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ORIGINAL ARTICLE

A novel sustained-release cysteamine bitartrate formulation for the treatment of cystinosis: Pharmacokinetics and safety in healthy male volunteers

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Abstract

The strict intake regimen of cysteamine bitartrate formulations, associated with side effects, is a concern for the treatment compliance in cystinosis therapy. Therefore, there is a need for a cysteamine formulation with an improved pharmacokinetic profile. This study investigated the pharmacokinetics, safety and tolerability of a new sustained-release cysteamine dosage form, PO-001, in healthy volunteers. This was a randomized, investigator-blinded, three-way cross-over study to compare single doses (600 mg) of PO-001 with Cystagon® (immediate-release) and Procysbi® (delayed-release). Collected blood samples were analyzed for plasma cysteamine concentrations and pharmacokinetic parameters were estimated by noncompartmental analysis. In addition, plasma cysteamine concentrations were analyzed using a population pharmacokinetic approach using NONMEM®. Pharmacokinetics showed clear sustained-release characteristics of PO-001 over time with a lower C_{max} and longer T_{max} compared to Cystagon® and Procysbi®. All treatment-emergent adverse events were of mild severity, with the exception of two subjects who reported moderate severity gastrointestinal problems including vomiting and diarrhea, which were related to Cystagon® intake. Population PK simulations showed a favourable PK profile based on C_{max} and C_{trough} concentrations at steady state. In conclusion, a single dose of 600 mg PO-001 was well tolerated with no findings of clinical concern. This new cysteamine bitartrate formulation showed pharmacokinetics of a sustained-release formulation, which may be beneficial for the treatment of cystinosis patients. This study supports advancing this type of sustained-release formulation into a subsequent

Abbreviations: AEs, Adverse Events; ECG, electrocardiography; MedDRA, Medical Dictionary for Regulatory Activities; WBCs, white blood cells.

The authors confirm that the Principal Investigator for this paper is I M C Kamerling and that she had direct clinical responsibility for the subjects. Co-author N B Klarenbeek had medical responsibility for the subjects.

study to confirm reduced dosing frequency with efficient control of white blood cells (WBCs) cystine levels. Netherlands Trial Registry (NTR) (NL67638.056.18).

KEYWORDS
compliance, cystagon, cysteamine, cystinosis, sustained-release

1 | INTRODUCTION

Cystinosis is a rare, inherited autosomal recessive disease caused by mutations in the lysosomal cystine carrier cystinosin, encoded by the CTNS gene (MIM 606272; GenBank NM_004937.2 17p13), leading to intralysosomal accumulation of cystine. The accumulation of cystine eventually results in intracellular crystal formation, leading to apoptosis and tissue damage.[1–3] Most patients develop nephropathic cystinosis and present between the age of 6–12 months with polyuria, polydipsia, and failure to thrive due to generalized proximal tubular damage in the kidney, i.e. renal Fanconi syndrome.[4,5]

The current mainstay of cystinosis treatment is cysteamine. Cysteamine reduces intracellular cystine concentrations by converting cystine into the transportable cysteine and cysteine-cysteamine mixed disulfide, which exit the lysosomes via an intact lysine and cysteine transport system, thereby bypassing the defective cystine carrier.[6] The level of cystine in white blood cells (WBCs) as a measure of cystine depletion, is a biomarker of cysteamine treatment efficacy (target <1 nmol hemicystine/mg protein).[7] Early initiation of cysteamine treatment can delay the progression of cystinosis and the development of renal failure.[8,9]

Cystagon® is a registered formulation of immediate-release cysteamine bitartrate for the treatment of nephropathic cystinosis in children and adults in the United States and Europe.[10,11] An important disadvantage of Cystagon® is the strict regimen of the intake of the capsules every 6 h to prevent a rapid rise in cystine levels and associated complications, which requires patients to wake up during the night.[6,12] Furthermore, cysteamine has several side effects; most patients experience gastrointestinal symptoms, such as vomiting and diarrhea, and treatment is associated with a persistent and unpleasant sulfurous body and breath odour (i.e. halitosis).[13,14] Therefore, Cystagon® therapy is often associated with impaired treatment compliance.

Even with optimal treatment compliance, the pharmacokinetic profile of Cystagon® is not optimal, with relatively large variations in peak-trough concentrations due to a short half-life of 4.8 (±1.8) h.[11] High concentrations can result in peak-related side effects, such as gastrointestinal symptoms and halitosis, whereas low concentrations can result in subtherapeutic concentrations. To minimize these large variations, a sustained-release dosage form can be of great benefit for releasing the drug slowly such that the peak-trough fluctuations are small which could potentially reduce gastrointestinal symptoms and the extent of halitosis, both of which may lead to improved patient compliance.

What is already know about this subject?

- The use of cysteamine is recommended for the treatment of cystinosis. However, there is an unmet need for a cysteamine formulation with a pharmacokinetic profile that allows for a reduced dosing frequency and therewith a reduced patient burden.

What this study adds?

- PO-001 showed clear sustained-release characteristics with a favourable pharmacokinetic profile based on the t_{max}, and both C_{max} and C_{trough} concentrations.
- PO-001 could be an advanced treatment for cystinosis patients and pharmacokinetic modeling shows promising possibilities for twice-daily dosing.

An alternative for Cystagon® is Procysbi®, an enteric-coated cysteamine bitartrate formulation, which has been registered in 2013 in the United States and Europe. Procysbi® is a delayed-release formulation and releases cysteamine gradually. Therefore, patients can take this medication less frequently (every 12 h). The enteric coating dissolves at a pH above 5.5 thereby bypassing the stomach.[15]

There is a pressing need for more continuous therapeutic blood levels, as opposed to a high peak-trough variability. Moreover, there is a need for an affordable, patient-friendly therapy for cystinosis without the mentioned side-effects and difficult treatment schedules.

In this study, we investigated a novel sustained-release cysteamine bitartrate formulation (PO-001) for the treatment of cystinosis. This drug is different from current treatment options as it is a sustained-release product by encapsulating the active ingredient with a coating that is non-pH-dependent.[16] In vitro time-dependent and pH-independent release were confirmed and therefore PO-001 is expected to be slowly released in the gastrointestinal tract over an extended period of time, which would decrease the peak-trough variability. As a consequence, patients will have to take PO-001 less than four times a day, which would be less disruptive for daily routines and will improve treatment adherence. In addition, lower peak levels of cysteamine may be associated with less halitosis.

This study was aimed to assess the pharmacokinetics, safety and tolerability of sustained-release cysteamine bitartrate (PO-001)

and to compare with Cystagon® and Procysbi® in healthy male volunteers.

2 | MATERIALS AND METHODS

The study was approved by an independent ethics committee (Stichting BEBO, Assen, the Netherlands) and registered in the Dutch Trial Register with identification number NL67638.056.18. The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. Written informed consent was obtained from all subjects before entry into the study and before the performance of any study-specific procedures. Study subjects were admitted to the Clinical Research Unit of the Centre for Human Drug Research (CHDR, Leiden, the Netherlands) for study execution.

2.1 | Study design

This was a randomized, investigator-blinded, three-way cross-over study in healthy male volunteers.

Subjects were randomly assigned to a treatment sequence in which they received all of the three cysteamine formulations (PO-001, Cystagon® or Procysbi®). Subjects received a single dose (600 mg cysteamine base) of each of the three treatments on three separate occasions, with a minimum washout of 7 days between administrations. The first three subjects were dosed using a sentinel approach (one subject receiving PO-001, one Cystagon® and one Procysbi®). Females were excluded from protocol to have a more homogeneous study population. All study drugs were administered orally following an overnight fast and subjects were instructed to swallow the drugs with the capsule intact. All doses were dispensed by the pharmacy of Leiden University Medical Centre (LUMC, the Netherlands). Subjects were offered a light snack 2 h after dosing and a standardized meal 4 h after dosing and during the study day.

2.2 | Investigational drug

The investigational drug PO-001 (sustained-release cysteamine bitartrate [molecular weight: 227.24 g/mol]; TioFarma, Oud-Beijerland, the Netherlands) was supplied as 150 mg capsules, with cysteamine bitartrate as active ingredient. The capsules contained coated pellets ensuring controlled release in the gastrointestinal tract. Pellet cores containing cysteamine bitartrate were produced using extrusion-spheronization. These cores were coated using fluidized bed coating. The coating consisted of a single type of polymethacrylate-based copolymer (Eudragit RS, Evonik, Germany), plasticized with triethyl citrate. Additionally, the coating contained talc as an anti-tacking agent, simethicone as a stabilizer, and hypromellose as a pore former.

Cystagon® (Orphan Europe) was supplied as 150 mg capsules and Procysbi® (Horizon Pharma, Inc.) as 75 mg capsules, and were acquired commercially.

2.3 | Study participants

Eligible participants were healthy male volunteers aged 18 to 55 years inclusive, with a body mass index from 18 to 27 kg/m^2. Eligibility was further assessed on subject's medical history, physical examination (including blood- and urine laboratory analyses), vital signs and electrocardiogram. Key exclusion criteria included clinically significant medical and/or psychiatric conditions that could have confounded the results of the study or posed an additional risk to the subject, or hypersensitivity to cysteamine (mercaptamine) or any other ingredients.

2.4 | Pharmacokinetic assessments

Blood samples for determination of plasma cysteamine levels were collected predose and at 30 min, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 14 and 24 h after dosing. Samples were chilled on wet ice immediately after collection into K2-EDTA containing tubes. Plasma was separated by centrifugation at 2000*g*, promptly transferred to an appropriately labeled polypropylene tube and frozen at approximately −70°C.

The samples were analyzed in bulk by Ardena Bioanalytical Laboratory (ABL, Assen, the Netherlands). Concentrations of cysteamine (molecular weight: 77.15 g/mol) in plasma were determined using a validated LC/MS/MS method. The assay range was 20.0 to 20 000 ng/ml for cysteamine in human K2-EDTA plasma. The overall accuracy (%bias) and precision (CV%) for the quality controls for analysis of cysteamine were within the 15% criteria, representing accuracy and precision for the analysis.

2.5 | Safety and tolerability assessments

Safety assessments were performed while subjects were in the clinical unit (24 h) and they were monitored during the entire study period. Safety was assessed through the evaluation of Adverse Events (AEs) classified by the Medical Dictionary for Regulatory Activities (MedDRA, version 21.1), physical examination, electrocardiography (ECG), vital signs (including systolic and diastolic blood pressure, pulse rate and body temperature). Blood chemistry and hematology tests were performed at the central laboratory of LUMC (Leiden, the Netherlands). The follow-up visit took place 7 to 21 days after the third dose for routine safety assessments.

During the study, abdominal symptom questionnaires were filled out by the subjects at various time points: predose, 30 min, 1, 1 h 30 min, 2, 3, 4, 5, 6, 8, 12 and 24 h after dosing. The following sensations in the abdominal region were quantitated using

10 cm visual analog lines: abdominal fullness (completely empty-intolerably full), nausea (no nausea-intolerable nausea), epigastric pain (no pain-unbearable pain), hunger (not at all- intolerable), desire to eat (very weak- intolerably strong). In addition, questions were asked whether subjects experienced changes in their natural odour (body, mouth and urine), at the following time-points: 1, 4, 8 and 24 h after dosing.

2.6 | Statistical analysis

Due to the exploratory nature of this study, no formal power analysis was conducted to inform the sample size. Nine subjects per group is a common sample size for estimation of the pharmacokinetic properties of a drug. Moreover, the interindividual variability in pharmacokinetic parameters for Cystagon® is relatively small (CVs for C_{max} and T_{max} of 25–50%, based on six healthy adult subjects) and therefore a group of nine subjects was chosen to be sufficient.[17]

For the pharmacokinetic parameters, a noncompartmental analysis was performed. The following pharmacokinetic parameters were assessed for each individual profile: area under the concentration–time curve from time 0 to the last quantifiable concentration ($AUC_{0\text{-last}}$) using the log-linear trapezoid rule, estimated area under the concentration–time curve from time 0 to infinity ($AUC_{0\text{–inf}}$), maximum observed concentration (C_{max}) and the time to reach C_{max} (T_{max}). In addition, the apparent elimination half-life ($t_{1/2}$), the time delay between drug administration and the last time point prior to first concentration above the LLOQ (t_{lag}), apparent clearance (CL/F) and the apparent volume of distribution (V_z/F) were calculated. PK variable programming was conducted with R 3.6.1 for Windows (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2019). All safety and statistical programming was done with SAS 9.4 for Windows (SAS Institute Inc.).

2.7 | Population pharmacokinetic (PK) analysis

The plasma cysteamine concentrations were analyzed using a population PK approach using NONMEM® Version 7.3 (ICON Development Solutions). The PK of cysteamine following oral administration with Cystagon® and/or Procysbi® has previously been modeled using a 1- or 2- compartment disposition model with first-order absorption and with or without lag time.[12,18,19] Therefore, 1- and 2- compartment disposition models were compared. First, the Cystagon® PK model was developed as immediate-release formulation and hereafter different release/absorption models for Procysbi® and PO-001 were evaluated. The final PK model was used to simulate the typical plasma cysteamine concentrations at a steady state following daily doses of 2 g/day divided over 2, 3 or 4 administrations (Q12H, Q8H and Q6H, respectively) for a typical subject of 70 kg. For a more detailed description, see Supplementary Material (Data S1).

3 | RESULTS

3.1 | Study population

A total of 11 subjects were enrolled in the study, demographic data are provided in Table 1. Two of the initially enrolled subjects did not complete the study, both not considered related to study drug administration. These two subjects discontinued after the first study visit, one received 600 mg PO-001 and the other received 600 mg Cystagon®. In total, nine subjects completed the entire study and received all of the three cysteamine formulations.

3.2 | Cysteamine pharmacokinetics

Plasma cysteamine concentration–time profiles for PO-001, Cystagon® and Procysbi® are shown in Figure 1. The concentration–time profile

TABLE 1 Baseline demographic characteristics

Subject	Sex	Age (years)	Race	Weight (kg)	BMI (kg/m^2)
1	Male	20	Asian/White	67.5	20.0
2	Male	21	White	81.4	21.9
3	Male	23	White	70.3	21.8
4	Male	24	White	75.0	23.5
5	Male	24	White	79.4	23.1
6	Male	25	White	77.7	24.8
7	Male	25	White	85.5	22.2
8	Male	25	White	89.2	24.8
9	Male	20	White	58.1	19.5
10	Male	25	Black or African American	69.1	22.2
11	Male	23	White	74.7	22.3
Mean	—	23	—	75.3	22.4

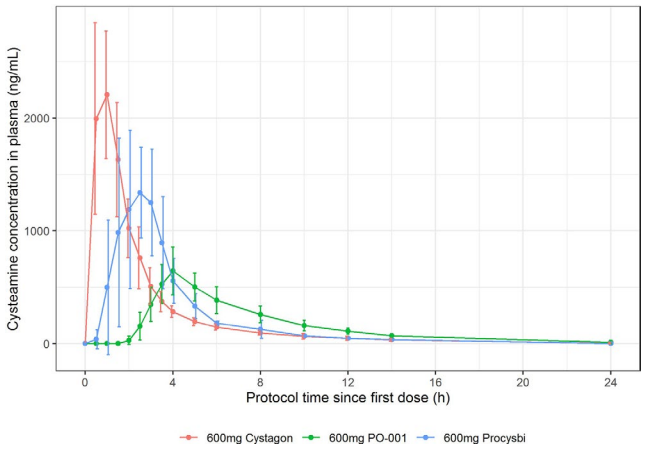

FIGURE 1 Mean plasma cysteamine levels (±SD) following oral administration of PO-001 (green), Cystagon® (red) and Procysbi® (blue) (ng/ml)

of PO-001 was characterized by slow absorption and disposition, and reached a lower C_{max} (675.6 ng/ml) and a longer T_{max} (4.0 h) compared to the two comparative treatments (C_{max}: 2512 and 1692 ng/ml, T_{max}: 1.0 and 2.5 h, respectively) (Table 2 and Figure 1—green line). Additionally, the t_{lag} was increased to a median of 1.5 h after PO-001 administration, indicating a delay in the absorption process. The plasma concentration–time profile of Cystagon® was characterized by rapid absorption and reached the highest C_{max} and shortest T_{max} (Figure 1—red line). The plasma concentration–time profile of Procysbi® was characterized by a slower absorption than Cystagon®, but more rapid compared to PO-001 (Figure 1—blue line). From 4 h onwards, the mean PO-001 concentrations were above the comparative treatments. The mean half-life ($t_{1/2}$) was similar between treatments (4.1, 4.8 and 4.9 h for PO-001, Cystagon® and Procysbi®, respectively).

PO-001 showed the lowest total drug exposure across time, compared to Cystagon® and Procysbi® ($AUC_{0\text{-inf}}$ =3662, 5600, and 4853 h*ng/ml, respectively). For each treatment, plasma cysteamine was below 50 ng/ml for all individuals at 24 h postdose. The CL/F and Vz/F was higher after PO-001 administration compared to Cystagon® and Procysbi®, potentially due to differences in bioavailability between the three cysteamine formulations. A selection of the pharmacokinetic parameters is graphically presented in Figure 2 and shows clear differences in C_{max}, T_{max} and $AUC_{0\text{-inf}}$ between treatments.

3.3 | Population PK analysis

Simulations of typical cysteamine concentrations with the final PK model are presented in Figure 3. The PK profile of both Cystagon® and Procysbi® showed large variations in peak-trough concentrations, as opposed to PO-001. Twice-daily to four times daily dosing of PO-001 showed reduced variation in the peak-trough concentrations. For a more detailed description of the final population models

TABLE 2 Pharmacokinetic parameters following oral administration (600 mg) of 3 cysteamine formulations in healthy volunteers

	Cystagon® (n = 10)			PO-001 (n = 10)			Procysbi® (n = 9)		
	Mean ± SD	Median	Range (Min-Max)	Mean ± SD	Median	Range (Min-Max)	Mean ± SD	Median	Range (Min-Max)
C_{max}	2512 ± 684.31	2545	1610–3800	675.6 ± 198.97	628	465–1060	1692 ± 434.45	1750	110–2390
T_{max}	—	1	0.5–1.5	—	4	3.5–5.02	—	2.5	1.5–3.02
AUCinf (h*ng/ml)	5600 ± 1246	5845	3250–6729	3662 ± 664	3495	2863–4925	4853 ± 1030	5125	3107–6651
CL/F (L/h)	114 ± 33.1	103	89.2–185	168 ± 27.8	172	122–210	129 ± 30.7	117	90.2–193
$t_{1/2}$ (h)	4.81 ± 1.89	4.55	1.73–7.88	4.13 ± 1.27	4.06	2.32–6.54	4.85 ± 1.66	4.36	3.27–8.84
tlag (h)	—	0	0–0	—	1.52	1.5–2	—	0.5	0–1
Vz/F (L)	740 ± 231	725	403–1143	971 ± 222	989	649–1435	870 ± 230	750	674–1408

AUCinf, Estimated area under the plasma concentration–time curve from time of dosing to infinity; CL/F, Apparent clearance; C_{max}, Maximum observed concentration during observation window); $T_{1/2}$, half-life (/h)); tlag, Time delay between drug administration and the last time point prior to first observed concentration above the LLOQ; T_{max}, Time after dose at which C_{max} was observed; V_z/F, Apparent volume of distribution.

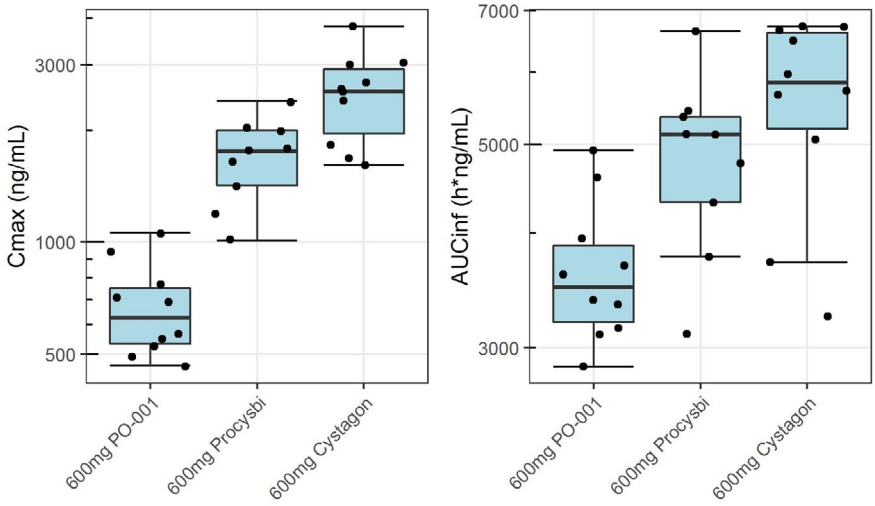

FIGURE 2 Summary boxplots of cysteamine in plasma. The median (horizontal solid), the 25%–75% distribution (IQR), with the largest value no further than 1.5× the IQR as whiskers are represented. Dots present the observations

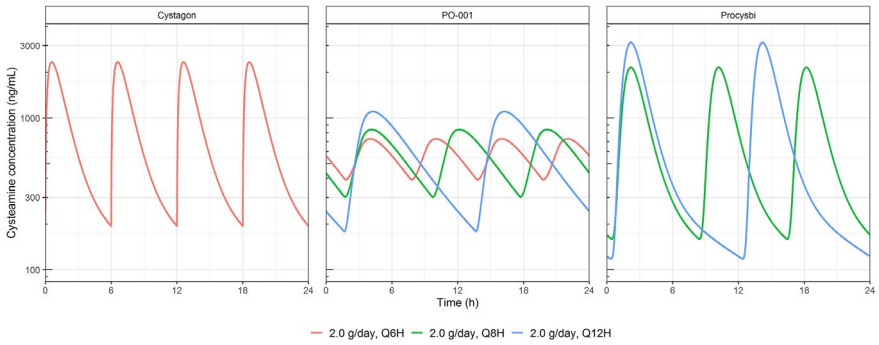

FIGURE 3 Simulations of typical Cysteamine concentrations of Cystagon®, PO-001 and Procysbi® at steady state (scenario: subject 70 kg ≥12 years; 2.0 g/day; dosing frequency every 6 h (red), 8 h (green), and 12 h (blue))

and PK parameter estimates, see Supplementary Material (Table S1, Figure S1 and Data S1).

3.4 | Safety and tolerability

Overall, no deaths, serious AEs, discontinuations due to AEs, or clinically significant changes in vital signs, clinical laboratory results or ECG occurred in any of the subjects. In total, 27 TEAEs were reported by nine (82.0%) subjects across all treatment visits, most commonly comprising of gastrointestinal disorders (Table 3).

After dosing with Cystagon®, gastrointestinal symptoms of nausea, vomiting and diarrhea were observed in two subjects, these findings started approximately 1.5 h after administration. No gastrointestinal disorders were reported by subjects after dosing with Procysbi®, and two subjects reported nausea after dosing with PO-001, which started approximately 5 h and 8 h after administration. All TEAEs were of mild severity, with the exception of two moderate severity gastrointestinal problems including vomiting and diarrhea, which were considered to be related to Cystagon®.

Based on the abdominal questionnaire, no clear effects on abdominal symptoms were observed for all treatments. A small

TABLE 3 Summary of treatment emergent adverse events (TEAEs)

	Cystagon® 600 mg (*N* = 10)		PO−001 600 mg (N = 10)		Procysbi® 600 mg (*N* = 9)	
System organ class/ preferred term	Events *N*	Subjects *N* (%)	Events *N*	Subjects *N* (%)	Events *N*	Subjects *N* (%)
Any events	15	3 (30.0)	10	4 (40.0)	2	1 (11.1)
Gastrointestinal disorders	7	2 (20.0)	2	2 (20.0)	—	—
Abdominal pain upper	1	1 (10.0)	—	—	—	—
Diarrhea	2	2 (20.0)	—	—	—	—
Nausea	2	2 (20.0)	2	2 (20.0)	—	—
Vomiting	2	2 (20.0)	—	—	—	—
General disorders and administration site conditions	3	3 (30.0)	2	2 (20.0)	1	1 (11.1)
Cold sweat	1	1 (10.0)	—	—	—	—
Fatigue	1	1 (10.0)	1	1 (10.0)	1	1 (11.1)
Feeling cold	1	1 (10.0)	—	—	—	—
Influenza-like illness	—	—	1	1 (10.0)	—	—
Procedural complications	—	—	2	2 (20.0)	—	—
Post procedural hematoma	—	—	1	1 (10.0)	—	—
Procedural site reaction	—	—	1	1 (10.0)	—	—
Nervous system disorders	5	2 (20.0)	4	2 (20.0)	1	1 (11.1)
Dizziness	2	2 (20.0)	—	—	—	—
Headache	1	1 (10.0)	4	2 (20.0)	1	1 (11.1)
Presyncope	2	2 (20.0)	—	—	—	—

increase in nausea was observed 2–3 h after Cystagon® administration, confirming the reported gastrointestinal symptoms. In the odour questionnaire, no changes in natural body and urine odour were observed directly after study drug administration. Two subjects indicated a change in mouth odour after Cystagon® administration (4, 8 and 24 h after dosing) and one subject after PO-001 administration (4 h after dosing). Subjects who indicated a change in mouth odour had an iron-like taste in their mouth.

4 | DISCUSSION

In this study, we compared a novel cysteamine formulation, PO-001, with two existing formulations (Cystagon® and Procysbi®). Compliance with cysteamine treatment is important to maintain WBC cystine levels below the threshold, however, even with optimal compliance, the pharmacokinetic profile of the existing formulations is not optimal with high peak-trough variability. This may impair optimal treatment for patients with cystinosis. Compliance is hampered by the strict regimen of intake, in the case of Cystagon® (every 6 h, even during the night), the size of capsules and complaints of halitosis.[6,11,13] This affects not only the quality of life for the (very) young patients, but also for their parents. Therefore, there is a clear need for an improved formulation, such as PO-001, with favourable sustained-release characteristics allowing a lower dosing frequency while not comprising optimal effect, thereby enhancing the patient's quality of life and improve compliance.

Pharmacokinetics of PO-001 showed a pattern of delayed and sustained-release of cysteamine concentration, compared to the conventional Cystagon® in healthy male volunteers. Procysbi® showed both a delayed-release pattern, based on the enteric coating preventing release in the stomach, and an extended-release pattern. Compared to Procysbi®, PO-001 further extended the time of sustained-release with a longer time to reach peak levels. Although PO-001 showed a lower C_{max} and a lower bioavailability compared to Cystagon® and Procysbi®, it showed reduced peak-trough variations. It would be worthwhile to investigate a slightly modified formulation, applying the same principle and coating but with a reduced delay in the release to increase the bioavailability. PO-001 and Procysbi® were well tolerated and the study revealed no important safety issues. Two subjects dosed with Cystagon® showed complaints of nausea, vomiting and diarrhea, which could be explained by the observed higher peak levels. These are known side effects of cysteamine therapy.[20]

Until now, Procysbi® is the only approved cysteamine formulation for twice-daily dosing in cystinosis patients.[15,17,21] However in clinical practice, twice-daily dosing seems not always sufficient. Our results show that PO-001 has pharmacokinetic parameters comparable to those of Procysbi®, but with a more prolonged absorption profile and increased concentration up from 4 h.

The study of Langman et al. showed maximum observed cysteamine concentrations (C_{max}) of 2730 ng/ml ±1360 and 3700 ng/ml ±1720 for Cystagon® and Procysbi®, respectively.[22] In the study of Belldina et al, the mean C_{max} for Cystagon® was 2800 ng/ml ±903 (36.3 ± 11.7 μM).[12] Previous studies have observed the effect of cysteamine on WBC cystine levels in cystinosis patients. The study of Belldina shows a mean C_{trough} around 309 ng/ml (4 μmol/L) at which the WBC cystine content is maintained below the target level of 1 nmol hemicystine/mg protein. [12]

In our study, PO-001 had a C_{max} of 675.6 ng/ml (9 μmol/L), which is just above this C_{trough} value. Therefore, an increased dose or bioavailability of PO-001 should be required, which would allow for a longer period above this concentration to control the WBC cystine content. However, limited information is available on the relationship between the pharmacokinetics of cysteamine and its pharmacodynamics, for instance WBC cystine levels. Based on our population PK simulations, PO-001 would be acceptable with our specifications in a subsequent study for twice-daily dosing (every 12 h). A slight increase in dose would be required to maintain C_{max} concentrations below the Q6 h Cystagon® C_{max} and to maintain C_{trough} concentrations above the Q6 h Cystagon® C_{trough}. A similar pharmacodynamic profile for PO-001 would be expected as for Cystagon® and this should be enough to reach the desired effect in patients (target <1 nmol hemicystine/mg protein).

Furthermore, cysteamine therapy is associated with an unpleasant sulfurous body odour and breath, negatively affecting quality of life. Halitosis is caused by metabolized dimethylsulfide in expired air and body secretes and is dependent on the level of cysteamine.[23] The lower peak levels of cysteamine due to the sustained-release of PO-001 may be associated with less halitosis. This study was not powered to detect such differences. The amount of dimethylsulfide in expired air after cysteamine intake should be further studied to better evaluate halitosis and body odour related to cysteamine treatment.

The results of this study support the further investigation of pharmacokinetics of this type of sustained-release dosage form (PO-001) after twice-daily dosing preferably with the biomarker WBC cystine levels to learn if these levels will be maintained to acceptable levels. Data generated in this study, combined with extensive PK/PD modeling, could guide the selection of dose level and regimen for a subsequent study. This together with the pharmacokinetic and pharmacodynamic relationship for cysteamine described in the public domain. In addition, the observed lower bioavailability of PO-001 could be further ameliorated for a following study by adjusting the dose or slightly reformulation the dosage form concept. Further improvements could include the development of a formulation that requires less active ingredient, thereby reducing the potential unused spillover and reducing the volume of the formulation. For the formulation of the drug also the size of the formulation and smoothness of intake should be taken into account for those patients who have difficulties with swallowing.

The main strengths of this study are the randomized three-way cross-over design including washout periods, allowing a head-to-head comparison between three different formulations. The relatively small sample size is appropriate for comparing the pharmacokinetic profile of the three formulations, but not to compare side effects in detail. In addition, the exposure of the three formulations differed, which is accounted for in the developed population PK model to provide a reliable comparison between formulations after multiple doses.

In conclusion, our study suggests that the new cysteamine bitartrate formulation, PO-001, has sustained-release characteristics and would allow for twice-daily dosing with acceptable C_{max} and C_{trough} concentrations. This would contribute to fewer disruptions to the daily routines of patients and improved treatment compliance.

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DISCLOSURES

The trial was initiated by Fair Medicine, a Foundation with the aim to develop affordable drugs and represented by its employees H Büller and F de Loos. Fair Medicine participates in Patient One BV. The trial was sponsored by Patient One BV, which is owned by a coalition of companies and institutions and represented by V van der Wel. L G J de Leede is the owner of Exelion BV, which participates in Patient One BV.

Medication was prepared by Tiofarma BV represented by its employees W S de Vries and H Waals. H Waals owns shares of Tiofarma BV, which participates in Patient One BV.

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AUTHOR CONTRIBUTIONS

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole and have given their approval for this version to be published.

MEDICAL WRITING ASSISTANCE

Medical writing and editorial support were provided by K Broekhuizen, an employee of Centre for Human Drug Research.

COMPLIANCE WITH ETHICS GUIDELINES

The study was approved by an independent ethics committee (Stichting BEBO, Assen, the Netherlands) and registered in the Dutch Trial Register with identification number NL67638.056.18. The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. Written informed consent was obtained from all subjects before entry into the study and before the performance of any study-specific

procedures. Study subjects were admitted to the Clinical Research Unit of the Centre for Human Drug Research (CHDR, Leiden, the Netherlands) for study execution.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during this study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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