

Correlation of Plasma Cysteamine and WBC Cystine Levels at Steady State in Patients Treated with Cysteamine Bitartrate.

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Abstract

Cystinosis patients on a stable dose of Cystagon® for at least 21 days prior to screening, enrolled in a pilot study to assess the safety, tolerability and pharmacokinetics (PK) and pharmacodynamics (PD) of cysteamine bitartrate delayed-release capsules (RP103), compared to cysteamine bitartrate capsules, (Cystagon®). Although patients were under variable doses of cysteamine bitartrate (350 mg x 4 times a day to 750 mg x 4 times a day) such that they were considered as effectively treated by their treating physician, with a WBC cystine level less than 1 nmol/half-cystine/mg protein, their single-dose pharmacodynamic cysteamine curves were all very similar, with a cysteamine concentration at 6 hours post-dose of Cystagon® (or pre-dose) at 5.08 ± 3.16 (mean + standard deviation) $\mu\text{mol/L}$ and a corresponding C_{max} at 48.46 ± 18.63 $\mu\text{mol/L}$. An examination of the individual plots of cysteamine plasma levels versus time and WBC cystine concentrations versus time indicated little or no delay between the maximum plasma and the minimum concentrations of cystine for the majority of individuals. This would be indicative of minimal hysteresis thereby negating the need to collapse the hysteresis using an appropriate model (e.g. effect compartment model). Thus, an inhibitory fractional sigmoid E_{max} PD model describing the relationship between plasma cysteamine and WBC cystine

$$\text{Cystine} = E_0 \cdot (1 - \text{Cysteamine}^{\gamma} / (\text{Cysteamine}^{\gamma} + EC_{50}^{\gamma}))$$

with $E_0 = 2.24$, $EC_{50} = 2.23$, $\gamma = 0.84$ provided a good fit to the data from the pilot study.

Knowing the difficulty of realizing WBC cystine measurements, with cell preparation happening as soon as possible after blood sampling, and the significant amount of blood necessary (~4 to 10 mL), the characterization of a relationship between the level of cysteamine and WBC cystine would lead to use instead an easy measurement like the plasma cysteamine concentration pre-dose for the determination of the dose of cysteamine bitartrate necessary to effectively treat patients.

Cysteamine for Nephropathic Cystinosis

Nephropathic cystinosis is a rare lysosomal storage disease due to an autosomal recessive genetic mutation affecting about 500 people in the United States. This mutation disables the mechanism for clearing the amino acid cystine, a breakdown product of cellular proteins. This results in an accumulation of cystine crystals in all organs and tissues.

The condition is usually diagnosed in early childhood when patients exhibit poor growth, vision problems (photophobia) and specific kidney problems (called Fanconi syndrome). If left untreated, cystinosis destroys major organ systems including the kidneys, eyes, liver, muscles, pancreas and the brain.

The goal of cysteamine treatment of cystinosis is to reduce cystine levels in cells. Studies have shown that cysteamine therapy may delay and/or prevent kidney transplant and other clinical manifestations of the disease.¹⁻⁴

However, patient compliance is challenging due to frequent dosing and gastrointestinal side effects.^{2,4-6} Unfortunately, it has been shown that decreased compliance, even reduction of dosing from 6 to 9 hours, with current formulation can result in poorer reduction of white cell cystine and worse renal function.¹

Pilot (Phase IIb) Study

A pilot study was recently completed in order to assess the pharmacokinetics (i.e., cysteamine C_{max} and T_{max}), pharmacodynamics (i.e., WBC cystine levels), safety and tolerability of Cysteamine Bitartrate Delayed-release Capsules (RP103) compared to Cystagon® in 9 patients with cystinosis.

Methods

Cysteamine: A Hydrophilic Interaction Liquid Chromatography (HILIC) HPLC electrospray tandem mass spectrometry (HPLC-MS/MS) was used to measure plasma free and total cyst(e)amine.

WBC Cystine: Cystine Determination was done by tandem mass spectrometer (Sciex API-4000). Tandem mass spectrometry, allows fast, sensitive and accurate analysis compared with the classical cystine binding protein (CBP) assay.

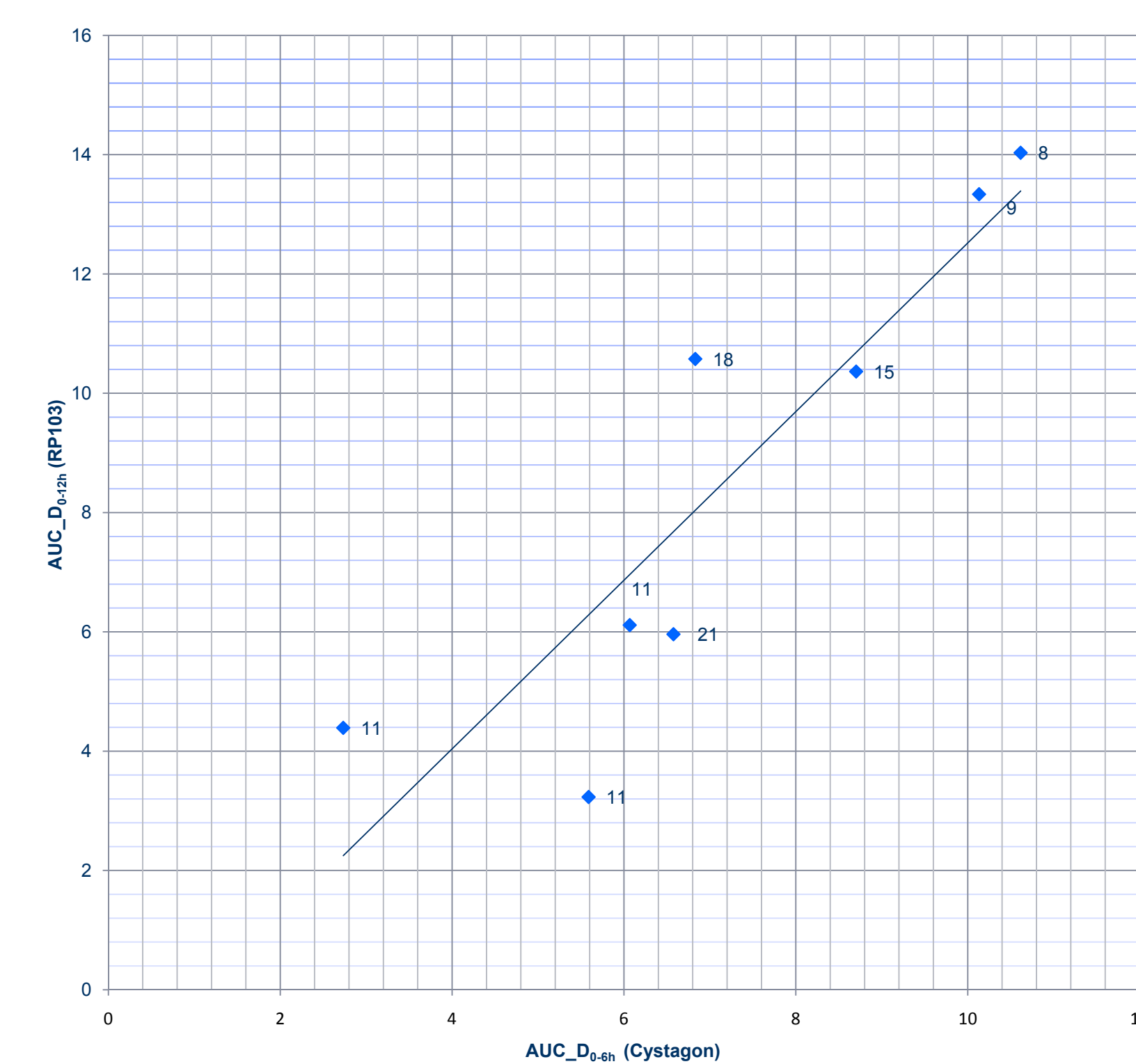
PK/PD Modeling: Phoenix WinNonlin v6.0 (Pharsight, CA) and SAS Software v9.2 (SAS Institute, NY) were used for pharmacokinetics and pharmacodynamics modeling.

WBC Cystine sample preparation (1-2 hours)

The flowchart details the following steps:

- STEP 1:** Collect 10 mL of blood into a heparinized tube. Centrifuge at 1000g for 10 minutes. Remove plasma and store at -20°C.
- STEP 2:** Wash red blood cells (RBCs) with distilled water to remove plasma. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 3:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 4:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 5:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 6:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 7:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 8:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 9:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 10:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.
- STEP 11:** Resuspend RBCs in distilled water. Centrifuge at 1000g for 10 minutes. Repeat until supernatant is clear.

Comparative Cysteamine Bioavailability



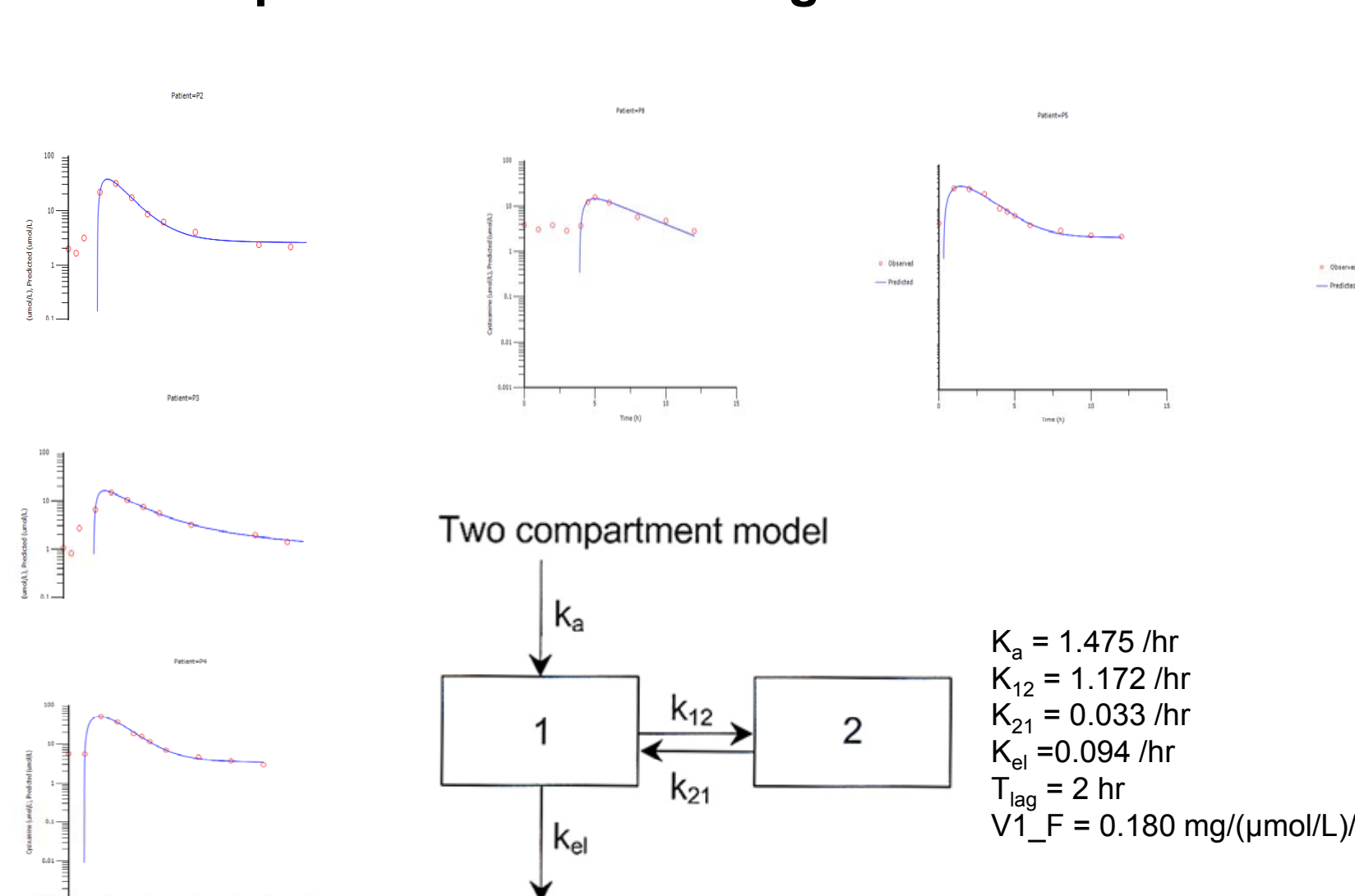
A regression of the AUC_{0-12h} normalized by dose in mg/kg ($AUC_{D_{0-12h}}$) for RP103 versus AUC_{0-6h} normalized by dose in mg/kg ($AUC_{D_{0-6h}}$) for Cystagon® suggests a linear relationship with a slope of 1.4136. Thus, to achieve an equivalent exposure of cysteamine over a 12 hour dosing interval as measured by AUC_{0-12h} , a single dose of RP103 will have to be equal to $2/1.4136 = 1.4148$ times a single dose of Cystagon®, which corresponds to a daily dose of RP103 equal to 70% of a daily dose of Cystagon®. As it can also be seen on this figure, when using the administered dose of Cystagon® for labeling individual AUCs, there is not a good relationship between dose and AUC, due to a high inter-patient variability in absorption/elimination of cysteamine (highest AUCs corresponding to lowest doses for example) despite a very low intra-patient variability (AUCs after RP103 being easily predictable from AUCs after Cystagon®).

DR-cysteamine (RP103) pharmacokinetics

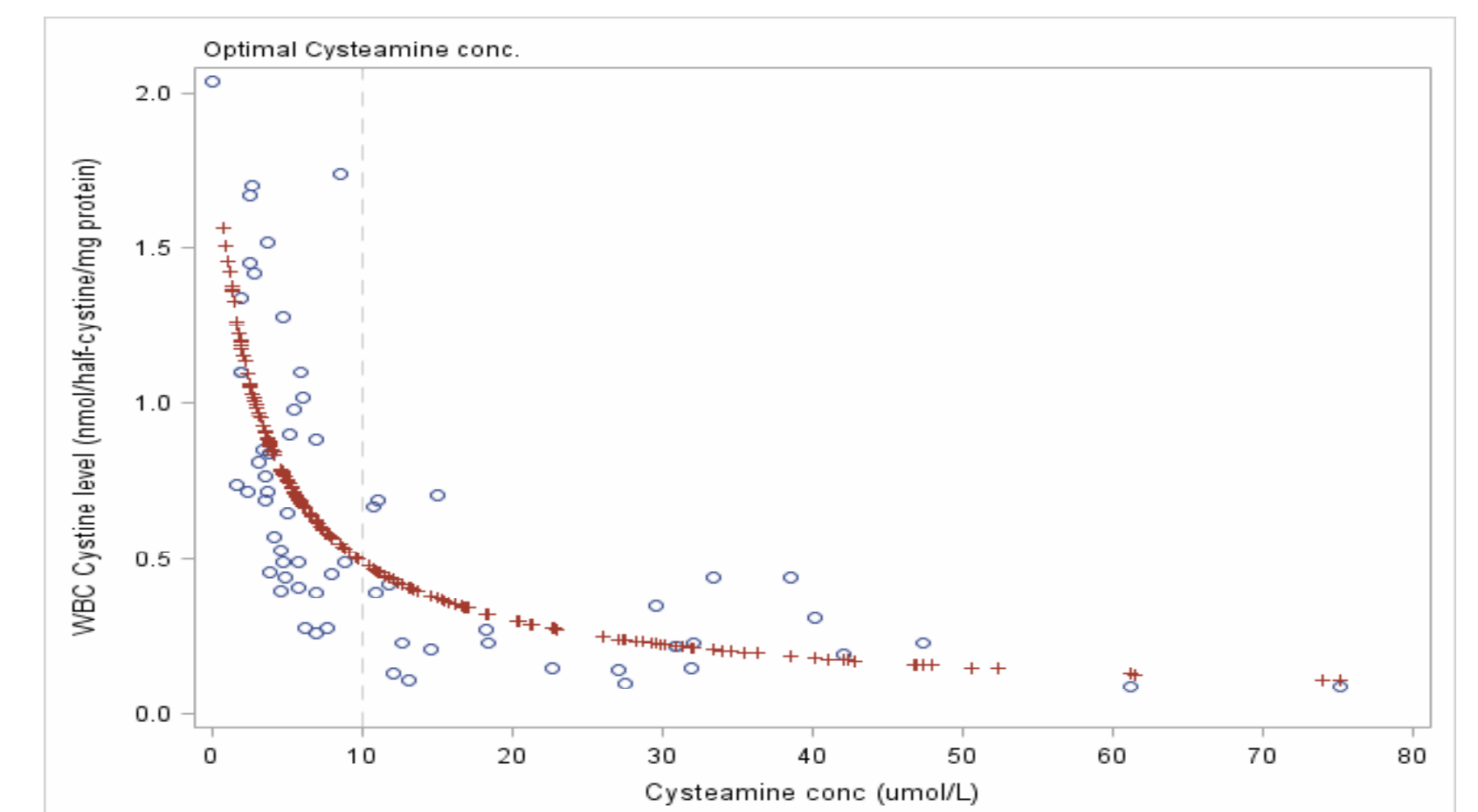
Non-Compartmental Analysis

Patient Identifier	Dose (mg)	C_{max} (nmol/L)	$C_{\text{max}}/\text{Dose}$ (nmol/L/mg)	T_{max} (h)	Terminal $t_{1/2}$ (h)	AUC_{0-12h} (nmol·h/L)	AUC_{0-12h}/Dose (nmol·h/L/mg)
P1	450	9.72	9.72	4	1.63	31.3	97.7
P2	350	30.9	42.55	3	4.44	83.4	156
P3	450	15	15	3	4.3	44.5	63
P4	450	50.6	50.6	2	6.17	127.2	153.6
P5	450	30.2	30.2	1	9.09	104.4	124
P6	750	61.2	36.72	4	5.4	137.9	186.4
P7	1050	31.9	13.67	0	11.31	82	121.7
P8	450	15.7	15.7	5	3.91	38.4	79.5
P9	750	32.3	31.38	3	6.4	157.7	197.4
Mean	566.7	33.66	27.79	2.78	6.88	89.6	118.2
SD	234.8	18.22	14.99	1.56	2.89	45.8	54.6

2-Compartment Model Fitting

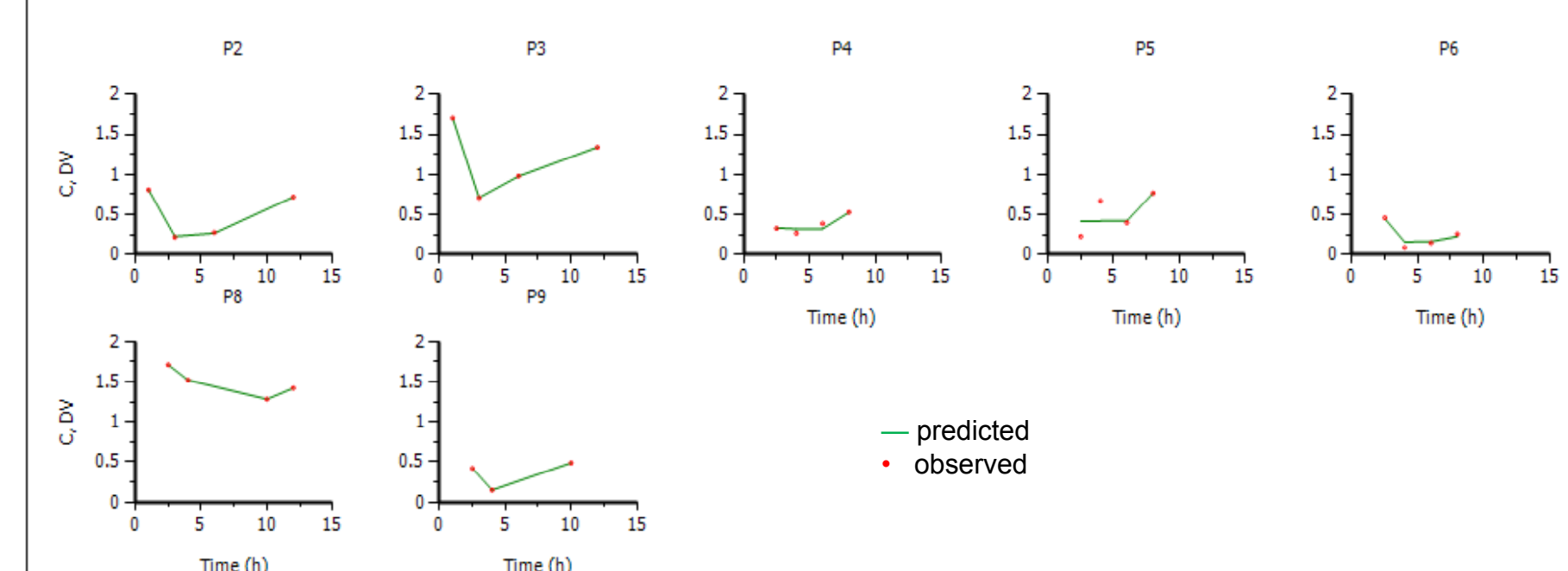


PK/PD relationship: WBC Cystine vs. Cysteamine



Inhibitory fractional sigmoid E_{max} PD model:
 $\text{Cystine} = E_0 \cdot (1 - \text{Cysteamine}^{\gamma} / (\text{Cysteamine}^{\gamma} + EC_{50}^{\gamma}))$
with $E_0 = 2.24$, $EC_{50} = 2.23$, $\gamma = 0.84$

PK/PD Model: 2-compartment PK model & Inhibitory E_{max} PD model combined



A PK/PD model provided a very good fit to the data, and even when there are some discrepancies (cf. Patients 5 and 6) around the C_{max} of cysteamine, the pre-dose (or 12-hour post-dose) WBC cystine level can be very well predicted based only on the pre-dose cysteamine concentration.

Conclusion

A PK/PD model combining a 2-compartment PK model for cysteamine and a fractional inhibitory E_{max} PD model for WBC cystine provides a very good fit to the data collected in 9 patients after single oral administration of a delayed-release formulation of cysteamine bitartrate (RP103). A 2-compartment PK model for cysteamine and a fractional inhibitory E_{max} PD model for WBC cystine have been described after single oral administration of a standard release formulation of cysteamine bitartrate.⁷

Based on this PK/PD model, pre-dose cysteamine concentration should be predictive enough to avoid using pre-dose WBC cystine as a surrogate marker, otherwise difficult to measure in standard clinical settings. Long-term studies will be necessary though to determine this minimum pre-dose cysteamine concentration for any specific drug formulation.

References

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