



New insights into the effects of dissolution profiles on the pharmacokinetics of trientine dihydrochloride

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Abstract

Purpose Trientine dihydrochloride (TETA-2HCl) is an established treatment for Wilson disease. We assessed different dissolution profiles of TETA-2HCl capsules on the pharmacokinetics (PK) of trientine (TETA) and on direct copper (Cu) parameters.

Methods In this open-label, randomized, two way cross-over study, 24 healthy subjects received two single oral doses of 600 mg TETA-2HCl with a washout of at least one week; one dose with a fast and one dose with a slow dissolution profile. Blood and urine samples were collected up to 48 h for analysis of plasma TETA and its metabolites, N1-acetyltriethylenetetramine (MAT) and N1-N10-diacetyltriethylenetetramine (DAT), serum Cu, ceruloplasmin (Cp) and urinary Cu excretion (UCE). The effect of dissolution profile on the PK was assessed through the ratio of geometric mean ratios (GMRs) and two-sided 90%-confidence intervals (CI) of the fast vs. slow dissolution profile.

Results The C_{max} of TETA was comparable for the two products (GMR 95.81%; CI 83.87-109.46%) while AUC_{0-inf} was slightly lower for the capsules with fast dissolution profile (GMR 90.65; 90% CI 78.32-104.91%). PK parameters were similar for the metabolites of TETA. Additionally, serum Cu and Cp concentrations remained stable over the 12 h period after dosing and were comparable between the two products, as was UCE.

Conclusion The pharmacokinetic profiles of TETA after administration of TETA-2HCl capsules with fast and slow dissolution characteristics were similar. Though the lower 90%-CI for AUC_{0-inf} was outside the formal bioequivalence ranges, differences were small (9%) and not considered clinically relevant. A difference in dissolution profile did not affect copper parameters and tolerability.

Keywords Wilson disease · Trientine dihydrochloride · Dissolution, pharmacokinetics

Introduction

Trientine dihydrochloride (TETA-2HCl) is a well-established treatment for patients suffering from Wilson disease (WD) intolerant to D-penicillamine. Wilson disease is a rare autosomal recessive disorder of copper (Cu) metabolism which results in accumulation of excess Cu [1–4]. TETA-2HCl is a chelating agent, forming a stable complex with free Cu in serum, thereby reducing the non-ceruloplasmin bound Cu (NCC) and promoting urinary excretion of the TETA bound Cu. Additionally, TETA reduces the Cu absorption by forming complexes with free Cu in the gastrointestinal tract. Accumulated copper can also be excreted in faeces when enterocytes are sloughed off into the intestinal lumen [5–9]. Importantly, the pharmacodynamic action of TETA-2HCl is only dependent on its Cu-chelating properties and

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does not interact with receptors, enzymes, or any other biological system that might cause off target effects [7, 9, 10].

The absorption rate of orally administered TETA-2HCl in human has previously been reported to be relatively slow (time to peak plasma concentration (T_{max}) between 0.8 h and 4 h) and highly variable [7, 9]. Maximum plasma concentration (C_{max}) differed significantly between individuals with WD who had been dosed with 2×300 mg TETA-2HCl (i.e., 300 mg dihydrochloride salt containing 200 mg TETA base), with C_{max} values ranging from 0.4 to 20 mg/L. The variation in C_{max} was less profound in a study of healthy subjects who received the same dose [9].

TETA is metabolized via acetylation, which is catalyzed by spermidine/spermine N^1 -acetyltransferase (SSAT), forming two major metabolites, N^1 -acetyltriethylenetetramine (MAT) and N^1, N^{10} -acetyltriethylenetetramine (DAT) [9]. Both metabolites, MAT and DAT, are found in human plasma and urine following TETA administration. MAT and DAT retain copper-binding capabilities [11]. However, their binding affinities are lower than the binding affinity of the parent compound TETA. It is believed that MAT, but not DAT, is pharmacologically active and thus still contributes meaningfully to copper chelation which would result in an influence on the therapeutic efficacy of TETA.

Results of a pharmacokinetic study conducted in 2018 by Pfeiffenberger et al., in 20 WD patients who received TETA-2HCl, showed a T_{max} of 0.5 to 4 h post-dose. When the pharmacokinetic (PK) parameters of TETA were dose-normalized (the dosage ranged from 300 mg to 900 mg for children and from 300 mg to 1500 mg for adults¹), an 11.6-fold range in area under the plasma concentration-time curve (AUC) from 0 h to the last quantifiable concentration in the profile normalized for dose (AUC_{0-t}/D) was observed; and a 6.7-fold range in maximum plasma/drug concentration (C_{max}/D) [12]. The exposure and variability of exposure observed in the Pfeiffenberger study in patients with WD were similar to that reported by Lu et al. in healthy subjects [9, 12].

Over the last couple of years, multiple TETA formulations have been introduced to the market with e.g. different salt forms of TETA or different capsule sizes. As recent studies reported non-uniform results on the dissolution rate of different TETA formulations (TETA-2HCl and trientine-tetrahydrochloride (TETA-4HCl)) and its impact on pharmacokinetics [12], the current study aimed to assess the effect of two different dissolution profiles of TETA-2HCl capsules on the pharmacokinetics of TETA and its metabolites in healthy subjects and its effect on direct Cu parameters.

¹ Doses mentioned throughout this manuscript are expressed as mg of TETA-2HCl salt (i.e. not in mg of the trientine base).

Materials and methods

This was a Phase 1, single-dose, open label, randomized, two period, cross-over, pharmacokinetic study in 24 healthy male and female subjects. The study was performed between July 11, 2018, and September 12, 2018, and conducted by QPS-Netherlands in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice, and the principles of the Declaration of Helsinki as well as with all national and local laws of the appropriate regulatory authorities, including archiving of essential documents. The trial protocol was approved by an independent ethics committee (St. BEBO, Assen, The Netherlands) and all study participants signed an informed consent form before any screening evaluation.

The primary objectives of the study were to characterize the pharmacokinetics of TETA after administration of TETA-2HCl capsules with either a fast or a slow dissolution profile (Univar Solutions B.V., Rotterdam, the Netherlands), both under fasting conditions, and to assess the effect of dissolution rate on the pharmacokinetics of TETA. The secondary objectives were to assess the pharmacokinetics of the metabolites of TETA, N^1 -acetyltriethylenetetramine (MAT) and N^1 - N^{10} -diacetyltriethylenetetramine (DAT) and to assess and compare the effect of TETA exposure on the short-term pharmacodynamics in serum and urine, following administration of TETA-2HCl capsules with different dissolution profiles.

The method used to determine the dissolution rate of the different products was in accordance with testing of dissolution for solid oral dosage forms as described in the European Pharmacopoeia [13]. The fast dissolution product in this study met the requirements for very rapid dissolution (i.e. >85% within 15 min). The slow dissolution product demonstrated 50% dissolution at 30 min and 90% dissolution at 90 minutes. The same dissolution testing method was used for the fast and the slow dissolution product of TETA-2HCl (Univar Solutions B.V., Rotterdam, the Netherlands) used in this study [13]. The qualitative composition of the fast and slow dissolution products was identical.

Study population and data collection

A total of 24 healthy subjects were enrolled in the study. No formal statistical sample size calculations have been made, but based on previous experience the number of subjects was considered to be adequate for the characterization of the pharmacokinetics of TETA and the comparison of the pharmacokinetics of the investigational products with different dissolution profiles [9, 12, 13]. Healthy subjects were

chosen as the primary population for bioequivalence studies, in line with the appropriate guidelines [14].

Inclusion/exclusion criteria and restrictions prior and during the study period

The inclusion and exclusion criteria are summarized and presented in Supplementary Table 1. Briefly, healthy adult subjects, aged between 18 and 75 years and having a BMI between 18 and 30 kg/m² at screening, were eligible for inclusion in the study if they were able to provide informed consent to take part in the trial, had normal serum Cu and ceruloplasmin (Cp) levels, and did not have an abnormal diet, were not anemic, pregnant, planning to become pregnant, breastfeeding, using any prescription or over the counter medications, recent blood donors, or smoked more than 5 tobacco products per day.

Furthermore, subjects were restricted in the consumption of beverages and foods containing alcohol, grapefruit and grapefruit containing products, or caffeine/xanthine; these were not allowed during each treatment period from 48 h prior to dosing until the last pharmacokinetic sample in that period had been collected (day 3). Tobacco, nicotine products or e-cigarettes were not allowed during confinement in the clinical research facility. Subjects were not allowed to engage in strenuous exercise from 48 h prior to the first dose of study medication until the follow-up visit.

Treatments and assessments

Subjects received in a randomized order once a single oral dose of 600 mg (2 × 300 mg capsules) of TETA-2HCl (Univar Solutions B.V., Rotterdam, the Netherlands) with a fast dissolution profile and once a single oral dose of 600 mg (2 × 300 mg capsules) of TETA-2HCl (Univar Solutions B.V., Rotterdam, the Netherlands) with a slow dissolution profile. Study medication was administered with 240 mL of water after a 10-hour fast. Fasting continued for an additional 4 h. There was a washout period of at least 7 days between the treatment periods. For each treatment period, subjects remained in the clinic from the afternoon of day - 1 (day before the study medication was administered) until the morning of day 3, after the last pharmacokinetic blood sample (48 h) had been collected. Between 7 and 10 days after the last drug administration, subjects visited the clinical research facility for a follow-up visit. Prior to each drug administration (on day - 1), vital signs, laboratory tests, drug screen, alcohol tests, and a pregnancy test (in urine) were repeated, and the inclusion and exclusion criteria were reviewed. During each treatment period, and taking into account the high variability in the absorption of trientine from the intestine [9, 15], blood samples of 2 mL

for the pharmacokinetic analysis of TETA, MAT and DAT in plasma were collected at pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 20, 24, 30, 36, and 48 h post-dose. With this design it was aimed to capture the variability in the different pharmacokinetic parameters assessed in this study. Blood was collected in K₂EDTA tubes, processed to plasma, and stored at -80 °C awaiting analysis.

Regarding the pharmacodynamic assessments a different sampling design was followed. In Wilson disease copper in urine is normally assessed over 24 h as one sample. We aimed at assessing Cu excretion in more detail over time, but sampling urine at the frequency of the PK sampling design would most likely not result in sufficient urine and/or copper content in the urine. Therefore, during each treatment period, all urine was collected at baseline (pre-dose void) and during the following collection intervals: 0–2, 2–4, 4–8 and 8–12 h post dose for Cu excretion analysis. From each collection interval, a sample of 5 mL was taken for Cu analysis.

For the assessment of serum Cu and Cp a simpler sampling design was chosen as for the pharmacokinetic assessment, because the pharmacodynamic assessment was not the primary focus of the study. Therefore, we aimed to keep the burden for the healthy volunteers as low as possible. Blood samples of 2 mL were taken pre-dose and at 2, 4, 8 and 12 h after each drug administration.

Given the very limited availability of pharmacokinetic studies with Trientine at that time, all sampling schemes were designed based on clinical experience with our other Phase I study in Wilson Disease patients [12].

Vital signs, Electrocardiogram (ECG) and safety labs were measured/recorded at protocol defined time points throughout the study.

Clinically relevant changes in vital signs, ECGs and laboratory parameters were reported as Adverse events (AEs).

Bioanalytical methods

Trientine and its metabolites MAT and DAT were analysed by QPS Netherlands B.V., Groningen, the Netherlands, using a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method with TETA-*d*₄-4HCL, MAT-*d*₄-3HCL MAT-*d*₄-2HCL as internal standard. The assay was validated and sample analysis was conducted in accordance with Good Laboratory Practice principles [16]. The assay validation and the evaluation of its results were conducted following the European Medicines Agency EMEA/CHMP/EWP/192,217/2009 Guideline on Bioanalytical Method Validation (Effective date: 01 February 2012). The assay involved sample preparation by extraction of TETA, MAT and DAT from human K₂EDTA plasma via protein

precipitation followed by derivatization with fluorenylmethyloxycarbonyl chloride. After quenching samples were analyzed using an API 400 LC-MS/MS system (Agilent). The validated lower and higher limits of quantification for all three analytes were 20 and 2000 ng/mL respectively. Precision and accuracy were set to 15%, except for the lowest concentration of 20 ng/mL, precision and accuracy were set to 20%. Incurred sample re-analysis (ISR) was performed on 9% of samples with 20% assay variability in at least 2/3 of samples. Samples were stored at -80°C for up to 45 days between sampling and bioanalysis, well within the confirmed long-term storage stability of 450 days at -80°C .

Pharmacokinetic parameters

For the pharmacokinetic profiles of TETA, MAT and DAT, the maximum plasma concentration (C_{max}) and related time (T_{max}), area under the curve from time zero to the last measurable concentration (AUC_{0-t}), area under the plasma concentration from time zero to infinity ($\text{AUC}_{0-\text{inf}}$), the terminal elimination half-life and the terminal elimination rate constant were calculated.

Statistical analysis was performed on the primary PK parameters C_{max} , AUC_{0-t} , $\text{AUC}_{0-\text{inf}}$ obtained after administration of capsules with either the fast or the slow dissolution profile treatments. These parameters underwent logarithmic transformation and were analyzed using a mixed effects model including terms for treatment, period and sequence as fixed effects and subject within sequence as a random effect. The appropriate pharmacokinetic parameters of TETA, MAT and DAT were derived from individual subject plasma concentration time data, using a non-compartmental method, and are listed and summarized by treatment using descriptive statistics: number (n), arithmetic mean (AM), arithmetic standard deviation (SD), geometric mean (GM), geometric SD, coefficient of variation (CV), median, minimum, and maximum.

The ratios of the geometric means for C_{max} , AUC_{0-t} and $\text{AUC}_{0-\text{inf}}$ for TETA, MAT and DAT from capsules with a fast or slow dissolution profile and corresponding 90% confidence intervals (CI) were calculated to determine the effect of the dissolution profile. Bioequivalence was concluded when the 90% confidence ranges fell within the pre-specified 80–125% range. Pharmacokinetic parameters were calculated for each treatment by non-compartmental methods (linear up/log down method) using Phoenix WinNonlin, version 6.3 (Certara, Princeton, NJ, USA). Actual elapsed blood sampling times were used for the estimation of the pharmacokinetic parameters. Descriptive presentations of plasma concentrations were provided by treatment group and nominal times.

Pharmacodynamic parameters

Pharmacodynamic parameters included urinary Cu excretion (UCE) and serum Cu and Cp concentrations. Serum Cu and Cp concentrations per collection point, and UCE (per collection interval and cumulative excretion) are presented per subject and were summarized descriptively: number (n), arithmetic mean (AM), arithmetic standard deviation (SD), geometric mean (GM), geometric SD, coefficient of variation (CV), median, minimum, and maximum. No statistical analysis was performed on the pharmacodynamic parameters.

Outcome measures for safety and tolerability

Treatment Emergent Adverse events (TEAEs) were coded using the current version of Medical Dictionary for Regulatory Activities (MedDRA version 21.0). All TEAEs, vital signs, ECG and clinical laboratory parameters were summarized overall and for TETA-2HCl with both fast and slow dissolution profile, by relationship, and by severity using descriptive statistics.

Statistical analysis

All statistical analyses were conducted using SAS[®] software, Version 9.3 of the SAS System for Windows 7.

Results

Study population

Subject demographics and baseline characteristics are summarized and presented in Table 1.

All 24 randomized subjects completed the study. All subjects were included in the pharmacokinetic, pharmacodynamic, and tolerability evaluation.

Pharmacokinetics of trientine, MAT and DAT and the effect of dissolution rate

TETA, MAT and DAT plasma concentration profiles and the derived pharmacokinetic parameters are shown in Fig. 1, and Table 2.

The mean plasma concentration profiles of TETA (Fig. 1) after both treatments were similar. Following oral dosing a mean T_{max} of 2.0 h (range, 0.5 to 6.1 h) was

Table 1 Demographics and baseline characteristics

	All subjects (N=24)
Age (years)	
n	24
Mean (SD)	45.2 (18.39)
Median (min, max)	51.5 (18, 73)
Sex	
Female	17 (70.83%)
Male	7 (29.17%)
Ethnicity	
Non-Hispanic/Non-Latino	24 (100%)
Race	
White	23 (95.83%)
Other	1 (4.17%)
Height (cm)	
n	24
Mean (SD)	172.27 (8.485)
Median (min, max)	174.20 (157.4, 187)
Weight (kg)	
n	24
Mean (SD)	73.77 (9.857)
Median (min, max)	73.80 (52.3, 92.5)
BMI (kg/m ²)	
n	24
Mean (SD)	24.82 (2.437)
Median (min, max)	24.95 (19.5, 29.2)

BM/body mass index, *SD* standard deviation

observed for capsules with a fast dissolution profile and a mean T_{max} of 2.5 h (range, 0.5 to 4.0 h) for capsules with a slow dissolution profile. Subsequently, TETA plasma concentrations declined with a mean $t_{1/2}$ of 4.4 h and 5.1 h for capsules with a fast and slow dissolution profile, respectively. The rate (C_{max}) and extent of exposure (AUC) as well as the time to reach maximum concentrations (T_{max}) were comparable between the two capsule formulations although the AUC was slightly lower for the fast dissolution profile (AUC_{0-t} and AUC_{0-inf} respectively were 8%, and 9% lower). There was a large between subject variation in the pharmacokinetic parameters as shown by rather high arithmetic coefficient of variation values (CV) which is equal to $SD/AM*100$ (CV%) values for the primary pharmacokinetic parameters: these were more than 60% for the AUC parameters and somewhat lower for C_{max} (Table 2).

Similarly to TETA, the mean plasma concentration profiles for MAT and DAT (Fig. 1) after both treatments were comparable. Mean T_{max} values of 5.5 h and 5.0 h were reported for MAT (for fast and slow dissolution profiles, respectively), whilst mean T_{max} for DAT was 6.0 h for both profiles. The C_{max} and AUC were again comparable between the two capsules, for both MAT and DAT. Similarly, MAT plasma concentrations had a mean $t_{1/2}$ of 14.1 h

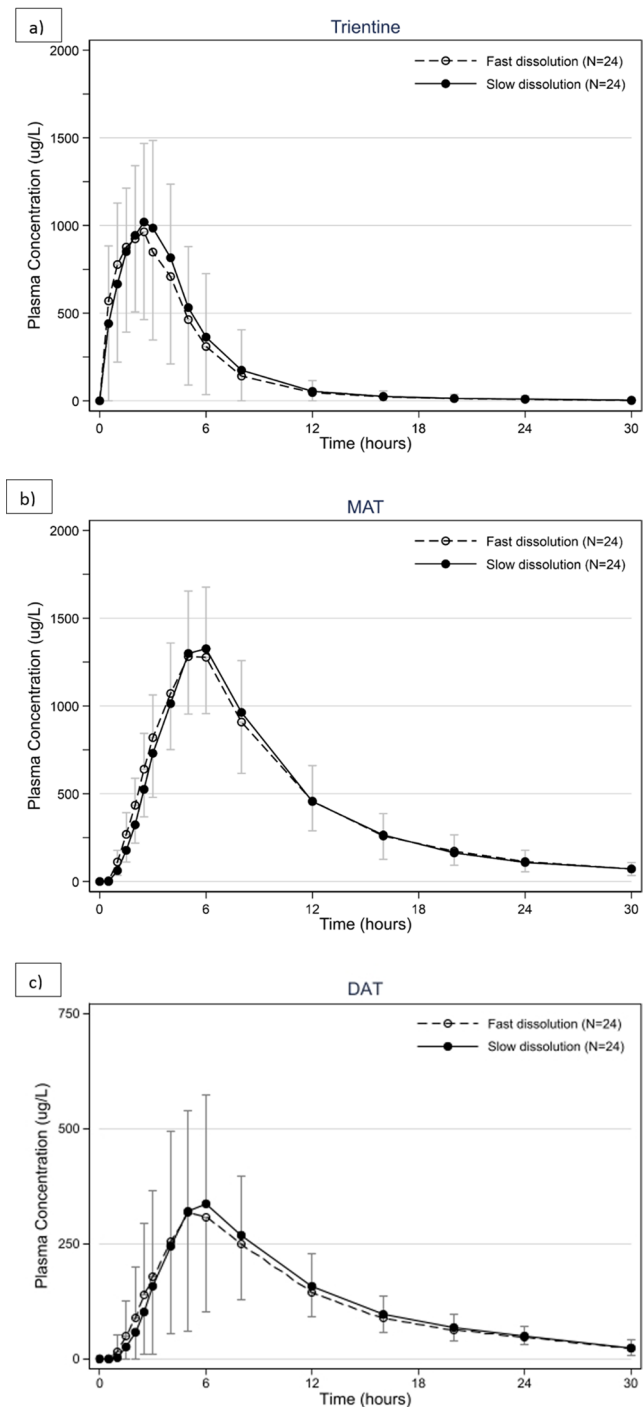


Fig. 1 Mean (SD) trientine plasma concentration-time profiles after administration of TETA-2HCl capsules with a fast and a slow dissolution profile: (a) trientine, (b) MAT, (c) DAT

and 14.4 h for capsules with a fast and slow dissolution profile, respectively, compared to 8.5 h (fast dissolution) and 8.2 h (slow dissolution) for DAT. The large between subject variation in the pharmacokinetic parameters seen for TETA was also observed for both MAT and DAT PK parameters (Table 2).

Table 2 Summary of trientine, MAT and DAT pharmacokinetic parameters after administration of TETA-2HCl capsules with a fast and a slow dissolution profile

Parameters	Fast Dissolution Profile		Slow Dissolution Profile	
	N	Mean (SD) (CV%)	N	Mean (SD) (CV%)
Trientine				
C_{max}^a (ng/mL)	24	1090 (551) (50.3)	23	1120 (495) (44.4)
$T_{max}^{a,b}$ (hr)	24	2.0 (0.5, 6.1) (53.8)	23	2.5 (0.5, 4.0) (39.0)
AUC_{0-t}^a (ng*h/mL)	24	5000 (3050) (61.1)	23	5420 (3510) (64.7)
$AUC_{0-inf}^{a,c}$ (ng*h/mL)	24	5170 (3140) (60.7)	21	5640 (3640) (64.5)
$t_{1/2}^{a,c}$ (hr)	24	4.42 (4.76) (107.7)	21	5.05 (7.48) (148.2)
MAT				
C_{max}^a (ng/mL)	24	1350 (321) (23.8)	23	1390 (372) (26.8)
$T_{max}^{a,b}$ (hr)	24	5.5 (4.0, 6.1) (12.1)	23	5.0 (5.0, 6.0) (9.3)
AUC_{0-t}^a (ng*h/mL)	24	13,300 (3970) (29.9)	23	13,200 (3560) (27.0)
AUC_{0-inf}^a (ng*h/mL)	24	14,000 (4230) (30.1)	23	13,900 (3710) (26.6)
$t_{1/2}^a$ (hr)	24	14.1 (3.74) (26.5)	23	14.4 (5.84) (40.5)
DAT				
C_{max}^a (ng/mL)	24	327 (255) (78.1)	23	351 (251) (71.4)
$T_{max}^{a,b}$ (hr)	24	6.0 (5.0, 8.0) (16.1)	23	6.0 (5.0, 8.0) (14.7)
AUC_{0-t}^a (ng*h/mL)	24	3540 (1850) (52.3)	23	3730 (1920) (51.4)
AUC_{0-inf}^a (ng*h/mL)	24	3850 (1880) (48.7)	22 ^d	4110 (1950) (47.3)
$t_{1/2}^a$ (hr)	24	8.46 (3.01) (35.6)	22 ^d	8.18 (2.44) (29.8)

^aPharmacokinetic parameters for one subject receiving TETA-2HCl with a slow dissolution profile could not be estimated due to insufficient data

^bMedian (min, max) is presented

^c AUC_{0-inf} , $t_{1/2}$ are not reportable for 2 subjects receiving TETA-2HCl with a slow dissolution profile due to adjusted $R^2 < 0.80$

^d AUC_{0-inf} , $t_{1/2}$ could not be estimated for one subject receiving TETA-2HCl with a slow dissolution profile due to percentage of extrapolated $AUC > 20\%$

Bioequivalence testing (see Table 3) on the primary parameters C_{max} , AUC_{0-t} and AUC_{0-inf} showed that for TETA, the 90% CI of C_{max} was within the pre-specified range of 80–125%. However, the 90% CIs for AUC_{0-t} and AUC_{0-inf} were slightly wider and the lower ends were outside the range of 80–125%: 78.96 and 78.32 respectively. Regarding both metabolites of TETA, bioequivalence was

Table 3 Statistical comparisons of plasma pharmacokinetic parameters

	Fast Dissolution Profile (N=24)	Slow Dissolution Profile (N=23 ^{a, b, c})	Fast vs. slow profile (dissolution rate)
	GLSM	GLSM	GMR (90% CI)
Trientine			
C_{max} (ng/mL)*	968	1010	95.81 (83.87,109.46)
AUC_{0-t} (ng*h/mL)*	4210	4580 ^a	91.95 (78.96,107.07)
AUC_{0-inf} (ng*h/mL)*	4360	4810 ^{a, b}	90.65 (78.32,104.91)
MAT			
C_{max} (ng/mL)*	1310	1340 ^a	97.22 (86.44,109.36)
AUC_{0-t} (ng*h/mL)*	12,600	12,800 ^a	98.42 (88.48,109.47)
AUC_{0-inf} (ng*h/mL)*	13,400	13,600 ^a	98.51 (88.76,109.33)
DAT			
C_{max} (ng/mL)*	275	290 ^a	94.76 (84.31,106.51)
AUC_{0-t} (ng*h/mL)*	3160	3280 ^a	96.11 (86.55,106.73)
AUC_{0-inf} (ng*h/mL)*	3480	3550 ^{a, c}	98.08 (90.14,106.73)

GLSM Geometric least squares mean, GMR Geometric Mean Ratios

*Back-transformed least squares mean and confidence interval from mixed effects model performed on natural log-transformed values

^a C_{max} , AUC_{0-t} and AUC_{0-inf} could not be estimated for one subject receiving TETA-2HCl with a slow dissolution profile due to insufficient data

^b AUC_{0-inf} are not reportable for two subjects receiving TETA-2HCl with a slow dissolution profile due to adjusted $R^2 < 0.80$

^c AUC_{0-inf} is not reportable for one subject receiving TETA-2HCl with a slow dissolution profile due to the percentage of extrapolated $AUC > 20\%$

proven according to the bioequivalence testing on the primary parameters.

Effect of dissolution rate on the pharmacodynamics

As shown in Fig. 2, the concentration of Cu in urine as well as the Cu excretion were transiently increased after dosing, returning to baseline values at approximately 10 h after dosing. The profiles were similar for the two treatments, which is further demonstrated by mean cumulative amounts of Cu excreted in urine up to 12 h after dosing: 149 μg (standard deviation [SD]: 73.2 μg) and 150 μg (SD: 57.4 μg) after dosing capsules with a fast and slow dissolution profile, respectively. Serum Cu and Cp

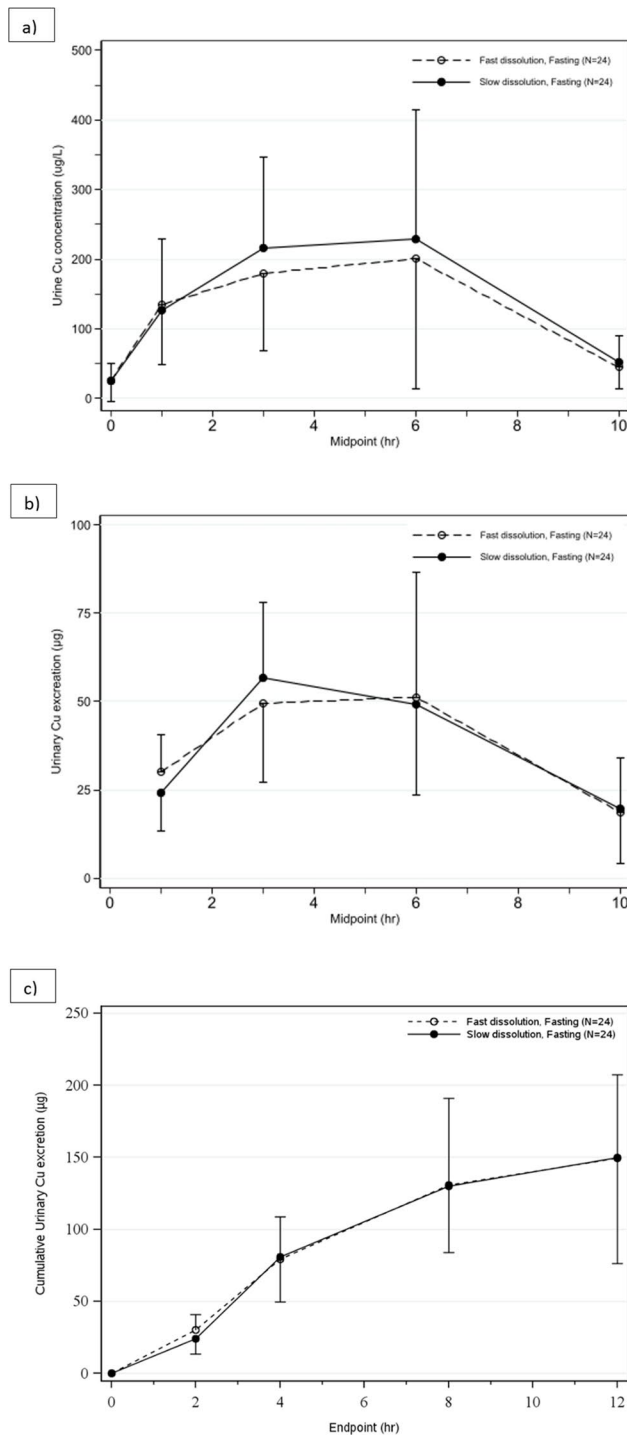


Fig. 2 Mean (SD) urinary copper concentration, excretion-time profiles and cumulative urinary copper excretion after administration of TETA-2HCl capsules with a fast and a slow dissolution profile (a) urinary copper concentration, (b) urinary copper excretion and (c) cumulative urinary copper excretion

concentrations remained stable over the period after dosing and did not show any notable differences between the two treatments (Fig. 3).

Tolerability results

Out of the 24 subjects, 9 [37.5%] reported a total of 14 TEAEs after administration of capsules with a fast dissolution profile and 4 subjects [16.7%] reported a total of 7 TEAEs after administration of capsules with a slow dissolution profile. Out of these 21 TEAEs, 5 were classified as treatment related (3 after administration of a capsule with a fast dissolution and 2 after administration of a capsules with a slow dissolution). No serious TEAEs were reported (Table S2).

The majority of TEAEs reported were mild; one moderate TEAE (abdominal pain) was reported by a subject who received TETA-2HCl with a fast dissolution profile. No severe TEAEs were reported (Table S3). The most frequently reported TEAEs were headache (6 subjects) and nausea (4 subjects) (Table S4).

No deaths, serious TEAEs, TEAEs leading to discontinuation, or other medically important TEAEs were reported in this study. There were no clinically significant abnormal laboratory values or vital signs, physical examination, or ECG findings in this study. Overall, there were no clinically significant differences in the tolerability profiles between the two treatments and both treatments were well tolerated.

Discussion

The objectives of this study were to characterize and compare the pharmacokinetics (PK) of TETA after a single oral dose of 600 mg TETA-2HCl (300 mg dihydrochloride salt containing 200 mg TETA base per 300 mg capsule); once as capsules with a fast and once as capsules with a slow dissolution profile, both administered under fasted conditions. Supportive pharmacokinetic data were collected for the two metabolites of TETA, MAT and DAT. Short term pharmacodynamic (PD) effects, as well as safety and tolerability data on the two different TETA-2HCl capsules were also assessed.

The 90% CI of C_{max} of TETA was within the bioequivalence range of 80–125%. Regarding the AUC_{0-t} and AUC_{0-inf} , the CIs included 100% but due to the large variation, the 90% CIs of TETA were wide, and the lower ends were slightly outside the range of 80–125%. As a result, formal bioequivalence between the two different capsules could not be claimed. For this study we did not perform a formal power calculation but based the sample size of 24 subjects on previous experience with trientine [9, 15]. In view of the large variability a higher number of subjects may have resulted in a smaller 90% CI, bringing it within the 80–125% bio-equivalence range. However, between-subject variability in pharmacokinetic parameters

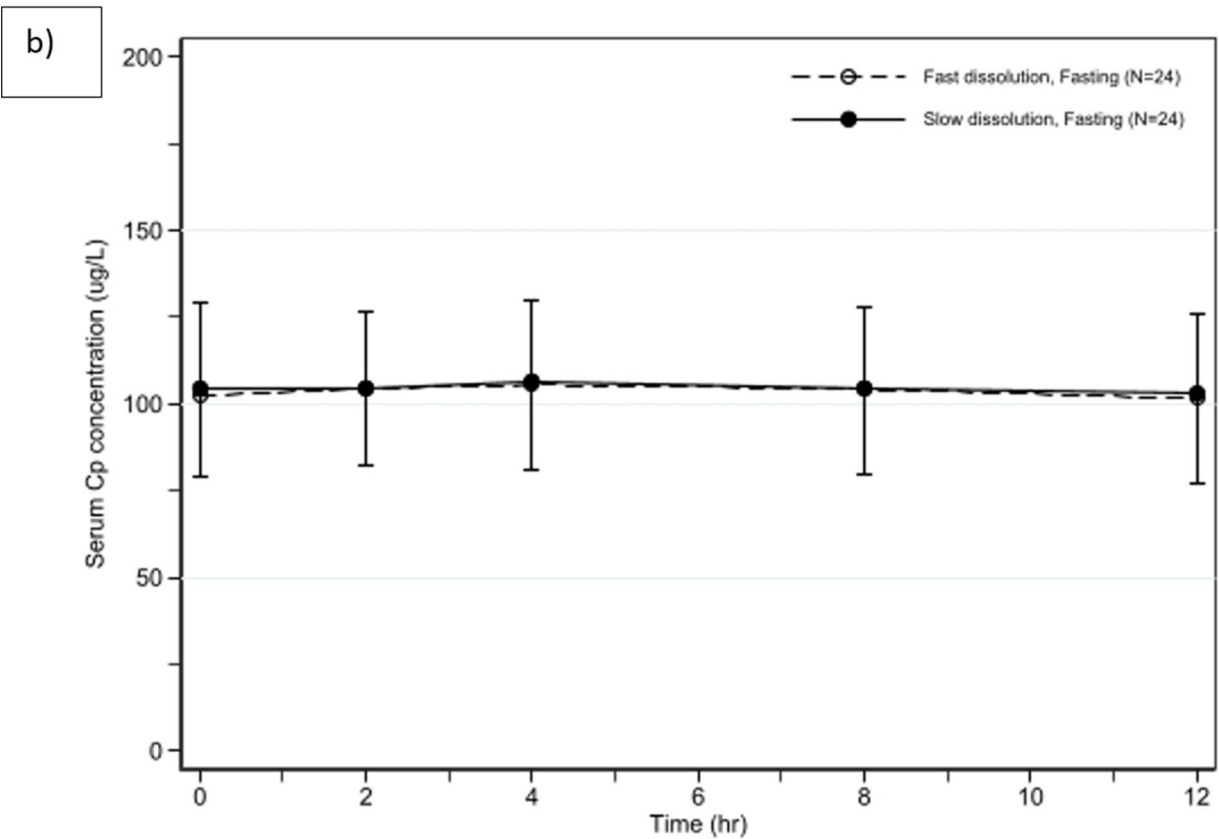
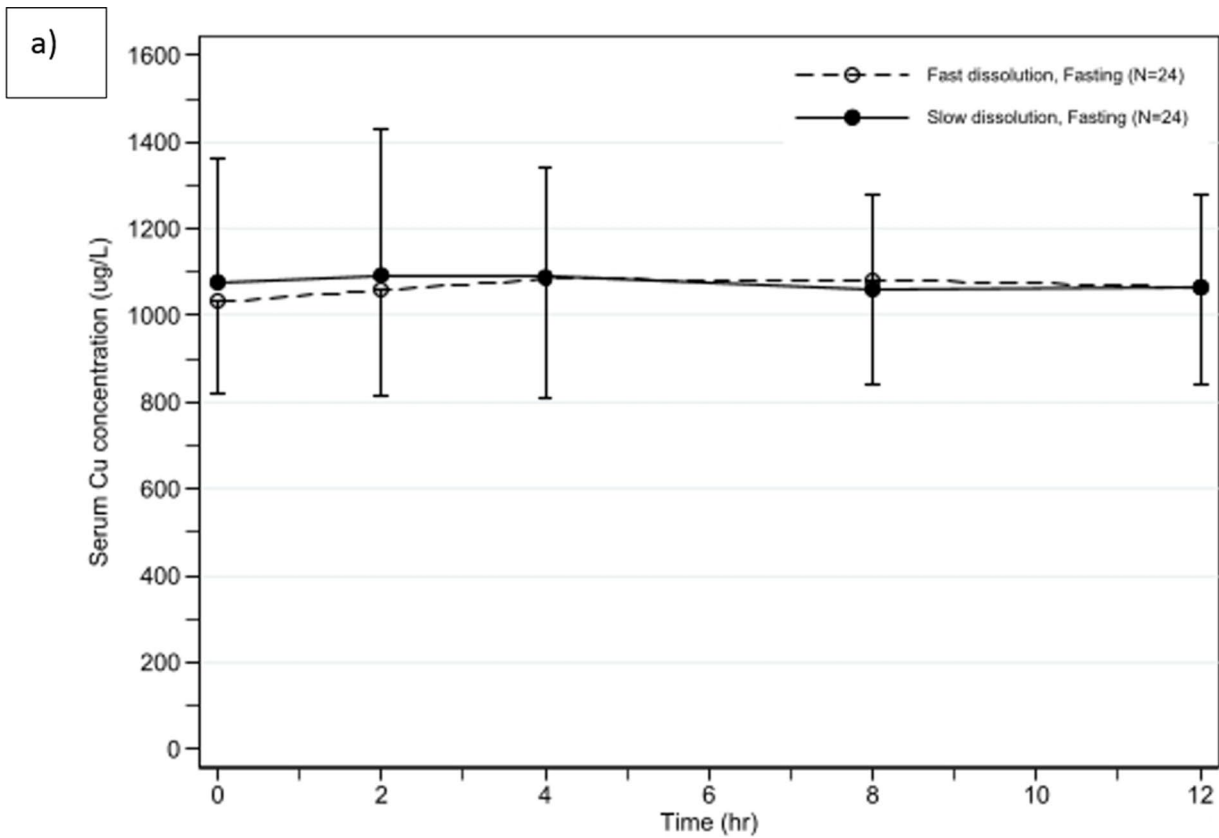


Fig. 3 Mean (SD) serum Cu and Cp concentration-time profiles after administration of TETA-2HCl capsules with a fast and a slow dissolution profile: (a) copper, and (b) ceruloplasmin

is frequently reported as high in both healthy individuals and WD patients [8, 9, 12]. Bioequivalence was shown for both metabolites (MAT and DAT) based on the primary parameters C_{max} , AUC_{0-t} and AUC_{0-inf} .

One of the main underlying reasons for the observed out of range CIs for AUC_{0-t} and AUC_{0-inf} for TETA is the large variation in PK parameters as observed between individuals in this study [8, 9, 12]. This was demonstrated by Cho et al. [15], who showed consistent single/multiple-dose PK and dose-proportional and serum concentration-proportional effects of TETA on enhancing Cu excretion in healthy subjects. The observed variability between healthy subjects in PK and PD parameters was considered high, although in this study linked to the relatively small numbers of participants per dose group [15]. In the study from Pfeifferberger et al. (2018) the steady state PK of TETA was determined in 4 paediatric WD patients (aged 12 years and over) as well as in 16 adult WD patients who were on a stable regimen of twice daily TETA-2HCl [12]. A variability in PK parameters for TETA was reported, with a 10-fold range in C_{max} (309 to 3100 ng/mL) and a 13.8-fold range in AUC_{0-t} (1240 to 17,100 ng*h/mL) [12]. This variability reduced slightly when values were normalized for dose to a 6.7-fold range in C_{max}/D and an 11.6-fold range in AUC_{0-t}/D [12]. Furthermore, they demonstrated that the steady state PK of TETA in WD patients were broadly similar to what had been reported in healthy subjects with no discernible differences in PK parameters between adult and paediatric WD patients [12].

The PK characteristics of TETA as obtained from the current study are in line with available data in healthy subjects published by Lu et al. (2010) which also shows detailed PK profiles of TETA, MAT, and DAT in human plasma following TETA-2HCl administration in healthy subjects [9].

It has previously been hypothesised that between-patient variability observed in the PK profile of TETA may be related to variations in bioavailability: Weiss et al. (2021) concluded that dissolution rate was a likely explanation for the differences in bioavailability of TETA observed. In this study, 23 healthy adult subjects received two different oral formulations of TETA in a single dose equivalent to 600 mg of TETA base; a TETA-2HCl reference formulation (three capsules containing 200 mg of TETA base each) and a TETA-4HCl formulation (four tablets containing 150 mg of TETA base each). The median time to reach maximum plasma concentration (T_{max}) was 3.0 h for TETA-2HCl and 2.0 h for TETA-4HCl. The C_{max} and AUC_{0-inf} of absorption of the active moiety, TETA, were greater by approximately 56% and 68% respectively, for TETA-2HCl

than for the TETA-4HCl formulation. It was suggested that a more rapid dissolution of the TETA-4HCl formulation might explain the faster and better absorption of TETA as reflected by C_{max} [13, 17]. However, in the current study, the effect suggested in Weiss et al. (2021) was not as evidently present. C_{max} values demonstrated bioequivalence for the two capsules and AUC_{0-inf} 90% CI values were only just outside the pre-specified limits for bioequivalence. Based on the outcome of this study, dissolution differences did not impact the absorption rate of TETA-2HCl. Further to the observation that only marginal effects of dissolution rate on the PK of the parent drug TETA were detected, no differences of dissolution rate resulted in clinically relevant effects on the rate and extent of exposure of the two metabolites of TETA, MAT and DAT.

In this study, the demographic characteristics of the study population show a predominant female and white study population. In general, it is not possible to fully exclude the possibility that this predominantly female and white study population might limit the generalizability of the results. Regarding differences in plasma levels of trientine in relation to sex, a higher plasma level was observed previously for males compared to females [17]. Furthermore, lower levels of MAT and DAT were observed in males. Therefore, absorption and metabolism may differ slightly between sexes. However, no differences in terminal half-life ($t_{1/2}$) of trientine was observed in relation to sex. Thus, elimination kinetics remain consistent without showing any sex related differences. Regarding the influence of race and ethnicity on the generalizability of the study data, no specific data exists. It is important to point out that most Phase I clinical trials have been conducted in relatively small and homogeneous populations, limiting the ability to detect such differences. Which might also be related to the high between-patient variability generally observed for PK parameters [9, 15].

No differences were observed in the pharmacodynamic parameters, i.e., UCE and the serum profiles of Cu and Cp, between the two capsules. The mean cumulative urinary Cu excreted up to 12 h after dosing was 150 μ g and 149 μ g for the slow and fast dissolution profile, respectively. However, it should be noted that the study duration was relatively short, and the healthy subjects did not have an excessive Cu pool or a disturbed Cu homeostasis, as seen in patients with WD. A greater variation in Cu parameters would also be expected in patients with WD, as these measurements are reflective of disease severity. Although a threshold of 100 μ g (1.6 μ mol)/24 hours UCE is generally used for WD diagnosis in symptomatic, non-treated patients [1], it is widely accepted that UCE levels may vary substantially between individuals, with guidelines recommending ranges for patients receiving chelation therapy from 3 to 8 μ mol/24 h) [3, 4].

The outcome of this study confirms the well-known and described safety profile of TETA-2HCl. While taking into account the small sample size and short duration of this study, the limited number of reported TEAEs including treatment related TEAEs as well as the nature of these reported TEAEs, indicate that a difference in dissolution rate of TETA-2HCl capsules does not affect the tolerability profile of TETA-2HCl. The most frequently reported TEAEs were nausea and headache. Nausea is a known side effect of TETA; additionally, headaches are commonly reported and considered non-specific in studies conducted with healthy subjects hospitalized in a research facility for some days.

In conclusion, this study shows that the pharmacokinetic profile of TETA after administration of TETA-2HCl capsules with a fast dissolution rate is comparable to the pharmacokinetic profile of TETA after administration of TETA-2HCl capsules with a slow dissolution rate. In other words, the differences in the *in vitro* dissolution profiles of the two investigated capsules in this study are considered to play no role on the *in vivo* pharmacokinetic behavior of TETA and its metabolites. The extent in which pharmacokinetic parameters were affected by the differences in dissolution rate were marginal and were much smaller than the between-individual differences observed in the TETA pharmacokinetics and were therefore not considered clinically relevant by the investigators. This is considered a further extension on the already existing knowledge of the pharmacokinetics of TETA. Additionally, no differences in short term pharmacodynamic effects and tolerability were observed between the two TETA-2HCl capsules, confirming their similarity between the two treatments. Overall, the two different capsules behave similar when it comes to their tolerability, pharmacokinetic and pharmacodynamic features.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests K.H. Weiss advises for Univar Solutions BV, Vivet Therapeutics, Alexion, Orphalan, Ultragenyx, Pfizer, AbbVie, Bayer and is on the speakers bureau of Alexion, Orphalan and Falk and received travel support from Alexion. For the remaining authors, no conflict of interests were declared.

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