



Pharmacokinetics of Amprelosetine, a Norepinephrine Reuptake Inhibitor, in Healthy Subjects and Adults with Attention-Deficit/Hyperactive Disorder or Fibromyalgia Pain

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Published online: 27 August 2020
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Abstract

Background and Objective Amprelosetine is a novel norepinephrine reuptake inhibitor in development for the treatment of symptomatic neurogenic orthostatic hypotension. The objectives of this analysis were to define the pharmacokinetics of once-daily oral amprelosetine and provide dose recommendations for clinical development.

Methods We fitted a population pharmacokinetic model to amprelosetine plasma concentrations from single- and multiple-ascending dose trials in healthy subjects and two phase II studies in adult subjects with attention-deficit/hyperactive disorder or fibromyalgia at doses of 2–50 mg.

Results Amprelosetine pharmacokinetics was best described by a two-compartment model with first-order absorption and elimination. The terminal half-life was 30–40 h, resulting in sustained drug concentrations for the entire 24-h dosing interval at steady state. Covariates of age, weight, or renal impairment did not impact amprelosetine exposure. Cytochrome P450 2D6 phenotype had no influence on amprelosetine exposure. Sex and smoking status were identified as statistically significant covariates, suggesting a role for cytochrome P450 1A2 in the elimination of amprelosetine. Despite statistical significance, differences in amprelosetine exposure in male vs female subjects and smokers vs non-smokers were not clinically meaningful at the recommended dose. At the 10-mg dose, > 75% norepinephrine transporter inhibition and < 50% serotonin transporter inhibition are anticipated for adult subjects.

Conclusions The population pharmacokinetic model effectively described the plasma concentration–time profile of amprelosetine after single and multiple doses. Population pharmacokinetic/pharmacodynamic analysis justified using a fixed dosing regimen with no dose adjustments across a broad population and can be used to inform dosing strategies in future clinical studies.

Clinical Trial Registration ClinicalTrials.gov identifier numbers NCT01693692 (fibromyalgia); NCT01458340 (attention-deficit/hyperactive disorder).

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Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s40262-020-00918-7>) contains supplementary material, which is available to authorized users.

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Key Points

Pharmacokinetic analysis of plasma amprelosetine exposure indicated that the terminal half-life was 30–40 h, reached steady state after 6 days, and accumulated three- to four-fold with once-daily dosing.

Population pharmacokinetic modeling identified sex and smoking status as statistically significant covariates indicating that cytochrome P450 1A2-based metabolism is the key mechanism for amprelosetine elimination.

Based on the pharmacokinetic/pharmacodynamic relationship, a once-daily amprelosetine dose of 10 mg is predicted to achieve sustained norepinephrine transporter engagement during the entire dosing interval.

1 Introduction

Ampreloxetine (previously known as TD-9855) is a novel small-molecule inhibitor of both norepinephrine (NE) and serotonin (5-HT) uptake, with selectivity for NE [1]. Based on the results of both non-clinical [2] and human [3] studies, ampreloxetine is anticipated to offer benefits for the management of symptomatic neurogenic orthostatic hypotension (nOH). Symptomatic nOH remains largely unaddressed by current therapies [4–7]. The pressor agents, fludrocortisone [5], a synthetic mineralocorticoid, midodrine, an α_1 -adrenergic receptor agonist approved for the treatment of orthostatic hypotension [6], and droxidopa, an NE precursor approved in the USA for the treatment of nOH [7], are effective in acutely treating declines in blood pressure but lack durability of effect in treating the debilitating symptoms of nOH [8–12]. Moreover, midodrine and droxidopa have short half-lives and require multiple doses per day [6, 7].

Pharmacological inhibitors of NE transporters (NETs) afford an opportunity to treat the core symptoms of nOH that are attributable to the variety of underlying neurological disorders (e.g., synucleinopathies, such as multiple system atrophy, Parkinson's disease, or pure autonomic failure) [4]. Norepinephrine transporter inhibitors are anticipated to be beneficial in the treatment of nOH through potentiation of residual sympathetic tone that support positional changes from supine to standing [2, 13]. Atomoxetine, an NET inhibitor approved in the USA for the treatment of attention-deficit/hyperactivity disorder (ADHD) [13], has been evaluated in a proof-of-concept clinical study for the treatment of nOH, with results suggestive of symptomatic benefits in patients with nOH [14].

The pharmacological and pharmacokinetic behavior of ampreloxetine has been studied in previous clinical trials. An absorption, metabolism, and excretion study of [^{14}C]-ampreloxetine was conducted in six healthy male volunteers after a single oral administration of 20 mg (100 μCi) of ampreloxetine [15]. Ampreloxetine elimination is primarily driven by metabolism, as less than 5% of the dose was recovered in urine and feces as unchanged drug. Furthermore, ampreloxetine has been evaluated in healthy male subjects to assess NET and serotonin transporter occupancy in the human brain using positron emission tomography after administration of oral doses from 4 to 20 mg [1]. The results of the positron emission tomography study demonstrated NET occupancy to be predominant (NET > serotonin transporter) and a concentration-dependent increase in NET occupancy with increasing ampreloxetine plasma concentrations over the tested dose range. Limited serotonin transporter occupancy was observed at the highest evaluated dose (20 mg), 25% ($\pm 8\%$) at a plasma concentration of 6.8

ng/mL [1]. The relationship between plasma ampreloxetine concentration and NET occupancy data was well described by an E_{max} model, with an ampreloxetine plasma concentration of 1.2 ng/mL (95% confidence interval 0.76–1.7 ng/mL) predicted to yield 50% occupancy of NET within the brain.

The objectives of this analysis were to define the pharmacokinetics of ampreloxetine in healthy volunteers and subjects with ADHD and fibromyalgia (FM) and identify any covariates or special populations to inform the dosing strategy for further clinical development.

2 Materials and Methods

2.1 Ethical Conduct of the Study

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, the 1964 Helsinki Declaration and its later amendments, and Good Clinical Practice [16]. The trial protocols were approved by independent ethics committees and/or institutional review boards (single- and multiple-ascending dose studies, Independent Investigational Review Board, Inc., 6738 West Sunrise Blvd, Suite 102, Plantation, FL 33313, USA; ADHD and FM studies, Western Institutional Review Board[®], 3535 7th Avenue, SW Olympia, WA 98502-5010, USA, and IntegReview 3001 S. Lamar Blvd, Suite 210, Austin, TX 78704, USA). All subjects provided written informed consent before initiation of any trial-related activities.

2.2 Study Designs

2.2.1 Single-Ascending Dose

A phase I, double-blind, randomized, placebo-controlled, single-ascending dose (SAD) study of ampreloxetine was conducted to evaluate the pharmacokinetic (PK) profile in healthy male and female subjects. A total of 40 subjects were enrolled in this study into five sequential cohorts ($n = 8/\text{cohort}$) to receive a single oral dose of either ampreloxetine (2, 4, 8, 20, or 50 mg) or placebo after 8 h of fasting, in a ratio of 6:2 active drug to placebo. No concomitant medications were allowed during the course of this study.

In all cohorts, blood samples for measurement of ampreloxetine concentration were collected pre-dose and at 0.5, 1, 1.5, 2, 3, 3.5, 4, 6, 8, 10, 12, 24, 36, 48, and 72 h post-dose. Based on plasma half-life and time to maximum concentration values observed in cohort 1, additional blood samples were collected at 14 and 96 h post-dose in cohort 2, and at 14, 96, and 144 h post-dose in cohorts 3, 4, and 5.

2.2.2 Multiple-Ascending Dose

A phase I, double-blind, randomized, placebo-controlled, multiple-ascending dose (MAD) study was conducted to evaluate the safety and pharmacokinetics of multiple doses of amprelosetine in healthy subjects aged 18–55 years and in an additional cohort of healthy subjects aged 56–65 years. Thirty-two subjects aged 18–55 years and eight subjects aged 56–65 years were enrolled in this study; thus, a total of 40 subjects were randomized into five sequential cohorts ($n = 8/\text{cohort}$). Within each cohort, subjects were randomized to receive either amprelosetine or placebo, in a ratio of 6:2 active drug to placebo. In cohorts 1, 2, and 3, amprelosetine doses of 4 mg, 10 mg, and 20 mg were administered orally once daily for 14 days, respectively, in subjects aged 18–55 years. Cohort 4 received amprelosetine 20 mg once daily for 14 days in subjects aged 56–65 years. In cohort 5, dose titration was explored, starting with 20 mg of amprelosetine administered once daily for 3 days followed by 40 mg administered once daily for 14 days, in subjects aged 18–55 years. No concomitant medications were allowed during the course of this study.

In cohorts 1–4, PK blood samples for assays of amprelosetine were collected on days 1 and 14 at pre-dose and at 0.5, 1, 2, 4, 6, 8, 10, 12, and 14 h post-dose. Additional samples were collected after the day 14 dose at 24, 48, 72, and 96 h post-dose. Trough samples were collected pre-dose on days 2, 4, 6, 8, 10, and 12. In cohort 5, during the 3 days at the 20-mg dose level, PK blood samples were collected at pre-dose and 10 h post-dose on day 1, and at pre-dose on day 2. Following escalation to the 40-mg dose level, serial, trough, and post-dose samples were collected on the same schedule as in cohorts 1–4.

To evaluate the effect of food on the pharmacokinetics of amprelosetine, following the 14 days of amprelosetine administration and an additional 14-day washout period, subjects in cohort 1 were administered a single oral dose of amprelosetine 30 min after eating a high-fat meal [17]. Pharmacokinetic sample times mirrored the sampling following the day 14 dose.

2.2.3 Attention-Deficit/Hyperactivity Disorder Study

A phase II, double-blind, placebo-controlled, parallel-group, three-arm, randomized study (NCT01458340) was conducted to compare 5 mg and 20 mg of amprelosetine with placebo in 285 adult subjects with clinically confirmed ADHD according to the Adult Attention-Deficit/Hyperactivity Disorder Clinical Diagnostic Scale (ACDS Version 1.2) [18]. Eligible subjects were randomized equally to receive either active treatment or placebo daily for 6 weeks. Subjects in the amprelosetine 5-mg or placebo arm received

amprelosetine 5 mg or placebo once daily for 6 weeks. Subjects in the amprelosetine 20-mg arm received an initial dose of 10 mg once daily for 1 week, followed by 20 mg once daily for 5 weeks. Each subject's dose was increased to 20 mg unless a safety or tolerability concern was identified during the 10-mg once-daily dosing week, in which case the subjects continued to receive 10 mg once daily for a total of 6 weeks. Blood samples for the measurement of amprelosetine plasma concentration were obtained on study days 8, 15, 29, and 43. On study days 15 and 43, the samples were obtained between 2 and 10 h post-dose. Cytochrome P450 (CYP) 2D6 genotype and phenotype were assigned using the AmpliChip CYP450 test (Roche Molecular Diagnostics, Branchburg, NJ, USA) based on the manufacturer's algorithm. Polymorphisms in CYP2D6 have been shown to be associated with significant differences in the PK profile for CYP2D6 substrate drugs [19].

2.2.4 Fibromyalgia Study

A phase II multicenter, randomized, double-blind, parallel-group, placebo-controlled study (NCT01693692) was conducted to compare a lower-dose regimen (2.5 mg once daily for 1 week, followed by 5 mg once daily for 5 weeks) and a higher-dose regimen (10 mg once daily for 1 week, followed by 20 mg once daily for 5 weeks) of amprelosetine vs placebo in approximately 375 adult subjects with FM according to the American College of Rheumatology diagnostic criteria for FM [20]. Blood samples for measurement of amprelosetine plasma concentrations were obtained on study days 8, 15, 29, and 43 (end of treatment). Blood samples were taken before dosing on days 8, 29, and 43 and at any time between 2 and 10 h post-dose on day 15.

2.3 Pharmacokinetic Analyses

2.3.1 Bioanalysis of Pharmacokinetic Samples

Plasma samples were assayed using a validated liquid chromatography with tandem mass spectrometry method at Advion/Quintiles (Ithaca, NY, USA). The amprelosetine assay quantification range was 0.05–100 ng/mL in all studies. Overall precision for the calibration standards and quality-control samples, as measured by coefficient of variation (%), was always within 10%.

2.3.2 Non-Compartmental Analysis

Non-compartmental analysis of the SAD and MAD studies was conducted using WinNonLin Version 5.2 (Pharsight Corporation, Sunnyvale, CA, USA). Reported PK parameters include maximum concentration (C_{max}), time

to maximum concentration, lag time, terminal elimination half-life, oral volume of distribution during terminal phase, oral clearance (CL/F), area under the concentration–time curve (AUC) from time 0 to 24 h (AUC_{0-24}), and 0 h to time t (AUC_{0-t}). The effect of food was assessed descriptively by examining the mean C_{max} and AUC_{0-24} in cohort 1 on day 29 (fed state) relative to the mean C_{max} and AUC_{0-24} values in cohort 1 on day 1 (fasted state).

2.3.3 Population Pharmacokinetic Analysis

Data from the phase I SAD/MAD and phase II studies were combined into a single dataset containing individual demographics and time course of plasma amprelosetine concentrations. A total of 2978 amprelosetine concentrations from 499 subjects were used to conduct a non-linear mixed-effects modeling analysis and identify sources of intra- and inter-individual variability. A two-compartment model best described the amprelosetine PK profile collected from the phase I SAD/MAD studies. The models assumed a log-normal distribution of the individual PK parameters and a mixed residual error model. Models were evaluated using the objective function value, computed as -2 times the log-likelihood, diagnostic plots, and the precision of parameter estimates. All analysis was conducted using NONMEM 7.2 (Icon plc, Dublin, Ireland) using the first-order conditional estimation method.

A covariate analysis was conducted to identify the effects of age, weight, creatinine clearance (as continuous covariates using exponential models of inter-individual variation), sex, and smoking status (as categorical covariates using an additive model) on the apparent CL/F of amprelosetine and volume of the central compartment (V_1/F). Identification of statistically significant covariates was conducted using a stepwise-forward selection with an $\alpha=0.01$ (a decrease of 6.63 points in the objective function) and a stepwise-backward elimination process with an $\alpha=0.001$ (an increase of 10.83 points in the objective function). Model fit was evaluated using goodness-of-fit plots and visual predictive checks.

The exposure to amprelosetine was simulated for each subject using the individual post hoc PK parameters from the final population PK model to determine the clinical significance of the statistically significant covariates. The post hoc exposures were also used to determine whether amprelosetine exposure remained consistent across the healthy, ADHD, and FM patient populations. Differences in amprelosetine plasma exposure between poor, intermediate, extensive, and ultra-rapid CYP2D6 metabolizers was evaluated using a one-way analysis of variance test of the log-transformed AUC values across each group.

2.3.4 Population Pharmacokinetic-Pharmacodynamic Simulation

The individually estimated PK parameters determined by the population PK analysis were used to simulate the steady-state concentration–time profiles for each subject in the dataset at two doses of amprelosetine (5 mg once daily and 10 mg once daily). The steady state AUC_{0-24} was calculated for each simulated subject using the linear trapezoidal rule.

Simulated individual plasma concentration–time profiles and the pharmacodynamic model established from the positron emission tomography study (half maximal inhibitory concentration = 1.2 ng/mL) were subsequently used to predict the time course of NET occupancy. The predicted NET occupancy after multiple doses of amprelosetine was averaged over the 24-h dosing interval to assess the steady-state level of NET inhibition.

3 Results

3.1 Amprelosetine Pharmacokinetic Profile in Healthy Volunteers

Amprelosetine PK assessments following single oral-dose administration based on non-compartmental analysis are presented in Table 1 and mean plasma concentrations of amprelosetine over time are plotted in Fig. 1a, b. Plasma concentration–time profiles were characterized by an initial lag in the absorption phase with a median lag time value range of 0.5–1.0 h, followed by a prolonged absorption phase with a median time to maximum concentration value range of 8–12 h post-dose (Fig. 1a, Table 1). Mean amprelosetine terminal half-life values had a range of 29.5–41.0 h. Exposure to amprelosetine in plasma increased in a dose-proportional manner across a dose range of 2–50 mg.

Amprelosetine PK assessments, following 14 days of once-daily dosing based on a non-compartmental analysis, are presented in Table 2 and mean plasma concentrations of amprelosetine after once-daily administration for 14 days are presented in Fig. 1b. Steady-state plasma concentrations of amprelosetine were achieved by day 6 in most subjects after once-daily doses of 4–40 mg (Table 2). Accordingly, the amprelosetine PK profile on day 14 represents steady-state behavior. Oral clearance estimates following multiple dosing are more robust than those determined after a single dose owing to a large percentage (> 20%) of the AUC_{0-inf} that was extrapolated in some subjects in the single-dose study. After reaching C_{max} , plasma concentrations of amprelosetine declined with the mean half-life range of 34.3–43.1 h at steady state. Consistent with the half-life, mean accumulation ratios were based on the AUC_{0-24} range of 3.18- to

Table 1 Summary of mean (standard deviation [SD]) plasma pharmacokinetic parameters for amprelosetine: single-ascending dose study

Dose level (mg)		C_{max} (ng/mL)	T_{max}^a (h)	T_{lag}^a (h)	AUC_{0-24} (ng*h/mL)	AUC_{0-t} (ng*h/mL)	$t_{1/2}$ (h)	CL/F (L/h)	V_z/F (L)
2	Mean	0.925	12	1	15.8	36.3	29.5	56.4	2397
	SD	0.335	8.00, 12.0	0.5, 1.5	5.6	11.1	4.6	22.3	916
	n	6	6	6	6	6	3	3	3
4	Mean	2.09	8	1	32.8	87.6	36.0	38.7	1983
	SD	0.34	8.00, 10.0	0.5, 1.0	4.3	11.4	6.4	6.4	353
	n	6	6	6	6	6	6	6	6
8	Mean	5.02	12	0.50	85.6	231	34.5	38.2	1659
	SD	1.4	8.05, 14.0	0, 1.0	22.5	98	12.8	17.4	386
	n	6	6	6	6	6	6	6	6
20	Mean	8.59	12	0.5	142	445	41.0	52.2	2745
	SD	2.45	8.00, 14.0	0, 2.0	43	195	12.2	33.8	1034
	n	6	6	6	6	6	6	6	6
50	Mean	26.4	12	0.5	461	1430	40.9	39.9	2337
	SD	5.7	8.00, 14.0	0, 1.0	105	530	8.8	15	1046
	n	6	6	6	6	6	5	5	5

In each cohort, 6 subjects were dosed with the active drug

AUC_{0-24} area under the concentration–time curve from time 0 to 24 h, AUC_{0-t} area under the concentration–time curve from time 0 to the time of last quantifiable concentration, CL/F oral clearance, C_{max} maximum concentration, $t_{1/2}$ terminal elimination half-life, T_{lag} lag time, T_{max} time to maximum concentration, V_z/F oral volume of distribution during terminal phase

^aMedian (minimum, maximum)

4.38-fold at steady state. Consistent with the single-dose PK profile, plasma exposure at steady state also increased in an approximate dose-proportional manner at a dose range of 4–40 mg (Table 2, Table 1 of the Electronic Supplementary Material [ESM], and Fig. 1b). Mean C_{max} and AUC_{0-24} were 9.4% and 6.5% lower, respectively, in the fed state compared with the fasted state. These differences are considerably lower than the magnitude of inter-individual variability in C_{max} and AUC_{0-24} and are therefore not anticipated to be clinically meaningful (Table 1 of the ESM).

At the 20-mg dose level, amprelosetine exposure in subjects aged 56–65 years was approximately 35% and 70% higher on days 1 and 14, respectively, when compared with adult subjects aged 18–55 years who were administered the same 20-mg dose (Table 2 and Table 1 of the ESM). However, the amprelosetine 20-mg older group consisted entirely of female subjects ($n=6$), compared with three male subjects and three female subjects in the amprelosetine 20-mg adult reference group. Accordingly, the impact of age or sex on amprelosetine pharmacokinetics could not be differentiated in this study.

3.2 Amprelosetine Pharmacokinetics in Subjects with Attention-Deficit/Hyperactivity Disorder and Fibromyalgia

After continuous once-daily dosing of amprelosetine in the phase II study in subjects with ADHD, mean amprelosetine plasma concentrations were generally consistent with

exposures observed in the SAD and MAD studies. Mean plasma concentrations at steady state had a range from 4.5 ng/mL (day 8) to 5.1 ng/mL (day 15) in the amprelosetine 5-mg treatment arm (Table 3). Subjects in the 20-mg treatment arm received a once-daily dose of 10 mg from day 1 to day 7. Subjects who were not escalated to 20 mg and continued to receive 10 mg up to day 42 had mean plasma concentrations at steady state from 12.0 ng/mL (day 29) to 13.4 ng/mL (day 8). Subjects who were escalated to 20 mg had a mean plasma concentration at the steady-state range from 18.2 ng/mL (day 43) to 20.3 ng/mL (day 29) (Table 3). No statistical difference in amprelosetine plasma exposure was observed between poor, intermediate, extensive, and ultra-rapid CYP2D6 metabolizers within each dose level at each timepoint based on a one-way analysis of variance test ($p>0.05$) (Fig. 1c).

After continuous once-daily dosing of amprelosetine in the phase II study in subjects with FM, amprelosetine concentrations were consistent with observed concentrations in the SAD, MAD, and phase II ADHD studies (Table 3). In the 5-mg and 20-mg treatment arms, mean amprelosetine plasma concentrations were 5.3 ng/mL (day 43) to 6.3 ng/mL (day 15) and 21.0 ng/mL (day 43) to 28.0 ng/mL (day 15), respectively, at steady state (Table 4).

3.3 Population Pharmacokinetic Analysis

The pooled PK dataset consisted of demographics and plasma concentrations from 499 subjects (Table 2 of the

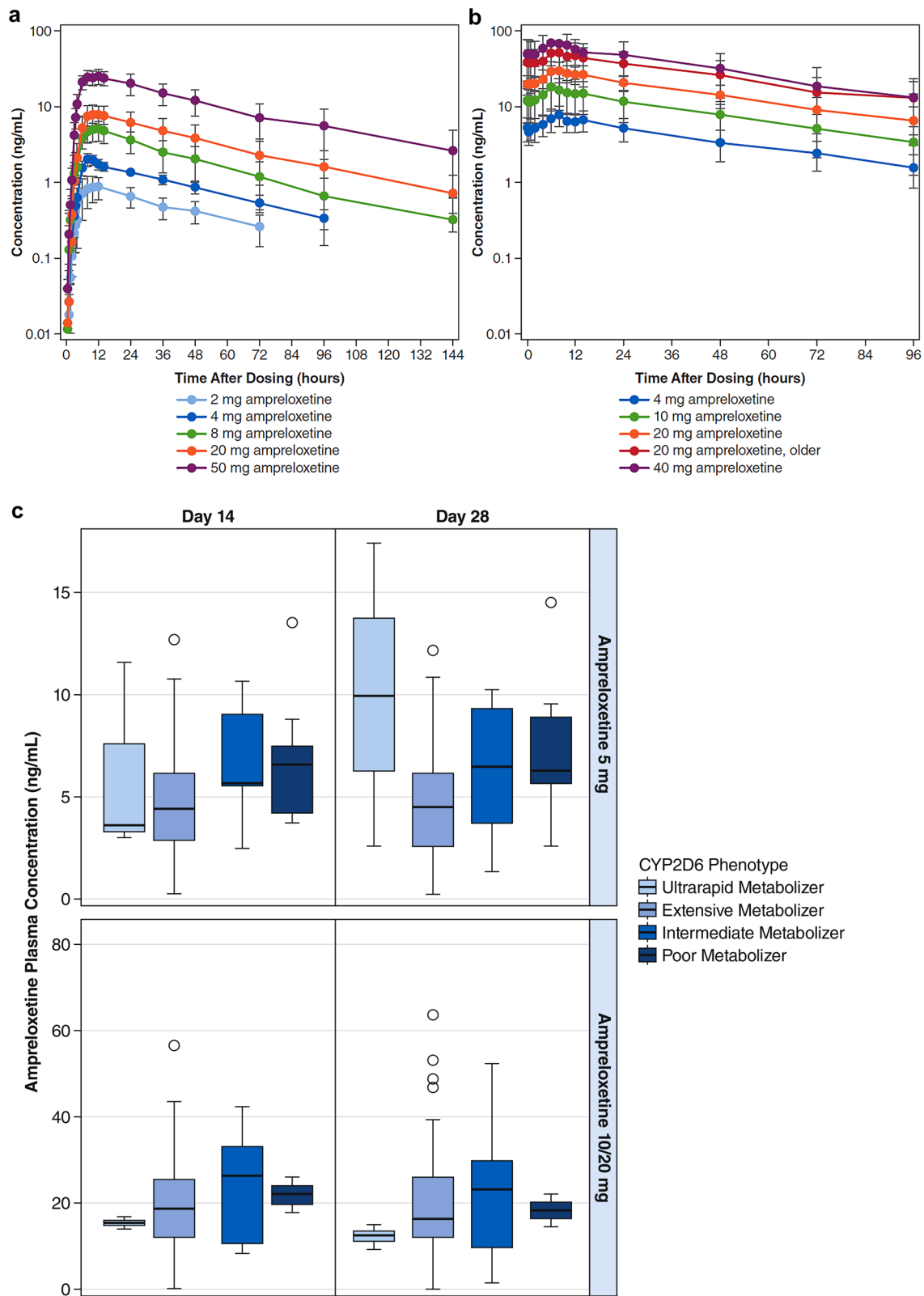


Fig. 1 Mean plasma concentrations of amprelosetine: **a** day 1 of single-ascending dose (SAD) study, **b** day 14 of multiple-ascending dose (MAD) study, and **c** attention-deficit/hyperactivity disorder study grouped by cytochrome P450 (CYP) 2D6 phenotype

Table 2 Summary of plasma pharmacokinetic parameters for amprelosetine: multiple-ascending dose study (day 14^a)

Dose level (mg)		C_{max} (ng/mL)	T_{max}^b (h)	AUC_{0-24} (ng*h/mL)	$t_{1/2}$ (h)	CL/F (L/h)	V_z/F (L)	AR	C_{trough} (ng/mL)				
									Day 4	Day 6	Day 8	Day 10	Day 12
4	Mean	7.88	8	150	43.1	29.9	1833	4.98	3.4	4.03	4.16	4.67	4.47
	SD	2.39	8.00, 8.00	47	4.1	13.2	737	0.84	1.09	1.46	1.39	1.61	1.47
	n	5	5	5	5	5	5	5	6	6	5	5	5
10	Mean	18.2	6.01	336	37.7	36.4	1880	3.18	8.36	12.3	13.1	12.1	11.5
	SD	8.7	6.00, 8.00	156	6.2	17.7	726	0.45	3.56	6.8	6.4	5.7	5.6
	n	6	6	6	6	6	6	6	6	6	6	6	6
20	Mean	30.5	8	600	37.6	35.7	2068	3.74	14.6	18.5	20.2	21.9	20.8
	SD	9.6	5.50, 12.0	169	3.3	10.1	614	0.78	4.9	7.1	6.6	5.4	5
	n	6	6	6	5	6	5	6	6	6	6	6	6
20 (older) ^c	Mean	52.5	7.03	1019	37.1	23.7	1528	4.38	21.9	28.5	29.2	29.5	30.8
	SD	22.8	6.00, 8.00	469	4.4	11.4	532	1.41	7.2	10.1	12.2	15.3	15.5
	n	6	6	6	4	6	4	6	6	6	6	6	6
40	Mean	74.5	8	1346	34.3	34.7	1611	NA	36.9	49.9	44.1	46.3	45
	SD	36.2	6.00, 10.0	570	7.3	15	496	NA	12	19.7	18.9	21.3	15.3
	n	6	6	6	6	6	6	NA	6	6	6	6	6

AR accumulation ratio (calculated using AUC_{0-24} on day 14 vs day 1), AUC_{0-24} area under the concentration–time curve from time 0 to 24 h, CL/F oral clearance, C_{max} maximum concentration, C_{trough} trough concentration (obtained prior to dosing on indicated days), NA not applicable, SD standard deviation, $t_{1/2}$ terminal elimination half-life, T_{max} time to maximum concentration, V_z/F oral volume of distribution during terminal phase

^aDay 14 of dosing for the 40-mg treatment arm corresponds to study day 17

^bMedian (minimum, maximum)

^cHealthy subjects aged 56–65 years

ESM). A two-compartment model with absorption lag, first-order absorption, and elimination provided the best structural fit to the data, based on the minimum value of the objective function, diagnostic plots (Fig. 1 of the ESM), and the precision of parameter estimates. The model was parameterized with CL/F , V_1/F , volume of distribution of the peripheral compartment, inter-compartmental clearance, and an absorption rate constant. The effects of covariates on the absorption lag, absorption rate constant, inter-compartmental clearance, and volume of distribution of the peripheral compartment were not characterized because of limited data from the sparsely sampled phase II studies.

Covariates of age (18–65 years), weight (43.1–157 kg), and creatinine clearance (45.2–201 mL/min) did not have statistically significant associations with either CL/F or V_1/F (Table 5). In contrast, both sex and smoking status had statistically significant associations with CL/F , and sex had a statistically significant association with V_1/F (Table 5). Male subjects had an increased CL/F and V_1/F , resulting in approximately 33% lower amprelosetine exposure compared with female subjects (Fig. 2a). Similarly, subjects who were smokers had increased CL/F compared with non-smokers, resulting in approximately 32% lower amprelosetine exposure in smokers compared with non-smokers for male as well as female subjects (Fig. 2a). No differences in amprelosetine exposure

were observed between the healthy volunteers vs subjects with ADHD or FM (Fig. 2b). Despite the statistical significance of sex and smoking covariates, the differences in amprelosetine exposure between male smokers, male non-smokers, female smokers, and female non-smokers demonstrated a significant overlap due to the inter-individual variability of amprelosetine exposure within each subgroup (Fig. 3a).

3.4 Population Pharmacokinetic-Pharmacodynamic Predictions

The individually predicted steady-state amprelosetine exposures following a 5-mg once-daily regimen and a 10-mg once-daily dosing regimen were simulated for 499 subjects (Fig. 3a). Predicted exposures were highest in the female non-smoker group, followed by comparable exposures between female smokers and male non-smokers, with the lowest exposures in male smokers. The predicted pharmacodynamic effect (steady-state NET occupancy) for the amprelosetine 5-mg group has a range of 71–95% in the female non-smoker group, 62–93% in female smokers and male non-smokers, and 63–85% in male smokers. However, at the 10-mg dose level, the predicted population pharmacodynamic effect was > 75% NET inhibition in nearly 100% of the patient population (Fig. 3b).

Table 3 Summary of mean plasma concentrations (ng/mL) of amprelosetine (attention-deficit/hyperactive disorder study)

Treatment arm	Dose level (mg)		PK collection day ^c			
			8	15	29	43
Amprelosetine 5 mg	5	Mean	4.53	5.11	4.85	4.91
		SD	2.18	2.86	3.43	3.91
		%CV	48	56	71	80
		Min	1.33	0.190	0	0
		Max	10.2	13.5	17.4	17.5
		<i>n</i>	93	91	85	81
Amprelosetine 20 mg	10 ^a	Mean	13.4	13.2	12.0	12.4
		SD	6.50	6.20	7.76	10.0
		%CV	48	47	65	81
		Min	4.72	4.74	1.27	3.55
		Max	27.5	24.1	27.9	41.0
		<i>n</i>	14	13	13	13
	20 ^b	Mean	9.29	20.1	20.3	18.2
		SD	4.50	11.2	13.4	14.9
		%CV	48	56	66	82
		Min	1.37	0.208	0	0
		Max	21.1	56.4	63.4	81.2
		<i>n</i>	79	75	66	66

CV coefficient of variability, *Max* maximum, *Min* minimum, *PK* pharmacokinetic, *SD* standard deviation

^aSubjects remained on amprelosetine 10 mg/day for the entire treatment period and were not escalated to 20 mg

^bSubjects received 10 mg/day on days 1 through 7 and then on day 8 were escalated to amprelosetine 20 mg/day for the remainder of the treatment period

^cCollection time was random prior to dosing, between 2 and 10 h

Table 4 Summary of mean plasma concentrations (ng/mL) of amprelosetine (fibromyalgia study)

Treatment arm	PK collection day			
	8	15	29	43
Amprelosetine 5 mg				
Mean	2.42	6.32	5.54	5.31
SD	1.43	3.39	3.67	3.63
%CV	59	54	66	68
Min	0	0	0	0
Median	2.45	5.99	5.07	5.15
Max	7.10	15.4	15.2	13.7
<i>n</i>	112	107	100	98
Amprelosetine 20 mg				
Mean	9.93	28.0	23.4	21.0
SD	6.06	16.1	16.1	15.5
%CV	61	58	69	74
Min	0	0	0	0
Median	9.33	26.6	22.6	18.0
Max	24.8	79.0	87.2	76.3
<i>n</i>	113	107	93	90

CV coefficient of variability, *Min* minimum, *Max* maximum, *PK* pharmacokinetic, *SD* standard deviation

Table 5 Population pharmacokinetic analysis

Description	Population estimate (%RSE)	Inter-subject variability %CV (%RSE)
Dose lag (h)	1.74 (1.52)	–
CL/F (L/h)	47.2 (3.72)	50.4 (10.9)
V_1/F (L)	2040 (6.25)	38.4 (31.9)
Q/F (L/h)	15.4 (20.1)	–
V_2/F (L)	357 (16.6)	–
<i>Ka</i> (L/h)	0.2 (7.17)	34.1 (48.1)
Sex effect on CL [if female] (L/h)	–16.9 (11.6)	–
Smoking effect on CL (L/h)	16.0 (16.1)	–
Sex effect on V_1 [if female] (L)	–720.0 (18.1)	–
Proportional residual error (%)	24.7 (8.7)	
Additive residual error (ng/mL)	0.199 (24.0)	

CL clearance, *CL/F* oral clearance, CV coefficient of variability, *Ka* absorption rate constant, *PK* pharmacokinetics, Q/F inter-compartmental clearance, *RSE* relative standard error, V_1 volume central compartment, V_1/F volume of distribution of the central compartment, V_2/F volume of distribution of the peripheral compartment

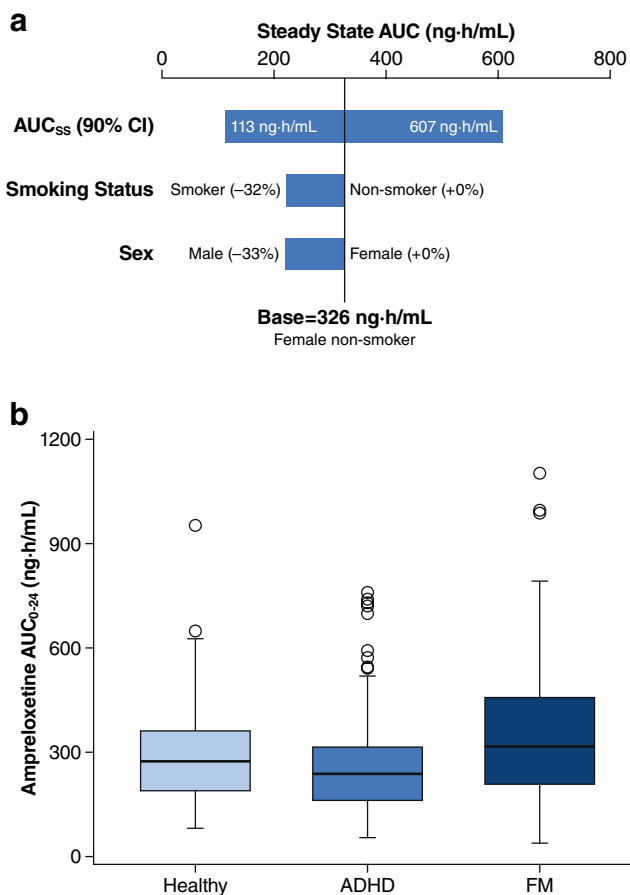


Fig. 2 Effect of covariates on the model-predicted amprelosetine exposure following repeated 10-mg once-daily dosing: **a** sensitivity plot comparing the effect of significant covariates, **b** grouped by disease state. Base, as represented by the black vertical line, refers to the predicted typical area under the concentration–time curve from time 0 to 24 h (AUC_{0-24}) in a female non-smoker from the population pharmacokinetic model. The top horizontal bar with values at each end shows the 5th to 95th percentile AUC_{0-24} range across the entire population. The middle and lower horizontal bars represent the influence of a single categorical covariate on AUC_{0-24} , with one subject created from each category with other covariates fixed at the typical values for the base subject. The solid black line denotes the median; the top and the bottom of the box denote the first and third quartiles; the whiskers denote 1.5 times the interquartile range. *ADHD* attention-deficit/hyperactivity disorder, AUC_{ss} AUC at steady state, *CI* confidence interval, *FM* fibromyalgia

4 Discussion

Dose selection for phase II and phase III studies is influenced by many factors, and drug pharmacokinetics plays a central role in the dose-selection rationale and concomitant medication strategy. The objectives of this population PK analysis were to define the pharmacokinetics of amprelosetine and identify covariates to inform dose selection for clinical development in subjects with symptomatic nOH.

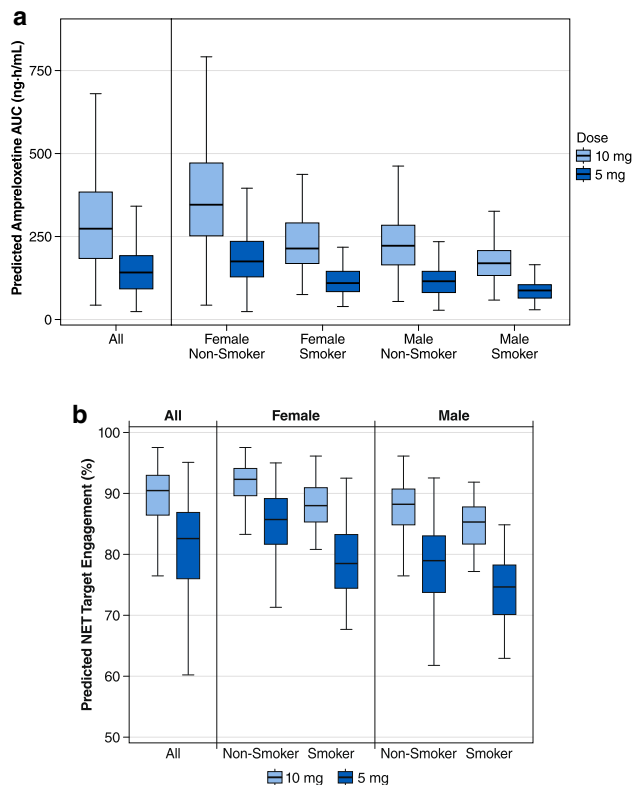


Fig. 3 Predicted amprelosetine steady-state pharmacokinetics and pharmacodynamics following repeated 10-mg and 5-mg doses: **a** area under the concentration–time curve (AUC). **b** Predicted brain norepinephrine transporter (NET) occupancy at an average plasma concentration of amprelosetine. The solid black line denotes the median; the top and the bottom of the box denote the first and third quartiles; the whiskers denote 1.5 times the interquartile range

Following oral administration, the pharmacokinetics of amprelosetine was best characterized by a two-compartment PK model with first-order absorption and elimination. Dose proportionality observed in the population PK model was consistent with the results from the non-compartmental analysis of the SAD and MAD studies. Pharmacokinetic profiles were similar in healthy subjects and subjects with ADHD and FM, allowing for direct translation of clinical pharmacology study results in healthy volunteers to patient populations. Following once-daily dosing, amprelosetine exposure reached steady state by day 6 and accumulated three- to four-fold, consistent with an amprelosetine elimination half-life of 30–40 h. Accordingly, amprelosetine exposure maintained a relatively flat drug concentration profile throughout the 24-h dosing interval at steady state. The low peak-to-trough ratio provides the potential for more sustained maintenance of stable orthostatic blood pressure throughout the entire 24-h dosing interval unlike the short-acting standard-of-care agents such as midrodrine [6] and

droxidopa [7] used for treating symptomatic nOH. Furthermore, the once-daily dosing regimen for amprelosetine is also anticipated to increase patient compliance [21] relative to midrodine [6] and droxidopa [7], which need to be administered three times a day owing to their short half-lives.

The population PK analysis of all the phase I and phase II clinical data indicates that the covariates of age, weight, or renal impairment (creatinine clearance in the range of mild-to-moderate renal impairment) did not impact amprelosetine exposure. No differences in amprelosetine exposure were observed based on the CYP2D6 phenotype and thus, the CYP2D6 enzyme is expected to have a negligible contribution towards amprelosetine metabolism. Accordingly, the same dose is recommended for subjects across all age groups, CYP2D6 phenotype, irrespective of baseline weight or body mass index and inclusive of subjects with mild and moderate renal impairment.

Sex and smoking status were statistically significant covariates shown to impact CL/F and V_1/F . Male subjects demonstrated an ~33% lower amprelosetine exposure compared with female subjects, while smokers demonstrated an ~32% lower amprelosetine exposure compared with non-smokers for both male and female subjects. These covariates indicate that CYP1A2-based metabolism is the key mechanism for amprelosetine elimination because smoking is known to induce CYP1A2 activity [22]. Accordingly, a clinical drug–drug interaction study with a CYP1A2 inhibitor and inducer will be conducted with amprelosetine.

Despite the statistical significance of the covariate effect, amprelosetine exposure in each of the subgroups including male smokers, male non-smokers, female smokers, and female non-smokers overlaps significantly, and predicted NET brain occupancy is still anticipated to be greater than 75% at the amprelosetine 10-mg dose for all subjects. Thus, no difference in the safety and efficacy profile is expected across the subgroups, and no dose adjustment based on sex or smoking status is recommended for clinical development.

5 Conclusions

The proposed population PK model effectively described the plasma concentration–time profile of amprelosetine after single and multiple doses in both healthy subjects and subjects with ADHD and FM. While the covariates included in the model were useful for interpreting inter-individual variability in the data, and providing a rationale for dose selection, dose adjustment in future clinical studies is not warranted. Based on the PK/pharmacodynamic profile of amprelosetine, a once-daily 10-mg dose is recommended as the optimal dosage to inhibit NET significantly in a majority of the intended patient population.

Author contributions AL, RMB, and JK designed the research, performed the research, analyzed the data, and wrote the manuscript. DB and RG designed the research, performed the research, and wrote the manuscript.

Compliance with Ethical Standards

Funding This study was funded by Theravance Biopharma, Inc. Kirsty Nahm MD of The Curry Rockefeller Group, LLC provided assistance with writing/formatting/proofreading/collation of the author comments and this assistance was funded by Theravance Biopharma, Inc. Open access publication of this manuscript was sponsored by Theravance Biopharma, Inc.

Conflict of interest Jitendra Kanodia, Arthur Lo, R. Michael Baldwin, Richard A. Graham, and David L. Bourdet are current or former employees of Theravance Biopharma US, Inc.

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to participate Informed consent was obtained from all individual participants in the study.

Data sharing Theravance Biopharma, Inc. (and its affiliates) will not be sharing individual de-identified participant data or other relevant study documents.

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