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Pharmacokinetics, Pharmacodynamics, and Metabolism of Triethylenetetramine in Healthy Human Participants: An Open-Label Trial

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The selective Cu^{II}-chelator, triethylenetetramine (TETA), is undergoing clinical trials for the treatment of heart failure in patients with diabetes. Recently, the authors showed that 2 acetylated metabolites, N_1-acetyltriethylenetetramine (MAT) and N_1,N_{10}-diacetyltriethylenetetramine (DAT), are formed in humans following oral TETA administration. Thus, it became necessary to determine whether the N-acetyltransferase (NAT) 2 phenotype has any effects on the pharmacological properties and safety profile of TETA. Twelve fast and 12 slow NAT2-phenotype healthy participants were recruited. After oral drug administration, the authors collected plasma and urine samples, measured plasma concentrations of TETA and its 2 metabolites along with concomitant urinary copper concentrations, and performed safety tests. They present, for the first time, the complete 24-hour pharmacokinetic profiles of TETA, MAT, and DAT in humans. There was no evidence for clear-cut differences in pharmacokinetic profiles between fast and slow acetylators. Pharmacodynamic analysis showed no significant differences in cupruresis between the 2 NAT2 phenotypes. Safety results were consistent with TETA being well tolerated, and no significant differences in safety profiles were observed between the 2 phenotypes. Based on these data, NAT2 phenotype does not affect TETA's pharmacokinetic, pharmacodynamic, or safety profiles. TETA may be acetylated via an alternative mechanism, such as that catalyzed by spermidine/spermine N_1-acetyltranferase.

Keywords: *acetylation; triethylenetetramine; metabolism; pharmacokinetics; pharmacodynamics*
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Diabetes is known to be a significant risk factor for cardiovascular morbidity and mortality.[1-3] Indeed, heart disease is the major cause of death in diabetes, wherein chronic hyperglycemia and cardiovascular complications are cardinal attributes. Estimated mortality rates attributable to cardiovascular disease among patients with type 2 diabetes may be as high as 50%,[4,5] and in the United States alone, there are an estimated 1 to 2 million patients with concomitant diabetes and congestive heart failure.[6]

We have shown previously that triethylenetetramine (TETA), a synthetic analog of endogenous polyamines that acts as an in vivo Cu^{II}-selective chelator, ameliorates left ventricular hypertrophy in diabetic rats[7] and diabetic patients.[8] TETA has long been used for the treatment of Wilson's disease, an inherited disorder of Cu metabolism.[9] We hypothesize that metabolic changes in diabetes, particularly those resulting in the accumulation of advanced glycation end products in the extracellular matrix, lead to or cause the accumulation of excess extracellular Cu^{II} in this compartment, and the resulting tissue-Cu imbalance could cause heart failure in the diabetic context.[7,10] TETA has the ability not only to extract

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excess extracellular Cu^{II} from tissues[10] but also to suppress the diabetes-evoked activation of the transforming growth factor-β1/Smad signaling pathway and to restore extracellular superoxide dismutase and heparan sulfate levels to control levels in the extracellular matrix of cardiovascular tissues.[11] In addition, we recently showed that TETA treatment can reverse several hallmarks of diabetic nephropathy, including nephromegaly, renal fibrosis, glomerulosclerosis, and albuminuria, without lowering hyperglycemia in an animal model of diabetes.[12] Taken together, these data show that TETA can break the link between hyperglycemia and tissue damage in diabetes.

TETA has been used for decades as a pharmacotherapy (administered in the form of its dihydrochloride salt, Trientine) for Wilson's disease.[9,13] However, there are only a few reports on aspects of TETA's human pharmacology in the literature[14-17] and no readily available detailed pharmacokinetic (PK) data for it and its metabolites. Most oral TETA is not absorbed but excreted unchanged in the feces.[18] The 5% to 18% that is systemically absorbed in humans is reported to be extensively metabolized, with the majority being excreted in urine as metabolite(s)[14,19]; results from rat studies have been consistent.[20-23]

We recently determined that 2 major TETA-derived metabolites appear in human plasma and urine following dosing, N_1-acetyltriethylenetetramine (MAT) and N_1,N_{10}-diacetyltriethylenetetramine (DAT), both of which are products of acetylation.[24] Many drugs, including dapsone, hydralazine, isoniazid, sulfasalazine, procainamide, nitrazepam, and caffeine, are acetylated in the liver by the *N*-acetyltransferase (NAT) 2 enzyme. Genetic polymorphisms dictate an individual's level of NAT2 activity, which in turn affects the metabolic rate of such drugs and, consequently, their toxicological and pharmacological properties.[25] Individuals can generally be classified as having either a fast or slow acetylator phenotype according to their genotype at the NAT2 locus, on the basis of their ability to convert a particular substrate such as caffeine to its *N*-acetyl derivative. Because TETA is metabolized to *N*-acetyl-containing metabolites, it is important to determine its pharmacokinetics and those of its metabolites, as well as pharmacodynamic (PD) and safety profiles of TETA dihydrochloride in participants having either fast or slow acetylator phenotypes.

Here, we used a recently developed and validated high-performance liquid chromatography (HPLC) method, which detected and measured TETA and its metabolites in one sample injection,[26] to study relevant PK and PD profiles in an open-label trial. Using a standard caffeine probe test, 12 volunteers were determined to have the fast acetylator phenotype and 12 the slow acetylator phenotype. In this study, TETA dihydrochloride was well tolerated at a relatively high dose for 7 days by participants in both acetylator groups. PK and PD data were collected on days 0 and 7 of the study. Different acetylation phenotypes had no measurable effects on any PK parameters, PD outcomes, or safety profiles.

MATERIALS AND METHODS

Authentic Standards and Other Reagents

TETA dihydrochloride (purity 99.93%), MAT trihydrochloride (purity 98.50%), and DAT dihydrochloride (purity 95.43%) were synthesized by CarboGen AG (Hunzenschwil, Switzerland) via a recently described novel organic synthesis route.[27] Borax buffer was prepared by dissolving 10 g of sodium tetraborate (Sigma-Aldrich, St. Louis, Missouri) in 200 mL of water (final pH 9.6). Ethylenediaminetetraacetic acid (EDTA) disodium salt, 9-fluorenylmethyl chloroformate (FMOC), and hexamethylenediamine (HDA) dihydrochloride were from Sigma-Aldrich.

Study Participants

Twenty-four healthy nonsmoking volunteers were enrolled who were not taking any over-the-counter or prescribed medications when they presented to the research clinic (a minimum of 12 hours free of such medications was required). Within 12 weeks before administration of study medication, participants completed a caffeine phenotype probe screening study to determine eligibility and acetylator status by analysis of urinary caffeine metabolites.[28,29] During the open-label, single-visit screening study, participants received a single oral dose of 200 mg caffeine (Vivarin) with 240 mL water following an overnight fast, having agreed to refrain from over-the-counter and prescription medications, as well as xanthine-containing food and beverage ingestion, for at least 12 hours. The restriction on additional caffeine intake was instituted primarily to address safety concerns rather than a complete washout from previous caffeine intake. Participants were expected to metabolize caffeine the same way (ie, by fast or slow acetylation) regardless of the time of last ingestion of caffeine, and the caffeine probe methodology

employed here was consistent with previous studies cited.

Participants remained in the research facility following dosing, and a urine sample (at least 10 mL) was collected 4 to 6 hours after caffeine administration. Urine samples were analyzed for 5-acetyl-amino-6-formyl amino-3-methyluracil[30] and 1-methylxanthine levels using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method (unpublished manuscript, Frontage Laboratories, Inc, Malvern, Pennsylvania). Values (ng/mL) were converted to nM, and the molar 5-acetyl-amino-6-formyl amino-3-methyluracil/1-methylxanthine ratio for each participant was calculated. A slow acetylator phenotype was defined by a ratio of ≤0.55 and a fast acetylator phenotype by one of >0.55.[28,29] The mean (range) of ratios of the 12 participants identified as fast acetylators (7 men and 5 women) enrolled into this study was 1.18 (0.749-1.669) and that of the 12 participants identified as slow acetylators (7 men and 5 women) was 0.184 (0.00-0.39). The median (range) of ages was 31.2 (19-46) years for all 24 participants, comprising 32.0 (21-46) years for the 12 fast acetylators and 30.4 (19-44) years for the 12 slow acetylators. The mean bodyweight was 76.7 kg (63.6-96.4) for all 24 participants, comprising 74.7 kg (64.1-85.3) and 78.7 kg (63.6-96.4) for the fast and slow acetylators, respectively.

Clinical Study

Informed consent was obtained from all participants prior to the screening study and again in eligible participants for the TETA study prior to initiation of any study-specific procedures. The Essex Institutional Review Board (www.essexirb.com) approved the research protocol and experimental use of trientine in this participant population (USFDA IND No. 70,722), and the study conformed to the Declaration of Helsinki.[31] The screening caffeine probe and TETA study protocols and consent forms were reviewed and approved by a duly constituted human ethics committee prior to study initiation and participant enrollment.

Participants reported to the research facility by 6 am on the day prior to the first dose of study medication (day –1). On day 0, at 8 AM, participants received a single oral dose of 600 mg of TETA dihydrochloride (two 300-mg capsules), followed by 48 hours of blood and urine collection. On days 2 to 6, participants received twice-daily oral doses of 600 mg of drug every 12 hours (1200 mg per 24 hours). On day 7, a single oral dose of 600 mg of drug was administered at approximately 8 AM, after which blood and urine were collected for 48 hours. On days 2 to 6, blood samples to measure trough drug and metabolite levels in plasma were obtained at time 0 (immediately before dosing). Participants remained resident at the research facility from day 0 to day 9, when they were discharged approximately 48 hours after the last dose of study medication.

Collection of Urine and Plasma Samples

On days 0 and 7, following a morning dose of TETA dihydrochloride, all urine was collected during the following periods: from –2 (2 hours before dosing) to 0 hours, then from 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 16, 16 to 20, and 20 to 24 hours postdose. Urine volumes were recorded for each period, and urine samples were used for copper analysis. Also on days 0 and 7, following a 600-mg morning dose of TETA dihydrochloride, serial blood samples were collected (approximately 7 mL each) to measure plasma concentrations of TETA and its metabolites at times 0, 10, 20, 30, 45, 60, 75, 90, and 120 minutes and thereafter at 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 30, 36, 42, and 48 hours postdose on days 0 to 1 and 7 to 8. On days 2 to 6, blood samples to measure trough drug and metabolite levels in plasma were obtained at time 0 on each day, immediately prior to administration of study medication.

Measurement of TETA, MAT, and DAT in Plasma

Analyses were performed in an accredited laboratory at the School of Biological Sciences, University of Auckland, by an analyst blinded as to treatment group and acetylator phenotype. Then, 25 μL of plasma was added into a reaction buffer containing 10 μL of 60 μM HDA (internal standard), 20 μL of 1 mM EDTA, 25 μL of water, and 25 μL of borax buffer (50 mg/mL). Then, 75 μL of acetonitrile was added and the solution was vortex-mixed and centrifuged at 10000 *g* for 3 minutes. Supernatants of 100 μL were removed and added into 100 μL of 7.5 mM FMOC (final pH 9.6). The mixture was vortex-mixed and stood for 10 minutes and then centrifuged at 4000 *g* for 2 minutes. Supernatants were removed and placed in glass HPLC vials. Then, 10 μL of each supernatant was injected onto an HPLC system (Shimadzu, Kyoto, Japan) comprising 2 LC-10ADvp pumps, a DGU-14AM degasser, a SIL-10ADvp temperature-controlled auto injector, a CTO-10ASvp column oven, and an RF-10AxL fluorescence detector. The chromatographic separation was achieved

using a Luna 3μ C18 150 × 3.0-mm reversed phase column (Phenomenex, Auckland) maintained at 25°C with a linear gradient mobile phase. The 2 mobile phases used were the following: A, 100% acetonitrile and B, 10 mM ammonium acetate in Milli Q water. The linear gradient program was as follows: 0 minutes, 50% A; 7 minutes, 70% A; 7.5 minutes, 80% A; 14.5 minutes, 80% A; 15 minutes, 100% A; 19 minutes, 100% A; 21 minutes, 50% A; and 35 minutes, 50% A. Overall runtime was 35 minutes with the flow rate being maintained at 0.5 mL/min.

The excitation and emission wavelengths of the fluorescence detector were 263 nm and 317 nm, respectively. Fully labeled TETA, MAT, DAT, and HDA were identified by mass spectrometry (MS) using a 2010A single quadrupole instrument equipped with an atmospheric pressure chemical ionization (APCI) interface in positive mode. The MS conditions were as follows: curved desolvation line (CDL) temperature, 200°C; heat block, 300°C; nebulizing gas, 2.5 L/min; interface voltage, 3.5 kV; CDL voltage, –20 V; polarity, positive; and Q-array voltage: DC 5.0 V, RF 150 V. Fluorescence-labeled analytes were identified using single-ion monitoring (SIM) detection with molecular mass settings of 1035 (for TETA labeled with 4 FMOC groups), 855 (for MAT labeled with 3 FMOC groups), 675 (for DAT labeled with 2 FMOC groups), and 562 (for HDA fully labeled with 2 FMOC groups).[26,32]

The following common medications and relevant compounds were tested for interference with this FMOC-based method: metoprolol succinate, metoprolol tartrate, captopril, glipizide, felodipine, warfarin, acetaminophen, cimetidine, digoxin, furosemide, insulin, magnesium salicylate, metformin hydrochloride, salicylic acid, simvastatin, diltiazem hydrochloride, and p-aminobenzoic acid, as well as spermidine, spermine, and EDTA.

Serial dilutions of TETA, MAT, and DAT standards were made in blank plasma samples containing HDA as the internal standard to create standard curves, such that each sample contained 1 aliquot of each standard. Data acquisition, peak integration with skim tangent, linear standard curve construction, and analyte concentration calculations were performed using Shimadzu LCsolution software. Area ratios of analyte peak versus internal standard peaks were used to construct standard curves. The ranges of calibration curves were 0.125 to 5 mg/L for MAT and DAT (for free-base analytes) and 0.0625 to 5 mg/L for TETA (TETA free-base, $r^2 = 0.99$ in each case). Relative recoveries were 85% to 115% of standard values, and coefficient of variation (CV) was <15%. Back-calculated values were within 15% of standard values for each concentration. CV values for inter- and intrabatch precision were below 15% in each case. Postderivatization solutions were stable at room temperature for at least 52 hours and for at least 56 hours at 4°C. Three freeze-thaw cycles during 24 hours did not affect the stability of postderivatization solutions.

Urinary Cu Analysis

Urinary Cu sample analysis was performed by atomic absorption spectrophotometry using a PerkinElmer Analyst 800 spectrometer, and assay dynamic range varied between 1 and 10 000 μg/dL.

Pharmacokinetic and Pharmacodynamic Analyses

The plasma PK profiles for the parent compound (TETA) and metabolites MAT and DAT, following single-dose and steady-state dosing of TETA dihydrochloride 600 mg (two 300-mg capsules), were determined by calculating values for areas under the curve (AUC), peak concentrations (C_{max}), time to peak concentrations (t_{max}), and terminal half-lives ($t_{1/2}$) for the first and last treatments and compared among groups of participants characterized as slow or fast acetylators. Plasma data were analyzed using WinNonlin v4.01 (Pharsight Corp, Mountain View, California). PK parameters were calculated using a standard noncompartmental analytical approach. The accumulation index for TETA dihydrochloride was calculated from the ratio of $AUC_{0\text{-}24}$ values on day 7 divided by those on day 0. Simple descriptive statistics, graphs, and participant listings were generated to summarize the data.

Safety Data

Clinical laboratory blood tests, including hematology, biochemistry, liver function, tests specific to iron deficiency, urinalysis, and other indicators (serum zinc, copper, calcium, magnesium, and ceruloplasmin), were performed on day –1 and day 9 to monitor safety. Hematology tests included hemoglobin, hematocrit, red blood cell count, platelets, white blood cell count, and differential measurement. Biochemistry tests included glucose, sodium, potassium, chloride, HCO_3^- (CO_2), blood urea nitrogen, and creatinine. Liver function tests included total

and direct bilirubin, albumin, and enzyme activities of alkaline phosphatase, gamma glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase. Measurements of iron status included serum iron, ferritin, and soluble transferrin receptors. Apart from the above tests, additional tests for pregnancy (as appropriate), a drug and alcohol screen, and glomerular filtration rate measurements were performed.

All adverse events were documented, including clinical signs, symptoms, or diagnosed disease, temporally associated with the use of medication, whether or not considered related to the investigational product. All adverse events reported by the participant or observed by the research staff were individually recorded. The signs and symptoms, time of onset (24-hour clock), duration, action taken, and follow-up procedures were also recorded. Sitting blood pressure, pulse rate, and temperature were measured at the screening visit, immediately prior to each dose (time 0), 3 hours following each morning dose, and prior to discharge. A resting 12-lead electrocardiogram (ECG) was performed at the screening visit, 24 hours prior to the first dose, 3 hours following each morning dose, and prior to discharge. A physical examination was performed at the screening visit, on day –1, and on day 9 prior to discharge. A follow-up telephone call was placed to all participants on day 14 to screen for postdischarge adverse events.

Statistical Analyses

The PK parameters, including AUC, C_{max}, t_{max}, and $t_{1/2}$, for fast and slow acetylator groups were analyzed using analysis of variance (ANOVA). Pearson's correlation coefficients from pairwise scatterplots were used to determine whether there was any correlation between numerical acetylator values and individual PK parameters. The PK concentration-time profiles of fast and slow acetylators at day 1 and day 7 were compared using 2-way ANOVA to determine whether there was an overall acetylator status effect and whether there was a significant difference between fast and slow acetylators on either day. A comparison of cumulative copper excretion during the 0- to 12-hour interval among participants identified with fast compared with slow acetylator phenotypes was performed by analysis of covariance (ANCOVA) using the generalized linear model (GLM) procedure, with baseline copper excretion included as a covariate in the model. Treatment was also included in the model. Comparisons of cumulative cupruresis during the 0- to 12-hour and 0- to 24-hour intervals on days 0 and 7 were also performed. A linear mixed effects model[24] was used to compare plasma drug/metabolite levels with cupruresis on days 0 and 7.

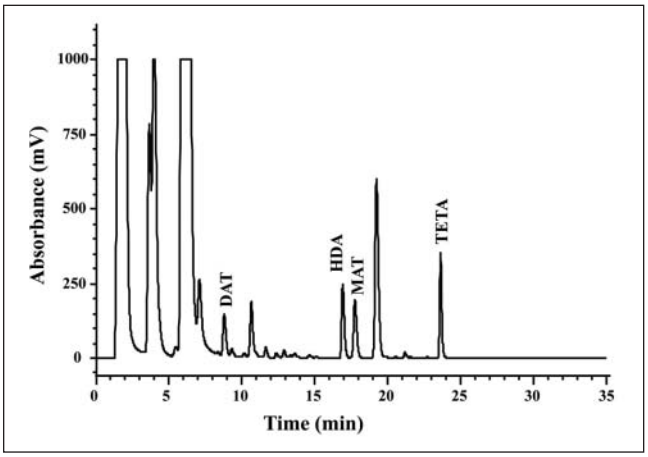

Figure 1. High-performance liquid chromatography chromatogram showing complete separation and detection of triethylenetetramine (TETA), N_1-acetyltriethylenetetramine (MAT), N_1,N_{10}-diacetyltriethylenetetramine (DAT), and hexamethylenediamine (HDA) by a 9-fluorenylmethyl chloroformate (FMOC)–based method in a representative plasma sample from a non-drug-treated participant to which equivalent masses of each compound had been added in vitro to respective final concentrations of 4.0 mg/L.

RESULTS

Plasma Drug and Metabolite Concentrations

TETA, MAT, DAT, and HDA were completely separated chromatographically (Figure 1), and their respective retention times were 23.7, 17.7, 8.6, and 16.9 minutes. None of the listed medications, spermidine, spermine, or EDTA, interfered with the detection of analytes by this HPLC method. Plasma drug and metabolite concentrations were calculated from the standard curves.

Pharmacokinetic Measurements

PK parameters for drug and metabolites on days 0 and 7 are summarized in Table I. Mean (SEM) plasma drug and metabolite concentration-time profiles are displayed for all participants on days 0 and 7 in Figure 2. Concentration-time profiles for participants by acetylator status on day 0 are shown in Figure 3A and on day 7 in Figure 3B. There were no significant between-group differences in any PK

Table I Summary Statistics for Plasma Pharmacokinetic Parameters of TETA, MAT, and DAT for All Participants in Fast and Slow Acetylator Groups

			TETA		MAT		DAT	
Variable	**Day**	**Acetylator Status**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
$t_{1/2}$, h	Day 0	All	2.48	1.63	5.33	4.27	10.8	6.17
	Day 0	Fast	2.50	1.30	6.89	5.56	14.0	6.43
	Day 0	Slow	2.47	1.97	3.77	1.41	8.48	5.23
	Day 7	All	3.28	1.15	9.04	5.11	13.9	6.56
	Day 7	Fast	3.14	1.35	9.95	5.72	14.1	5.74
	Day 7	Slow	3.41	0.938	8.14	4.48	13.7	7.64
t_{max}, h	Day 0	All	1.82	0.813	5.38	1.17	4.71	1.36
	Day 0	Fast	2.08	0.634	5.42	1.56	5.00	0.756
	Day 0	Slow	1.56	0.912	5.33	0.651	4.44	1.74
	Day 7	All	1.98	1.17	4.96	0.908	4.62	3.13
	Day 7	Fast	2.31	1.53	4.75	1.055	5.75	2.83
	Day 7	Slow	1.65	0.538	5.17	0.718	3.39	3.08
C_{max}, mg/L	Day 0	All	0.798	0.330	0.748	0.306	0.176	0.164
	Day 0	Fast	0.866	0.318	0.711	0.230	0.130	0.104
	Day 0	Slow	0.729	0.343	0.784	0.373	0.222	0.203
	Day 7	All	0.950	0.660	1.04	0.331	0.294	0.176
	Day 7	Fast	1.20	0.840	1.14	0.378	0.218	0.0892
	Day 7	Slow	0.700	0.265	0.937	0.252	0.369	0.211
AUC_{0-24}, mg·h/L	Day 0	All	2.97	1.67	5.23	2.13	1.01	1.07
	Day 0	Fast	3.47	1.37	5.39	1.77	0.719	0.829
	Day 0	Slow	2.47	1.85	5.07	2.50	1.30	1.24
	Day 7	All	4.16	2.38	10.8	3.43	3.11	2.19
	Day 7	Fast	4.90	2.65	11.9	3.67	2.59	1.57
	Day 7	Slow	3.41	1.89	9.72	2.92	3.63	2.63
$AUC_{0-\infty}$, mg·h/L	Day 0	All	3.25	1.80	6.69	3.17	4.11	1.48
	Day 0	Fast	3.77	1.43	7.66	3.61	4.50	1.75
	Day 0	Slow	2.73	2.03	5.72	2.44	3.82	1.32
	Day 7	All	5.61	2.87	14.0	6.49	6.53	3.91
	Day 7	Fast	5.93	2.87	16.0	7.46	5.94	2.44
	Day 7	Slow	5.29	2.97	12.0	4.85	7.12	5.08

TETA, triethylenetetramine; MAT, N_1-acetyltriethylenetetramine; DAT, N_1,N_{10}-diacetyltriethylenetetramine.

parameters and no significant correlations between fast and slow acetylator status and any of the PK parameters. There was no significant effect of acetylation status on the concentration-time profiles of TETA, MAT, and DAT on day 1 (P = .61, .69, and .21, respectively) and TETA and MAT on day 7 (P = .12 and .11, respectively). There was a weak association between the DAT concentration-time profile and acetylator status (P = .048). However, there was no significant difference in plasma concentrations for each of the 3 analytes between fast and slow acetylator groups at any sampling time point, except for 2: TETA values at 75 minutes on day 1 (P < .05) and at 2 hours on day 7 (P < .01). The mean (SD) trough values of TETA, MAT, and DAT for all participants during days 2 to 6 were 0.097 (0.098), 0.497 (0.113), and 0.181 (0.122) mg/L, respectively. The trough values of TETA, MAT, and DAT for fast acetylators were 0.105 (0.094), 0.542 (0.090), and 0.126 (0.042) mg/L, respectively, and for slow acetylators were 0.089 (0.094), 0.456 (0.083), and 0.237 (0.145) mg/L, respectively.

The mean accumulation index for TETA, calculated as the ratio of the AUC_{0-24} on day 7 to that on day 0, was 1.68. There were no clear-cut between-group differences in the pharmacokinetic profiles of TETA, MAT, and DAT between fast and slow acetylators following single and repeated oral doses of TETA dihydrochloride 600 mg twice daily.

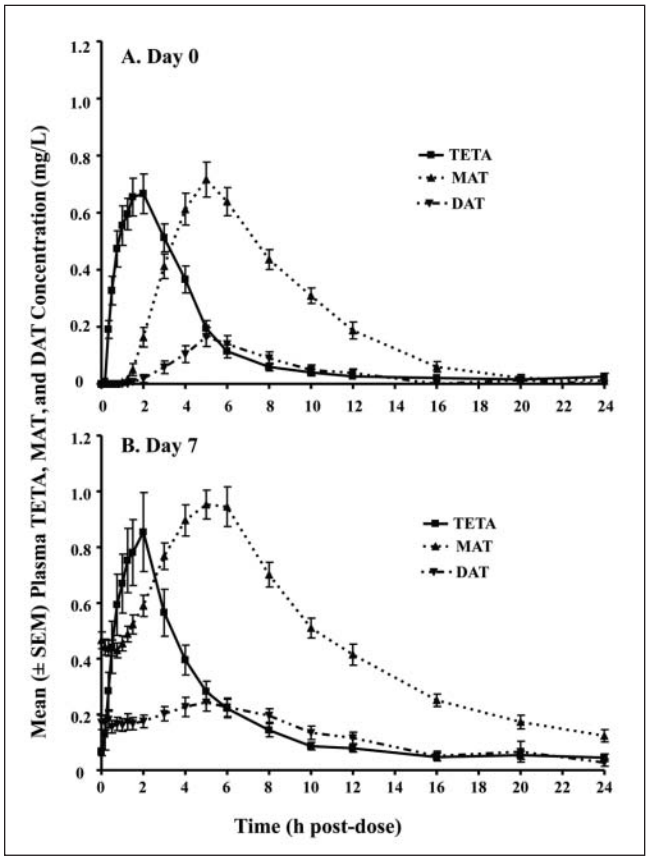

Figure 2. Mean plasma concentration-time profiles for triethylenetetramine (TETA), N_1-acetyltriethylenetetramine (MAT), and N_1,N_{10}-diacetyltriethylenetetramine (DAT) on study days (A) 0 and (B) 7, in all participants (n = 24) who took single 600-mg oral doses of TETA dihydrochloride on these days and twice-daily doses intercurrently on days 2 through 6. Values are mean ± SEM.

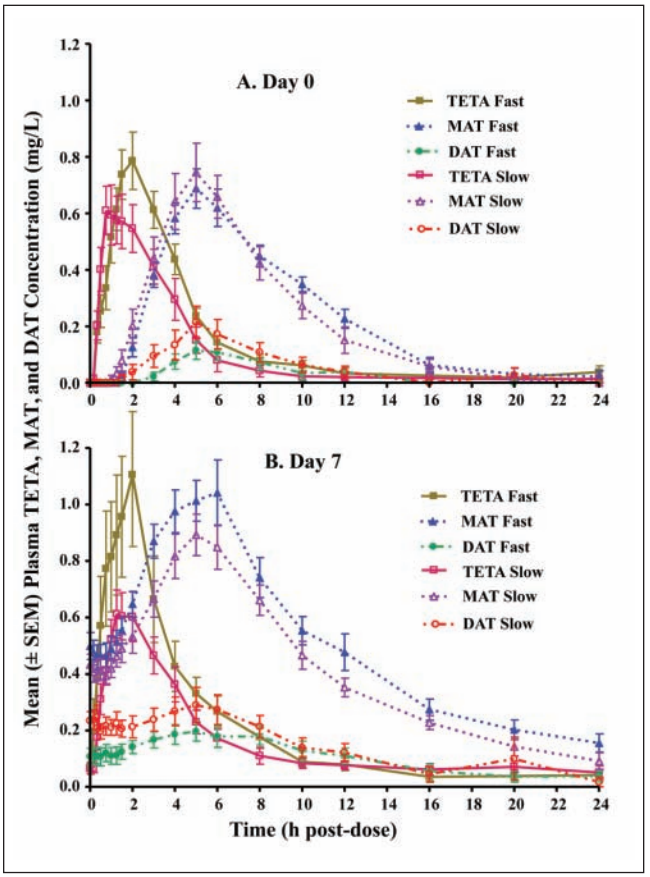

Figure 3. Mean plasma concentration-time profiles for triethylenetetramine (TETA), N_1-acetyltriethylenetetramine (MAT), and N_1,N_{10}-diacetyltriethylenetetramine (DAT) in groups (n = 12) of fast and slow acetylator participants on study days (A) 0 and (B) 7 who took single 600-mg oral doses of TETA dihydrochloride on these days and twice-daily doses intercurrently on days 2 through 6. Values are mean ± SEM.

Pharmacodynamic Profiles

Increased cupruresis was most evident throughout the 0- to 12-hour postdose intervals, wherein the highest rates occurred during the 0- to 4-hour postdose period in both acetylator groups. Among all participants, mean (SD) cumulative cupruresis during this 4-hour postdose period was 24.0 (11.8) and 18.7 (11.3) μg on days 0 and 7, respectively. Mean (SD) cumulative cupruresis during this period was 22.2 (9.0) and 18.4 (10.2) μg on days 0 and 7, respectively, in the fast acetylator group and 25.8 (14.3) and 18.9 (12.7) μg on days 0 and 7, respectively, in the slow acetylator group; these values are not significantly different. Data for cumulative cupruresis are shown in Figure 4 and cupruresis by individual urine collection periods in Figure 5.

A comparison of cumulative copper excretion during the 0- to 12-hour postdose interval between the groups of fast and slow acetylators was performed using ANCOVA with baseline cupruresis as the covariate and yielded P values of .480 and .710 on days 0 and 7, respectively. The same comparison of cumulative cupruresis during the 12- to 24-hour and 0- to 24-hour intervals on days 0 and 7 did not reveal any statistically significant differences ($P >$ .05) based on acetylator phenotype. Comparison of drug/metabolite plasma concentrations with cupruresis revealed that the change in urinary copper excretion was more closely related to [TETA + MAT] ($P <$ .0001, $r^2 = 0.8639$ on day 1 and $P = .0003$, $r^2 = 0.8207$ on day 7) than to either [TETA] ($P = .0439$, $r^2 = 0.4165$ on day 1 and $P = .0014$, $r^2 = 0.7424$ on day

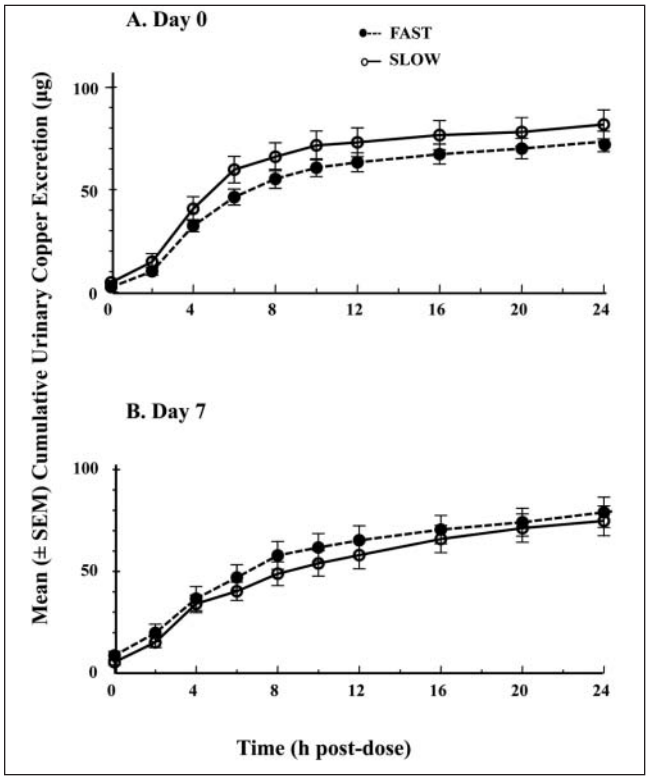

Figure 4. Cumulative cupruresis (µg) on days (A) 1 and (B) 7 in fast and slow acetylator participants (n = 12/group) who took single 600-mg doses of triethylenetetramine (TETA) dihydrochloride on days 0 and 7 and twice-daily doses intercurrently on days 2 through 6.

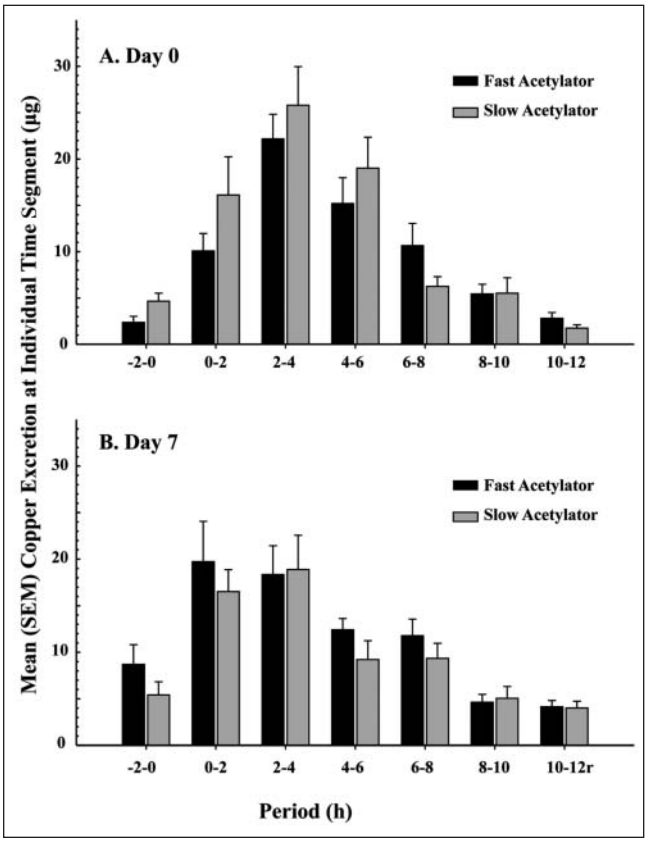

Figure 5. Cupruresis (µg) during indicated periods on days (A) 0 and (B) 7 in fast and slow acetylator participants (n = 12/group) who took single 600-mg doses of triethylenetetramine (TETA) dihydrochloride on days 0 and 7 and twice-daily doses intercurrently on days 2 through 6.

7) or [MAT] ($P = .0034$, $r^2 = 0.6775$ on day 1 and $P = .0269$, $r^2 = 0.4774$ on day 7) alone.

Safety Profiles

Oral doses of 600 mg TETA dihydrochloride taken once daily on days 0 and 7 and twice daily from days 2 to 6 inclusive were safe and well tolerated by these 24 healthy adult male and female participants. There were no evident between-group differences in the safety or tolerability of TETA dihydrochloride between fast and slow acetylators. Adverse events were reported by a total of 7 (29.2%) participants: 3 (25.0%) participants in the fast acetylator group and 4 (33.3%) participants in the slow acetylator group. Headache, reported by 4 (16.7%) participants, was the most common treatment-emergent adverse event. Two participants each (16.7%) in the fast and slow acetylator groups reported headache. No participants discontinued study participation due to an adverse event, and no serious adverse events were reported in this study. TETA dihydrochloride administration did not result in significant overall changes in any clinical biochemistry or hematology laboratory test parameters, except for 1 participant who experienced a mild, reversible elevation in liver enzyme levels (alanine aminotransferase and gamma glutamyl transferase).

Several male and female participants entered the study with borderline indicators of early iron deficiency, and there was a trend toward worsening in 6 of the 24 trial patients during the trial. None of the abnormal laboratory values for indicators of iron deficiency at the end of the study in these 6 patients were considered clinically significant. Serial blood sampling carried out in this study following the first and last dose of study medication may have exacerbated this tendency. Results were similar between participants in fast and slow acetylator groups. No clinically significant hemodynamic or

electrocardiographic effects attributable to TETA dihydrochloride were observed based on sitting blood pressure, pulse rate, and resting 12-lead ECG measurements obtained during this study. No differences were observed when comparing participants characterized with fast or slow acetylator phenotypes.

DISCUSSION

This is the first detailed study to be reported of the 24-hour pharmacokinetics in humans of TETA and its 2 metabolites, MAT and DAT. Despite a long history of clinical usage, detailed PK data for TETA and its metabolites are sparse. Miyazaki et al[15] applied an HPLC method using fluorescamine derivatization to study concentration-time profiles of TETA in 8 patients with Wilson's disease administered oral TETA dihydrochloride (~8.3 mg/kg). However, blood collection in that study was within the first 8 hours after dosing, and PK parameters were not reported. Tanabe[17] studied plasma concentration-time profiles of TETA, TETA + metabolite(s), and metabolite(s) in 8 Wilson's disease patients orally administered 25 mg/kg TETA dihydrochloride, using the HPLC method described by Miyazaki et al.[15] They reported that TETA's plasma kinetics were subject to first-pass effects. Again, however, blood collection was within 8 hours following dosing, and most PK parameters were not reported, except for $AUC_{0\text{-}6}$ values. Furthermore, the metabolite(s) measured in that study were not identified or characterized; thus, it was unclear which metabolite(s) had been measured. Nakano et al[16] measured the 24-hour TETA concentration-time profiles in 1 healthy volunteer under fasted and nonfasted conditions after oral TETA dihydrochloride administration at 25 mg/kg; however, PK parameters were not reported. Kodama et al[14] identified 1 major metabolite, MAT, in human urine samples, and we more recently showed that 2 major metabolites, MAT and DAT, exist in human plasma and urine.[24] It is important to study PK profiles of all 3 major compounds found in human blood following TETA dihydrochloride administration to understand more fully the pharmacological properties of the drug.

Our PK results show that on days 0 and 7, mean (SD) $t_{1/2}$ values for TETA were 2.48 (1.63) hours and 3.28 (1.14) hours, respectively. However, the fact that TETA was still detectable in plasma after 15 to 18 hours suggests that a noncompartment approach may not provide the closest estimate. Our previous study showed that the linear 2-compartment model with first-order absorption might better characterize the data and provide closer $t_{1/2}$ estimation, which would be longer than the current estimations.[33] We estimate from published concentration-time profiles that the average $t_{1/2}$ value for TETA following oral drug administration in humans is around 2 to 4 hours, which is close to our estimations. Animals, such as rats and rabbits, have shorter reported $t_{1/2}$ values, generally <2 hours. No $t_{1/2}$ values for MAT or DAT are available in the literature to compare with our current findings. Modest accumulation effects for TETA, MAT, and DAT were apparent because twice-daily oral administration of TETA dihydrochloride in this study caused significant increases in $t_{1/2}$ and AUC values. The day 7/day 1 AUC ratios were 1.68, 2.34, and 3.24 for TETA, MAT, and DAT, respectively, in this study. Both metabolites had higher ratios, probably because they had longer $t_{1/2}$ values. Given the long history of safe use for TETA in Wilson's disease, this accumulation is not expected to be of clinical significance.

Our PD study shows that increased cupruresis was associated with TETA dihydrochloride administration. Increased cupruresis was most evident during the first 12-hour postdose interval, wherein the highest rate of cupruresis occurred during the first 4 hours postdose. This observation is consistent with previous reports.[9,14]

Here, cumulative cupruresis was observed to be lower on day 7 than on day 1, whereas TETA exposure was higher on day 7. We previously reported that TETA treatment may well extract chelatable Cu^{II} from an extracellular pool, where it is likely to be bound to fibrous molecules in the extracellular matrix—for example, collagen covalently modified by addition of advanced glycation end products.[8,10,11] The fall in cupruresis between days 1 and 7 is interpreted to reflect the partial depletion of the chelatable Cu^{II} pool after 7-day chelator treatment in healthy participants.

Our safety study showed that oral TETA dihydrochloride was safe and well tolerated by these healthy adult male and female participants, who were given a single 600-mg dose on day 0, followed by daily doses of 600 mg twice daily on days 2 to 6 and a single 600-mg dose on day 7. Previous clinical reports have been consistent with these findings.[9,34,35] There are previous reports of acquired sideroblastic anemia in 2 patients treated for Wilson's disease with TETA dihydrochloride,[36,37] so hematological changes must be monitored carefully.

Our clinical laboratory tests showed that several participants entered the study with laboratory indicators of early iron deficiency, possibly the consequence of dietary factors, and that there was a trend

toward worsening in 6 patients during this trial. However, none of the abnormal laboratory values for indicators of iron deficiency in these patients were considered clinically significant at the end of the study. Serial blood sampling carried out in this study following the first and last doses of study medication (approximately 360 mL of blood in total for each participant) may have exacerbated this effect, which was consistent with acute blood loss due to phlebotomy.[38] Furthermore, we have found similar apparent changes in hematological parameters in both drug- and placebo-treated groups in an ongoing placebo-controlled PK/PD study in a group of healthy volunteers given escalating doses of TETA dihydrochloride up to 3600 mg/d (unpublished data). The hematological effects noted here were thus judged unlikely to be TETA related.

The main aims of the current study were to determine whether the NAT2 phenotype has any effect on PK, PD, or safety profiles of TETA dihydrochloride. Our current results show that there was no significant difference in relevant variables between males and females identified with fast compared with slow NAT2 phenotypes. However, the present study appears to have insufficient statistical power to discriminate differences in metabolic ratios, such as MAT/TETA or DAT/TETA for C_{max} or $AUC_{0\text{-}24}$ between the 2 groups on days 0 and 7. An increased pool of participants would be needed to ascertain whether there are any differences in metabolic ratios. NAT2 is one enzyme that might mediate TETA acetylation. Polymorphism is known to affect expression of NAT2 markedly and thereby to modify its ability to metabolize drugs and other xenobiotics.[39] The current results are consistent with our previous suggestion that NAT2 is unlikely to be responsible for systemic TETA acetylation. Because SSAT is an enzyme that specifically catalyzes the acetylation of spermine and spermidine[40,41] and TETA is a structural analog of these 2 endogenous polyamines, it is possible that SSAT might also catalyze TETA acetylation, which has also been indicated in our previous report.[24] We are currently undertaking studies to determine which enzyme(s) might be responsible for metabolic transformation of TETA into MAT and DAT.

In our current study, TETA-evoked cupruresis did not differ significantly between 2 groups of healthy human participants with different NAT2 phenotypes. Our prior studies have shown that increased urinary Cu excretion in healthy participants was primarily correlated with urinary elimination of TETA itself, whereas increased urinary Cu excretion in matched type 2 diabetic patients was more closely correlated with the combined urinary excretion of [TETA + MAT] rather than [TETA] alone.[24] Comparing the PK profiles of TETA and its metabolites with cupruresis revealed that urinary Cu increase in these healthy participants was mainly correlated with plasma [TETA + MAT]. However, urinary profiles for drugs or their metabolites are usually delayed from their corresponding plasma profiles. Because urinary copper excretion has been shown to be related to urinary [TETA] in healthy humans,[24] direct comparisons between drug or metabolites levels and those of urinary copper would probably be more informative. Another of our prior studies has indicated that the mechanism by which TETA elevates urinary Cu in healthy participants resulted mainly from drug-mediated increases in its uptake from the gut contents, whereas in type 2 diabetic patients, it acted mainly via extraction of systemic Cu.[10]

In the current study, plasma MAT and DAT peaked at similar times following TETA administration (Figures 2-3). The PK data reported herein are not necessarily consistent with the existence of a sequential metabolic pathway, whereby TETA is first acetylated to form MAT, which then serves as the substrate in a second acetylation reaction that yields DAT (ie, TETA→MAT→DAT). An alternative schema might exist, whereby TETA can be transformed directly into either MAT (TETA→MAT) or DAT (TETA→DAT), possibly via distinct enzyme-mediated pathways, without MAT having to serve as an obligatory intermediate in DAT formation. The enzyme systems whereby TETA is transformed into MAT and DAT remain to be elucidated.

In summary, we performed PK, PD, and safety studies of TETA dihydrochloride by administering it to healthy human participants in an open-label clinical trial, to investigate the pharmacological effects of the NAT2 phenotype. Here we report for the first time detailed PK profiles of all 3 major compounds—TETA, MAT, and DAT—which occur in human plasma following TETA dihydrochloride administration. TETA dihydrochloride was well tolerated by all participants, and the bulk of cupruresis occurred in the first 4 hours after dosing. There were no significant differences in PK, PD, or safety profiles between healthy participants with fast or slow NAT2 acetylator phenotypes. These results indicate that NAT2 is unlikely to be responsible for TETA metabolism and point toward SSAT as one alternative candidate for the enzyme that catalyzes TETA acetylation in humans. To our knowledge, the SSAT enzyme system has not previously been associated

with drug metabolism. This would be the first instance that a drug is metabolized via SSAT instead of traditional CYP/NAT routes.

Declaration of conflicting interests: The authors declared a potential conflict of interest (eg, a financial relationship with the commercial organizations or products discussed in this article) as follows: JL, SDP, AO, TS, and GJC disclose past associations with Protemix Corporation Limited.

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