of 20 mg/kg body weight for this study because rats have a faster metabolic rate than humans, and this dose was deemed also to be safe for both [20,21].

The pretreated and naïve groups were each subdivided into five groups (see *Table I*). Animals in groups 1A and 1B had a portal vein (PV) catheter and those in groups 2A and 2B had a jugular vena cava (JVC) catheter inserted. To estimate the hepatic first-pass effect on cysteamine, prehepatic blood was drawn from the PV and post-hepatic blood from the JVC for blood sampling. A single dose of cysteamine was delivered through the intraduodenal catheter, and blood samples (250 μL) were obtained in half of the animals at 0, 10, 22.5, 37.5, 60, 120, 480 min and at 5, 15, 30, 45, 90, 180 min in the other half. Blood was drawn in heparinized syringes and spun down at 4 °C. Animals in group 3 had CSF and tissue samples (liver, kidney, muscle) taken at 0, 22.5, 37.5, 60, 180, 360, and 1440 min post test drug. For collection of samples at these time points, animals were anesthetized using ketamine/xylazine; CSF was obtained via the cisterna

Table I The cysteamine pretreated (n = 33) and naïve groups (n = 33) were further subdivided as shown above. Animals that were pretreated received intraduodenal cysteamine twice daily for 48 h, with the last predose 12 h before staring the study.

Dose groups	Number of rats per time point	Dosing route	Blood sampling site	Blood sampling time points for plasma	Tissue collection time points (CSF, liver, kidney, muscle from hind legs)
Group 1A (3 rats total)	3	Duodenal	Portal vein	0, 10, 22.5, 37.5, 60, 120, and 360 min	N/A
Group 1B (3 rats total)	3	Duodenal	Portal vein	5, 15, 30, 45, 90, and 180 min	N/A
Group 2A (3 rats total)	3	Duodenal	JVC	0, 10, 22.5, 37.5, 60, 120, and 360 min	N/A
Group 2B (3 rats total)	3	Duodenal	JVC	5, 15, 30, 45, 90, and 180 min	N/A
Group 3 (21 rats total)	3	Duodenal	N/A	N/A	0, 22.5, 37.5 min, 60, 180, 360, and 1440 min

magna before they were exsanguinated and killed, and liver, kidney, and hind leg muscle were collected.

Plasma, CSF, and tissue were immediately frozen on dry ice and stored at −70 °C until analysis.

Plasma and cerebrospinal fluid cysteamine measurement

Plasma or CSF (25 μL) was mixed with 50 μL of 12.5 μL $^{2}H_4$-cysteamine, as internal standard. Disulfide bonds were selectively and quantitatively reduced by incubation with tris(2-carboxyethyl)phosphine for 1 h at 37 °C. Deproteinization was achieved by adding 1% formic acid in acetonitrile, and the supernatant collected after centrifugation for 10 min at 15 000 rpm. The supernatant was diluted fourfold with HPLC-grade water, 20 μL of which was injected into an API 4000 triple quadruple mass spectrometer (AB Sciex, Foster City, CA, USA) to determine total cysteamine concentration. Samples were delivered in a 70% acetonitrile in 2 mM ammonium formate +3.6 mM formic acid mobile phase and isocratically separated at 37 °C on a TSKgel Amide-80 HILIC column and guard cartridge containing carbamoyl (amide) covalently bonded silica at a flow rate of 0.5 mL/min. Positive electrospray ionization was employed, and specific multiple reaction monitoring transitions were tailored for cysteamine and $^{2}H_4$-cysteamine, using a collision energy of 22 eV and unit mass resolution. Cysteamine concentrations were calculated from an 8-point calibration curve (1, 2, 7.5, 22.5, 75, 112.5, 135, and 150 μM) constructed by supplementing plasma with the appropriate amounts of cysteamine, and quality control samples were run with each batch.

Tissue cysteamine measurement

The validated analytical method available for total plasma cysteamine was adapted to the different tissue matrixes (liver, kidney, muscle (hind leg), and CSF). Briefly, 25–50 mg of flash-frozen tissue was added to 0.5 mL HPLC-grade water. Samples were homogenized using a Precelly® 24-Dual homogenizer with 12.5 μM $^{2}H_4$-cysteamine, as internal standard. An optimized volume of TCEP was added and incubated for 1 h at 37 °C. The samples were then deproteinized using 1% formic acid in acetonitrile. The supernatant was collected and transferred to a new microtube and stored refrigerated.

Cysteamine levels were analyzed as indicated above for plasma samples. Protein levels were assayed using the Lowry method.

Pharmacokinetics and statistical analyses

The following pharmacokinetic parameters were calculated (Pharsight WinNonlin 6.2, St Louis, MO, USA) by standard non-compartmental methods:

- AUC_{0-t}: area under the concentration-time curve from time zero to the last measurable concentration.
- C_{max}: maximum observed concentration.
- T_{max}: time of observed C_{max}.

The statistical comparisons of AUC_{0-t} and C_{max} between groups were carried out using linear mixed models on their ln-transformed. Mixed model ANOVA was used to compare absolute concentrations in tissue and CSF. Tukey method to control for overall significance level was used for multiple pair-wise comparisons.

However, to take into account the limited data, both in blood and in tissues, the following pharmacokinetic parameters were calculated (Pharsight NLME 1.1, St Louis, MO, USA) using a population pharmacokinetic approach.

- V: apparent volume of distribution.
- CL: apparent clearance (volume of plasma cleared of cysteamine per unit time).

Models were estimated by a first-order conditional estimation extended least squares. Although PK of cysteamine in humans, as determined by population pK analyses, was described by a two-compartment model [22], this rat dataset suggested that the pK of cysteamine in rats could also use a one-compartment model. Effects of covariates (pretreated/naïve, site of blood/tissue collection) on both apparent clearance and volume were assessed simultaneously because of correlations between CL and V. Models were compared using the Akaike information goodness-of-fit criterion (AIC) and graphical diagnostic plots.

RESULTS

Sixty-six healthy male rats (mean weight 303 g, range 279–387 g) were studied (*Table I*).

Plasma cysteamine levels

These were measured in six rats in each of the naive and pretreated groups. Because of frequent blood sampling, only three rats were sampled at each time point. The C_{max} from the portal vein occurred at the first sampling (5 and 10 min). The mean PV-C_{max} levels, for all six rats, in the naive and pretreated groups were 281.4 (SEM ± 142.7) and 147.6 (±72.2) μM,

respectively, ($P = 0.08$). The mean PV-AUC_{0-t} measurements for naive and pretreated groups were 6433 (±1603) and 5171 (±1104) min*μM, respectively, ($P = 0.16$). The C_{max} from JVC (JVC-C_{max}) samples was achieved between 5–22.5 min (T_{max}) after drug delivery. The JVC-C_{max} for naive and pretreated groups was 81.9 μM (±22.9) and 53.4 μM (±7.2), respectively, ($P = 0.02$). The JVC-AUC_{0-t} for naive and pretreated groups was 3761 (±813) and 2800 (±383), respectively, ($P = 0.03$). Plasma cysteamine levels returned to baseline by 120 min in all groups studied. See *Figure 1* and *Table II*.

To compensate for sparse sampling and to better understand the pharmacokinetics of cysteamine in rats, population PK modeling was applied. Both naïve and pretreated PV levels were higher than the corresponding JVC concentrations (*Figure 1*). Pretreatment did not impact PV levels but JVC pretreated cysteamine levels were significantly lower than JVC naïve levels. The one-compartment model with the lowest AIC value is the model that includes both covariates (pretreated/naïve and PV/JVC), with PV/JVC being correlated with the volume of distribution V and the clearance CL, and pretreated/naïve being correlated with CL only. The

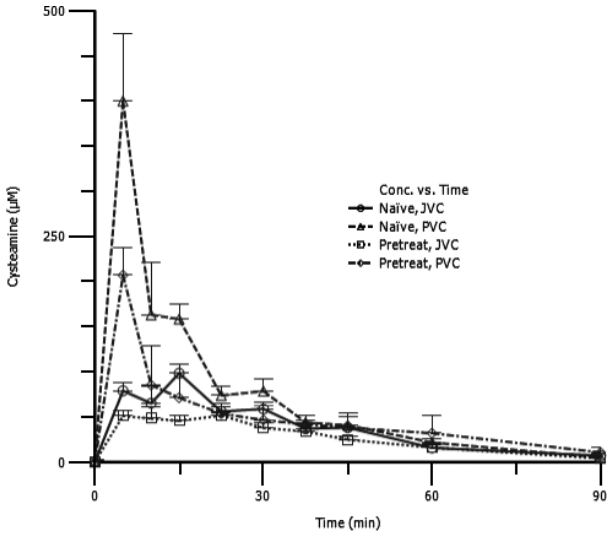

Figure 1 Mean plasma cysteamine levels following intraduodenal cysteamine (20 mg/kg body weight). Samples were taken from the portal vein (PV) and jugular vena cava (JVC) in rats that were either pretreated with or naïve to cysteamine therapy. The C_{max} was achieved in 5–10 min from PV and 5–22.5 min from JVC. The PV-C_{max} ($P = 0.08$), PV-AUC_{0-t} ($P = 0.16$), JVC-C_{max} ($P = 0.02$), and JVC-AUC_{0-t} ($P = 0.03$) were higher in naive than in pretreated animals.

Table II Mean pharmacokinetic parameters following intraduodenal cysteamine (20 mg/kg) delivery in rats that were naïve and pretreated with cysteamine.

	Portal vein		Jugular vena cava	
	Naive	Pretreated	Naive	Pretreated
AUC (min*μM)	6433 (±1603) $P = 0.16$	5171 (±1104)	3761 (±813) $P = 0.03$[a]	2800 (±383)
C_{max} (μM)	281.4 (±142.7) $P = 0.08$	147.6 (±72.2)	81.9 (±22.9) $P = 0.02$[a]	53.4 (±7.2)
T_{max} (min)	5–10		5–22.5	
AUC_{JVC}/ AUC_{PVC}	54%		58%	

The ratio AUC_{JVC}/AUC_{PVC} would suggest a hepatic first-pass effect of about 40%.

[a]Denotes statistical significance between naïve and pretreated groups.

difference between JVC and PV levels can potentially be explained by the hepatic first-pass effect on cysteamine, which does not seem to be influenced by pretreatment, as shown by the ratios of the AUCs determined by non-compartmental analyses: naïve $AUC_{JVC}/AUC_{PVC} = 2800/5170 = 54\%$ and pretreated $AUC_{JVC}/AUC_{PVC} = 3761/6433 = 58\%$, that is, ~40% reduction in both cases. The impact of pretreatment, as determined by population PK, corresponds to a difference in naïve CL_{JVC} (0.066 L/(kg*min)) vs. pretreated CL_{JVC} (0.090 L/(kg*min)) and to a lesser extent in naïve CL_{PV} (0.041 L/(kg*min)) vs. pre treated CL_{PV} (0.055 L/(kg*min)), that is, faster cysteamine elimination or metabolism when pretreated.

Tissue and CSF cysteamine levels

The peak tissue (kidney, liver, muscle) and CSF cysteamine levels were measured at 22.5 min, but may have occurred earlier (*Table III*, *Figures 2 and 3*). There was no significant difference between mean plasma cysteamine and mean CSF levels measured at the same time points in naïve and pretreated groups ($P = 0.2$). In the naïve group, the mean peak cysteamine concentrations in liver, kidney, and muscle were 0.90, 2.56, and 0.24 nmol/mg protein, respectively, and the overall mean concentrations were 0.31, 0.69, and 0.05 nmol/mg protein, respectively. In the pretreatment group, the mean peak cysteamine concentrations in liver, kidney, and muscle were 1.20, 4.61, and 0.26 nmol/mg protein, respectively, and the overall mean concentrations were 0.36, 1.51, and 0.08 nmol/mg protein, respectively. There was no statistical difference between these groups except for the naïve and pretreated kidney group ($P = 0.01$). Tissue cysteamine

Table III Mean tissue cysteamine concentrations in nmol/mg protein following intraduodenal delivery of cysteamine (20 mg/kg).

	Mean tissue cysteamine concentrations in nmol/mg protein						
	Baseline	22.5 min	37.5 min	60 min	180 min	360 min	1440 min
Liver-Naive	0.15 (0.02)	0.9 (0.21)	0.34 (0.07)	0.29 (0.04)	0.17 (0.01)	0.2 (0.02)	0.11 (0.02)
Liver-Pretreated	0.15 (0.01)	1.2 (0.39)	0.69 (0.21)	0.2 (0.02)	0.13 (0.007)	0.09 (0.01)	0.06 (0.009)
Kidney-Naive	0.24 (0.009)	2.26 (1.2)	0.74 (0.09)	0.53 (0.09)	0.23 (0)	0.23 (0.01)	0.31 (0.12)
Kidney-Pretreated	0.5 (0.04)	4.61 (0.72)	2.8 (0.23)	1.3 (0.17)	0.51 (0.05)	0.47 (0.13)	0.34 (0.05)
Muscle-Naive	0 (0)	0.24 (0.02)	0.07 (0.01)	0.04 (0.009)	0 (0)	0 (0)	0 (0)
Muscle-Pretreated	0.007 (0.003)	0.26 (0.08)	0.2 (0.03)	0.07 (0.003)	0.007 (0.003)	0.003 (0.003)	0.01 (0)

Standard error of the mean is shown in parenthesis.

levels returned to baseline between 60–180 min in all tissues for naïve and pretreated groups. Although tissue measurements were not taken at 120 min, by using population PK modeling, we estimated that the tissue half-life for cysteamine was 25–29 min and that >95% of cysteamine would be eliminated by 150 min.

DISCUSSION

There are very few published data on the bioavailability and biodistribution of cysteamine bitartrate, particularly following oral ingestion [23]. In patients with cystinosis, a number of factors may impact the optimal absorption of cysteamine such as intestinal dysmotility, which can be caused by the disease or by the drug itself [24].

Cysteamine has been reported to slow gastric motility, and this may result in erratic delivery to the SI where drug absorption is most effective [15,19]. A recent study in cystinosis showed that cysteamine delivered directly into the SI had a significantly higher C_{max} and AUC (plasma concentration-time gradient) than with gastric or colonic delivery [15]. Therefore, to avoid the effect of erratic drug delivery from stomach to SI, we administered cysteamine through an indwelling intraduodenal catheter. Our study shows that cysteamine is well absorbed from the SI and, as expected,

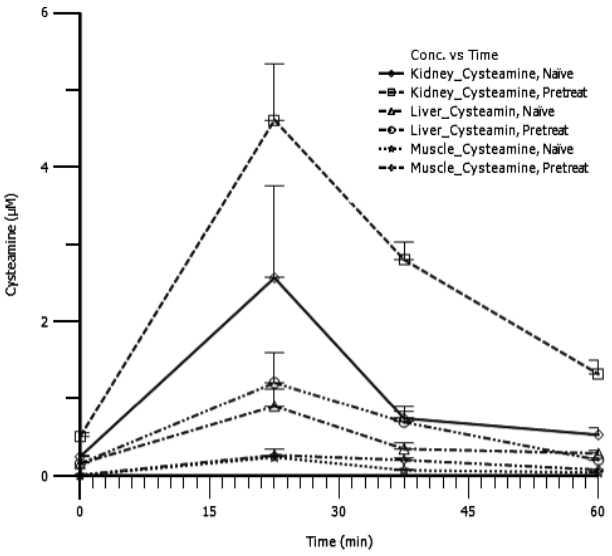

Figure 2 Mean cysteamine concentrations (nmol/mg protein) in kidney, liver, and muscle measured at predetermined time points in rats that were either pretreated with or naïve to cysteamine therapy. There was a statistical difference between the naïve and pretreated groups for kidney ($P = 0.01$), but not for liver ($P = 0.55$) or muscle ($P = 0.24$).

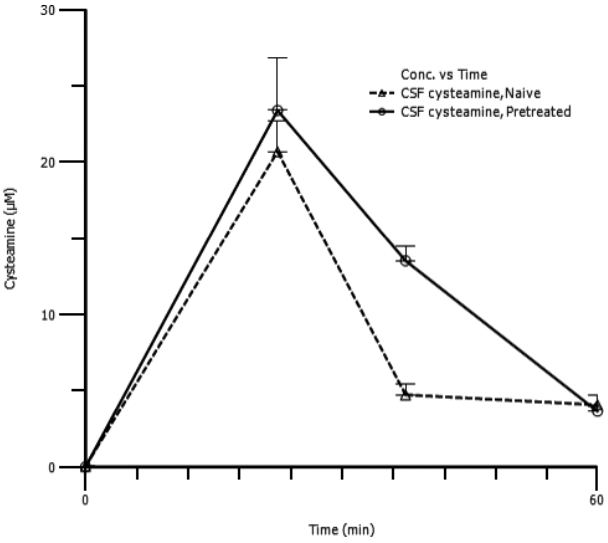

Figure 3 Mean cysteamine levels in cerebrospinal fluid measured at predetermined time points in rats that were either pretreated with or naïve to cysteamine therapy. There was no statistical difference between the naïve and pretreated groups for CSF ($P = 0.22$).

those measurements taken from the prehepatic PV were higher than those from the JVC at the same time points. Over 40% of the delivered drug was metabolized during hepatic first pass in both the naïve and pretreated groups. The JVC-C_{max} ($P = 0.02$) and AUC ($P = 0.03$) measurements were significantly higher in naïve than pretreated groups possibly because drug absorption was higher in the naïve groups and/or tissue metabolism of cysteamine was higher in the pretreated group. Better absorption in the naïve group may be explained by a recent animal study, which reports that cysteamine uptake from the intestinal tract is a saturable carrier-mediated process that involves organic cationic transporters [25]. It is unlikely that lower cysteamine levels in the pretreated group are due to increased renal clearance of the drug as it was previously shown in humans with cystinosis that <1% of the ingested dose of cysteamine was found in urine [26]. From a clinical perspective, this might explain why patients can tolerate cysteamine better, with fewer GI and other symptoms, when the dose is increased slowly over 4–6 weeks. It may also explain why children treated with cysteamine for NAFLD initially complained of GI symptoms when the therapeutic dose was achieved rapidly, but as the study progressed, the frequency of reported symptoms diminished [5].

Extrapolating animal model pharmacokinetics to humans may be difficult as it is usually based upon the difference in size between the two and does not include other factors such as rates of absorption or elimination or bioavailability (e.g., the different protein and metabolizing enzyme profiles that exist in different species). Our own previous human studies have shown that plasma cysteamine levels clear 4–6 h after cysteamine is delivered directly to the SI [20]. In rats, the cysteamine cleared from the plasma within 2 h. This may be because rats have a faster metabolic rate than humans. It should be noted, however, that although the rats in our study received about three times as much cysteamine (20 mg/kg in rats vs. 6 mg/kg body weight in adult humans), a number of the pharmacokinetic parameters were similar. Following SI drug delivery, the mean AUC_{0-t}, mean C_{max}, and mean T_{max} from JVC levels in naïve group rats were very similar to that in naïve humans with levels of 3761 min*μM vs. 3987 min*μM, 81 μM vs. 51 μM, and 15 min vs. 21 min, respectively [20].

In our present animal study, the cysteamine levels returned to baseline in CSF, liver, kidney, and muscle within 2–3 h following drug delivery. Rapid clearance of cysteamine from plasma and tissues has ramifications for the treatment of cystinosis. In this lysosomal storage disorder, oral cysteamine is ingested every 6 h to deplete tissue cystine. If we assume that our animal pK data estimate those seen in humans, then cysteamine may only be detectable above baseline levels for 8–12 h/day. This may help to explain the findings in a recent study in which patients with cystinosis, despite maintaining low WBC cystine levels on regular long-term cysteamine therapy, continued to show large amounts of cystine in there intestinal mucosal tissue [27]. This might then also explain why these patients continue to have deterioration in renal function despite adequate WBC cystine levels. So far, cysteamine has been shown to deplete cystine levels only in the cornea and in circulating WBCs. Corneal cystine depletion requires hourly topical cysteamine application at a much higher concentration than would be achievable or tolerated with oral therapy [28]. Although it is difficult to directly compare plasma and tissue levels of cysteamine, it is likely that drug levels are higher in plasma than in tissue. Circulating WBCs may be exposed to higher levels of cysteamine and therefore have lower cystine levels than in body tissues. For this reason, WBC levels may not accurately reflect tissue cystine levels.

Cysteamine's antioxidant effect may help to reduce cell dysfunction and apoptosis in cystinosis [8,9,29]. Cysteamine is presently also being evaluated as a potential therapeutic agent for conditions such as HD and NAFLD [5,6]. In HD, cysteamine may inhibit transglutaminase activity, which catalyzes polyglutamine expansion of insoluble protein aggregates and eventually neuronal death [12,14]. NAFLD is associated with increased oxidative stress [30], and a recent pilot study showed that cysteamine was effective in normalizing some surrogate markers of NAFLD [5]. Our study data therefore have potential implications for disorders other than cystinosis. Prior to our study, it was thought that cysteamine might remain (protein bound) in the liver after being absorbed [31]. However, our data show that liver cysteamine levels follow similar trends to that seen in other organs and in CSF with C_{max} levels occurring ≤ 20 min and then falling to near baseline within ≤ 120 min. This has implications for cysteamine's therapeutic efficacy in diseases such as cystinosis, NAFLD, or HD and may suggest that a sustained-release formulation would be more effective than the presently available rapid-release cysteamine bitartrate (Cystagon®).

Our study provides pK data following a single intraduodenal bolus of cysteamine. The drug is rapidly absorbed from the SI, undergoes significant hepatic first-pass metabolism, crosses the blood brain barrier, and is almost undetectable in plasma, CSF, or body tissues 2–3 h after ingestion. This information may help in developing cysteamine formulations that would be more effective than currently available rapid-release cysteamine. It is possible that a sustained-release formulation of cysteamine may result in prolonged exposure to specific organs and therefore more effective in conditions such as cystinosis, NAFLD, and HD.

CONFLICT OF INTEREST

The University of California, San Diego, has a financial interest in Raptor Pharmaceutics, the company sponsoring this research. Patrice Rioux is Chief Medical Officer of Raptor Pharmaceuticals. Dr Dohil and the University of California may financially benefit from this interest if the company is successful in developing and marketing a cysteamine product for the treatment of cystinosis and NAFLD. The terms of this arrangement have been reviewed and approved by the University of California, San Diego, in accordance with its conflict of interest policies. The other investigators have no conflicts of interest. Support was provided by Raptor Pharmaceuticals, Novato, CA, and The Cystinosis Research Foundation, Irvine, CA.

REFERENCES

1 Gahl W.A., Thoene J.G., Schneider J.A. Cystinosis. N. Engl. J. Med. (2002) **347** 111–121.
2 Gahl W.A., Reed G.F., Thoene J.G. et al. Cysteamine therapy for children with nephropathic cystinosis. N. Engl. J. Med. (1987) **316** 971–977.
3 Markello T.C., Bernardini I.M., Gahl W.A. Improved renal function in children with cystinosis treated with cysteamine. N. Engl. J. Med. (1993) **328** 1157–1162.
4 Kleta R., Bernardini I., Ueda M. et al. Long-term follow-up of well-treated nephropathic cystinosis patients. J. Pediatr. (2004) **145** 555–560.
5 Dohil R., Schmeltzer S., Cabrera B.L. et al. Enteric-coated cysteamine for the treatment of paediatric non-alcoholic fatty liver disease. Aliment. Pharmacol. Ther. (2011) **33** 1036–1044.
6 Dedeoglu A., Kubilus J.K., Jeitner T.M. et al. Therapeutic effects of cystamine in a murine model of Huntington's disease. J. Neurosci. (2002) **22** 8942–8950.
7 Lesort M., Lee M., Tucholski J., Johnson G.V. Cystamine inhibits caspase activity. Implications for the treatment of polyglutamine disorders. J. Biol. Chem. (2003) **278** 3825–3830.
8 Kessler A., Biasibetti M., da Silva Melo D.A. et al. Antioxidant effect of cysteamine in brain cortex of young rats. Neurochem. Res. (2008) **33** 737–744.
9 Kessler A., Biasibetti M., Feksa L.R. et al. Effects of cysteamine on oxidative status in cerebral cortex of rats. Metab. Brain Dis. (2008) **23** 81–93.
10 Maher P., Lewerenz J., Lozano C., Torres J.L. A novel approach to enhancing cellular glutathione levels. J. Neurochem. (2008) **107** 690–700.
11 Wood P.L., Khan M.A., Moskal J.R. Cellular thiol pools are responsible for sequestration of cytotoxic reactive aldehydes: central role of free cysteine and cysteamine. Brain Res. (2007) **1158** 158–163.
12 Lorand L. DRPLA aggregation and transglutaminase, revisited. Nat. Genet. (1998) **20** 231.
13 Jeitner T.M., Delikatny E.J., Ahlqvist J., Capper H., Cooper A.J. Mechanism for the inhibition of transglutaminase 2 by cystamine. Biochem. Pharmacol. (2005) **69** 961–970.
14 Hoffner G., Andre W., Vanhoutteghem A., Soues S., Djian P. Transglutaminase-catalyzed crosslinking in neurological disease: from experimental evidence to therapeutic inhibition. CNS Neurol. Disord. Drug. Targets (2010) **9** 217–231.
15 Dohil R., Fidler M., Barshop B.A. et al. Understanding intestinal cysteamine bitartrate absorption. J. Pediatr. (2006) **148** 764–769.
16 Dohil R., Fidler M., Gangoiti J.A., Kaskel F., Schneider J.A., Barshop B.A. Twice-daily cysteamine bitartrate therapy for children with cystinosis. J. Pediatr. (2010) **156** 71–75.
17 Dohil R., Gangoiti J.A., Cabrera B.L., Fidler M., Schneider J.A., Barshop B.A. Long-term treatment of cystinosis in children with twice-daily cysteamine. J. Pediatr. (2010) **156** 823–827.
18 Langman C.B., Greenbaum L.A., Sarwar M. et al. A randomized controlled crossover trial with RP103 for nephropathic cystinosis. Clin. J. Am. Soc. Nephrol. (2012) **7** 112–120
19 Lichtenberger L.M., Szabo S., Reynolds E.S. Gastric emptying in the rat is inhibited by the duodenal ulcerogens, cysteamine and propionitrile. Gastroenterology (1977) **73** 1072–1076.
20 Fidler M.C., Barshop B.A., Gangoiti J.A. et al. Pharmacokinetics of cysteamine bitartrate following gastrointestinal infusion. Br. J. Clin. Pharmacol. (2007) **63** 36–40.
21 Szabo S. Duodenal ulcer disease. Animal model: cysteamine-induced acute and chronic duodenal ulcer in the rat. Am. J. Pathol. (1978) **93** 273–276.
22 Belldina E.B., Huang M.Y., Schneider J.A., Brundage R.C., Tracy T.S. Steady-state pharmacokinetics and pharmacodynamics of cysteamine bitartrate in paediatric nephropathic cystinosis patients. Br. J. Clin. Pharmacol. (2003) **56** 520–525.
23 Eldjarn L., Nygaard O. Cysteamine-cystamine: intestinal absorption, distribution among various organs and excretion. Arch. Int. Physiol. Biochim. (1954) **62** 476–486.
24 Sonies B.C., Almajid P., Kleta R., Bernardini I., Gahl W.A. Swallowing dysfunction in 101 patients with nephropathic

cystinosis: benefit of long-term cysteamine therapy. Medicine (Baltimore) (2005) **84** 137–146.
25 Khomenko T., Kolodney J., Pinto J.T. et al. New mechanistic explanation for the localization of ulcers in the rat duodenum: role of iron and selective uptake of cysteamine. Arch. Biochem. Biophys. (2012) **55** 60–70
26 Levtchenko E.N., de Graaf-Hess A., Blom H.J., Monnens L.A. Negligible urinary cysteamine loss in cystinosis patients with Fanconi syndrome. Clin. Nephrol. (2002) **57** 349–351.
27 Dohil R., Carrigg A., Newbury R. A potential new method to estimate tissue cystine content in nephropathic cystinosis. J. Pediatr. (2012) **161** 531–535.
28 Kaiser-Kupfer M.I., Fujikawa L., Kuwabara T., Jain S., Gahl W.A. Removal of corneal crystals by topical cysteamine in nephropathic cystinosis. N. Engl. J. Med. (1987) **316** 775–779.
29 Park M., Helip-Wooley A., Thoene J. Lysosomal cystine storage augments apoptosis in cultured human fibroblasts and renal tubular epithelial cells. J. Am. Soc. Nephrol. (2002) **13** 2878–2887.
30 Sanyal A.J., Campbell-Sargent C., Mirshahi F. et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology (2001) **120** 1183–1192.
31 Gangoiti J.A., Fidler M., Cabrera B.L., Schneider J.A., Barshop B.A., Dohil R. Pharmacokinetics of enteric-coated cysteamine bitartrate in healthy adults: a pilot study. Br. J. Clin. Pharmacol. (2010) **70** 376–382.