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Effect of a High-Calorie, High-Fat Meal on the Bioavailability and Pharmacokinetics of PA-824 in Healthy Adult Subjects

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PA-824 is a novel nitroimidazo-oxazine being developed as an antituberculosis agent. Two randomized studies evaluated the pharmacokinetics and safety of a single oral dose of PA-824 administered to healthy adult subjects 30 min after a high-calorie, high-fat meal (fed state) versus after a minimum 10-h fast (fasted state). A total of 48 subjects were dosed in the two studies in a randomized crossover design with PA-824 at dose levels of 50, 200, or 1,000 mg in the fed state or fasted state. After the administration of PA-824, the geometric mean ratios of C_{max} and $AUC_{0-\infty}$ revealed an increase in exposure with the addition of a high-calorie, high-fat meal compared to the fasted state by 140 and 145% at 50 mg, 176 and 188% at 200 mg, and 450 and 473% at 50, 200, and 1,000 mg, respectively. The median T_{max} in the fed state was 4 h for the 50-mg dose and 5 h for the 200- and 1,000-mg doses. In the fasted state, the median T_{max} was 4 h for the 50- and 200-mg doses and 6.5 h for the 1,000-mg dose. All doses were well tolerated, and no serious adverse events occurred in either study. (This study has been registered at ClinicalTrials.gov under registration numbers NCT01828827 and NCT01830439.)

PA-824 is a new chemical entity of the nitroimidazo-oxazine class that possesses significant antitubercular activity against replicating and nonreplicating/persistent *Mycobacterium tuberculosis* via a unique mechanism of action (1–3). It is also active against all single-drug-resistant and multidrug-resistant clinical *M. tuberculosis* isolates tested (2), and novel PA-824-containing regimens have been effective in shortening treatment time in a murine model of drug-sensitive tuberculosis (TB) (4). PA-824 was also highly active as monotherapy in a phase IIa 14-day early bactericidal activity (EBA) study in humans with similar efficacy observed with all doses assessed (200 to 1,200 mg/day) (5). In a follow-up phase IIa study exploring a lower dose range (50 to 200 mg/day), a dose-response trend was detected, with 50 mg/day being the least efficacious dose and doses of 100, 150, and 200 mg/day showing similar efficacy profiles (6). In addition, in a subsequent 14-day EBA phase IIa study of novel antituberculosis drug combinations, the combination of PA-824 (200 mg/day), moxifloxacin (400 mg/day), and pyrazinamide (25 mg/kg/day) demonstrated EBA activity comparable to the current World Health Organization-recommended, first-line TB treatment regimen of rifampin, isoniazid, pyrazinamide, and ethambutol (7). Based on the results of these EBA studies, the expected therapeutic doses of 100 and 200 mg/day dosed with moxifloxacin (400 mg/day) and pyrazinamide (1,500 mg/day) for 2 months are being evaluated in a phase IIb trial in patients with pulmonary TB.

Initial reports of the pharmacokinetics (PK) of PA-824 have been presented previously (8). The goal of the current study was to determine whether the PK of PA-824 would be altered after a high-calorie, high-fat meal (fed state) compared to administration after a minimum 10-h fast (fasted state). PA-824 oral tablet formulations were evaluated in two phase I clinical studies. We provide here a detailed summary of the PK characteristics and safety of PA-824 following a single dose administered to healthy volunteers in a fed or fasted state.

MATERIALS AND METHODS

Study design. Two phase I studies were conducted to assess the safety, tolerability, and PK of PA-824 when dosed in healthy adult male and female subjects after a high-calorie, high-fat meal versus when administered after a minimum 10-h fast. Study designs were derived from Food and Drug Administration (FDA) industry guidelines, i.e., FDA Food-Effect Bioavailability and Fed Bioequivalence Studies (9). (This study has been registered at ClinicalTrials.gov under registration numbers NCT01828827 and NCT01830439.)

The initial clinical study (PA-824–CL-003) was conducted to evaluate the food effect on 1,000 mg of PA-824 (five 200-mg tablets). Subsequently, upon determining that the therapeutic dose would be ≤200 mg/day, a later second clinical study (PA-824–CL-009) was conducted to evaluate the food effect on lower doses of PA-824: 50 mg (one 50-mg tablet) or 200 mg (one 200-mg tablet).

Both clinical studies, PA-824-CL-003 (CL-003) and PA-824-CL-009 (CL-009), were randomized, balanced, single-dose, two-treatment, two-period, two-sequence, crossover, open-label studies conducted by Celerion, Inc. (formerly MDS Pharma Services, Inc.), in Lincoln, NE, for the TB Alliance (the study sponsor). The studies were identical in design and evaluated separately. Subjects were randomized to treatment sequences to minimize assignment bias, and a crossover design was used to increase precision for the comparison between the fed-state and fasted-state PK parameters. Each subject was given a single dose of PA-824 after either a high-calorie, high-fat meal or after a minimum 10-h fast. Based on prior experience with PA-824 in healthy volunteers, an 8-day washout between doses (a washout period in excess of five PA-824 half-lives) was included to allow for adequate clearance of the drug and thereby eliminate PA-824 carryover effects from the fed- or fasted-state treatment period.

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For the fasted state, the dose was administered with 240 ml of water after a minimum 10-h overnight fast. For the fed state, the dose was administered with 240 ml of water within 30 min after a high-calorie, high-fat breakfast provided after a minimum 10-h overnight fast. The test meal followed the FDA guidance on high-fat (ca. 50% of total caloric content of the meal) and high-calorie (800 to 1000 cal) content and consisted of two eggs fried in butter, two strips of bacon, two slices of toast with butter, four ounces of hash brown potatoes, and eight ounces of whole milk.

Subjects. Healthy male ($n = 9$) and female ($n = 7$) volunteers were recruited for CL-003. Similarly, to assess two PA-824 doses in CL-009, healthy male ($n = 16$) and female ($n = 16$) volunteers were recruited with a balanced approach per gender for each dose. Inclusion and exclusion criteria were nearly identical for both studies and are further detailed below. All subjects were 19 to 50 years of age, with a body mass index (BMI) of 18 to 29 and were medically healthy as deemed by the Principal Investigator via assessment of screening results, including medical history, clinical laboratory results, 12-lead electrocardiograms (ECGs), and physical examination. At both screening and check-in, subjects had negative urine test results for alcohol and other drugs of abuse, such as amphetamines, cannabinoids, and cocaine metabolites. Subjects were excluded if they had a history of peptic ulcer disease, gastritis, esophagitis, gastroesophageal reflux disease, any cardiac abnormalities, or any relevant drug or food allergies. Subjects were also excluded if they were positive for hepatitis C virus, hepatitis B virus, or human immunodeficiency virus. Female subjects were excluded if they were pregnant (positive test for serum human chorionic gonadotropin at screening or check-in) or breast-feeding. In CL-009, subjects were also excluded if they had any evidence of lens opacity on slit-lamp examination, if a female subject was planning to conceive a child within 1 week of cessation of treatment, or if a male subject was planning to father a child within 12 weeks of cessation of treatment.

All subjects provided written informed consent prior to the initiation of the study in which they were a participant. Study protocols and consent forms were reviewed and approved by Celerion's Institutional Review Board, and the studies were conducted in accordance with U.S. Code of Federal Regulations (21 CFR Part 50, 56, and 312) principles and requirements and with International Conference on Harmonisation guidelines (ICH E6).

Sampling. In CL-003, blood samples (10 ml) were collected during all treatment phases prior to dosing and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 24, 30, 36, 48, 72, 96, 120, 144, and 168 h after each 1,000-mg dose. Urine samples were collected predose and from 0 to 2 h, 2 to 4 h, 4 to 8 h, 8 to 12 h, and 12 to 24 h. Urine was then collected in 24-h intervals through 192 h after each dose.

In both treatment phases in CL-009, blood samples (10 ml) were collected prior to dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 h after a 200- or 50-mg dose. Urine samples were collected predose and from 0 to 4 h, 4 to 8 h, and 8 to 24 h.

Bioanalytical methods. Blood samples were collected and centrifuged, and plasma was separated and stored at −20°C. Urine samples were divided into aliquots and also stored at −20°C. Plasma and urine samples were sent to Covance Laboratories for analysis. PA-824 and the internal standard (added during sample processing), triazolam, were extracted from human plasma and urine samples using liquid-liquid extraction. After evaporation under nitrogen, the residue was reconstituted and analyzed using liquid chromatography with tandem mass spectrometric detection (8).

PK analysis. PA-824 plasma PK parameters were calculated for each subject in both studies by applying a noncompartmental approach using WinNonlin Professional Version 5.0 (CL-003) and Version 5.2 (CL-009) (Pharsight Corp., Mountain View, CA). The plasma PK parameters were derived from each subject after a single dose of PA-824 while in the fed and fasted states. The key PK parameters determined in both studies included C_{max} (maximum observed concentration), T_{max} (time at which C_{max} occurs), k_{el} (terminal elimination rate constant), $t_{1/2}$ (elimination half-life), AUC_{0-t} (area under the concentration-time curve up to the last observed plasma concentration), and $AUC_{0-\infty}$ (area under the concentration-time curve extrapolated to infinity). PA-824 urine PK parameters were also calculated, including Ae_{0-t} (amount excreted in urine) and CL_R (renal clearance).

AUCs were calculated using linear trapezoidal summation from time zero to the specified time point (either the last available time point, or infinity). k_{el} was estimated by unweighted log-linear regression of the last portion of the plasma concentration profile as follows: the log-linear regression was fitted according to the least-squares approach using concentrations from the time period beginning 24 h postdose and ending with the last concentration prior to the first assay that was below the limit of quantification. The elimination half-life ($t_{1/2}$) was calculated from k_{el}, using the formula $\ln(2)/k_{el}$. The amount excreted in urine (Ae_{0-t}) was calculated from the sum of the products of the analyte concentrations in urine and the urine volumes for all collection intervals. CL_R was calculated by dividing Ae_{0-t} by AUC_{0-t}.

All descriptive and inferential statistics were calculated in SAS Version 8.2 (CL-003) and Version 9.1.3 (CL-009). The PK endpoints AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} for PA-824 were compared between the fed and fasted state using an analysis of variance (ANOVA) model. The ANOVA model using SAS PROC mixed procedure included treatment, period, and sequence as fixed effects, and subject-within-sequence as a random effect. In addition, the ANOVA was repeated for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}, using treatment (fed and fasted states) and gender as fixed effects. Geometric least-squares means (LSM) were calculated by exponentiating the LSM from the ANOVA to compare the fed and fasted states.

Safety evaluation. Safety assessments included physical examinations, vital signs, ECGs, hematology, serum chemistry, coagulation, urinalysis, and ophthalmology (visual acuity and slit lamp in the CL-009 study) exams posttreatment. The frequency and severity of treatment-period adverse events (AEs) were assessed on a continual basis throughout the study via safety assessments, observation, direct participant reporting, and specific AE inquiry ("How do you feel?" questions) at various points during the study.

RESULTS

A total of 48 healthy male and female subjects participated in the two clinical studies evaluating the safety, tolerability, and PK of PA-824 after a single dose in the fed (after a high-calorie, high-fat meal) state versus in the fasted state (after a minimum 10-h fast). The demographics are summarized in Table 1.

Pharmacokinetics. Mean plasma concentrations in the fed state and fasted state for CL-003 and CL-009 are shown in Fig. 1. Key PK parameters are provided in Table 2. There were no issues with carryover since all predose PA-824 concentrations for all doses were <3.5% of the observed C_{max} of the following dose.

50-mg dose. As seen in Table 2, after a single oral 50-mg PA-824 dose (CL-009), the arithmetic mean exposures and percent geometric mean ratios (100 · [fed exposure/fasted exposure]) for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ differed between the fed and fasted state. The percent geometric mean ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 140, 147, and 145%, respectively (Table 3). T_{max} did not differ between the fed and fasted state. $t_{1/2}$ in the fed state (19.2 h) was not significantly longer compared to the fasted state (18.9 h; $P = 0.6685$). Renal clearance was not affected by food intake for the 50-mg dose. The percentage of the dose excreted in urine as unchanged drug over the first 24 h after the 50-mg dose was <.2% under both the fed and fasted states.

200-mg dose. As seen in Table 2, after a single oral 200-mg PA-824 dose (CL-009), arithmetic mean exposures increased for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ between the fed and fasted state. The percent geometric mean ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ (see

TABLE 1 Demographic characteristics by study

Characteristic	CL-003	CL-009		
	1,000 mg (n = 16)	200 mg (n = 16)	50 mg (n = 16)	Overall (n = 32)
Male/female (no. of subjects)	9/7	8/8	8/8	16/16
Ethnicity, no. of subjects (%)				
Black	2 (12.5)	0 (0)	2 (12.5)	2 (6.3)
White	12 (75)	15 (95.2)	12 (75)	27 (84.4)
Hispanic	2 (12.5)	1 (4.8)	1 (6.3)	2 (6.3)
Asian	0	0	1 (6.3)	1 (3.1)
Mean age, yrs (range)	26.9 (19–40)	30.5 (20–50)	34.6 (21–48)	32.5 (20–50)
Mean BMI, kg/m^2 (range)	23.5 (19.4–28.6)	24 (19.9–28.0)	23.9 (19.2–28.0)	24 (19.2–28.0)

Table 3) were 176, 188, and 188%, respectively. As noted in Table 2, T_{max} occurred later under the fed state (5 h) than under the fasted state (4 h) ($P = 0.0507$). The $t_{1/2}$ following the fed state (17.4 h) was not significantly longer than in the fasted state (16.9 h; $P = 0.2979$). CL_R was not affected by food intake for the 200-mg dose. The percentage of the dose excreted in urine as unchanged drug over the first 24 h after the 200-mg dose was <0.2% in both the fed and the fasted states.

1,000-mg dose. As seen in Table 2, after a single oral 1,000-mg PA-824 dose (CL-003), arithmetic mean exposures for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were higher in the fed compared to the fasted state. The percent geometric mean ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ fed versus fasted were 450, 374, and 373%, respectively. T_{max} did not differ under the fed state (5.04 h) than the fasted state (6.50 h) ($P = 0.0676$). The apparent elimination half-life ($t_{1/2}$) of PA-824 following the fed state was marginally longer (19.74 h) when compared than following the fasted state (18.94 h) ($P = 0042$).

In contrast to the results with the 50- and 200-mg doses, administration of 1,000 mg of PA-824 in the fed state was associated with a lower mean renal clearance compared to a dose administered to the same subjects in the fasted state ($P = <0.0001$, Table 2). Comparisons of PA-824 PK parameters for urine showed that the LSM ratios of Ae_{0-t} and CL_R following dosing of 1,000 mg in the fed state were 52 and 14%, respectively, of those in the fasted state. CL_R reflects the ~2-fold difference between the two treatment conditions for Ae_{0-t} and the ~3.5-fold difference between the two treatment conditions for AUC_{0-t}. However, renal excretion of intact PA-824 is a minor route of elimination with less than 0.4% of the dose excreted after dosing in the fasted state, and ca. 0.2% excreted in urine after dosing in the fed state. The 1,000-mg fasted renal excretion data are consistent with previous data.

The apparent elimination half-life ($t_{1/2}$) and T_{max} were similar under the fed state and fasted states for all doses. Considering the fact that the $t_{1/2}$ was not affected by food intake, the differences observed in PA-824 exposure in the fed versus the fasted state were most likely caused by an increased bioavailability in the fed state. For all doses, gender did not appear to have a statistically significant effect on the bioavailability (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) of PA-824 in either the fed or fasted state. Consistent with previous studies, in both of the current studies the plasma PA-824 levels increased less than dose proportionally in the fasted state. The lack of dose proportionality was not apparent in the fed state.

Safety and tolerability. PA-824 was well tolerated at all dose levels studied, with no serious adverse events (SAEs), and no AEs

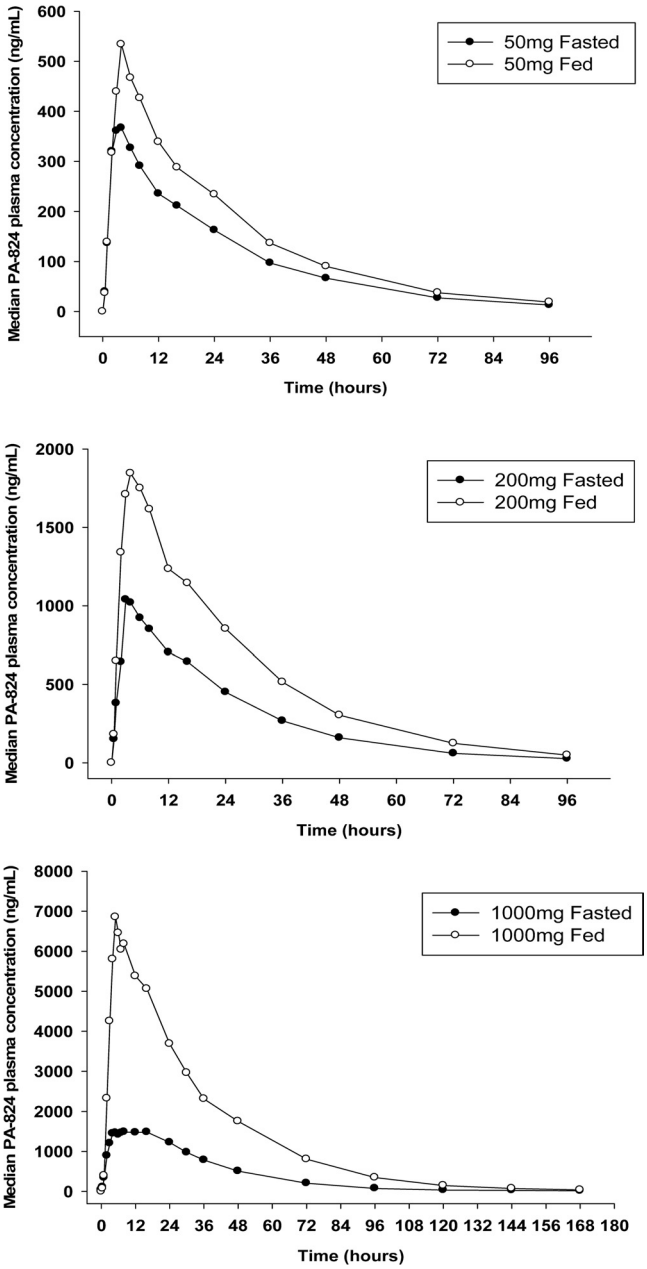

FIG 1 Median PA-824 plasma concentrations over time after the administration of 50 mg (study CL-003 [top]), 200 mg (study CL-009 [middle]), and 1,000 mg (study CL-003 [bottom]) in the fed or fasted state.

TABLE 2 PK parameters following a 50-, 200-, or 1,000-mg dose administered under fed and fasted states (CL-003 and CL-009)

	Fasted state (mean ± SD)			Fed state (mean ± SD)		
	CL-009		CL-003	CL-009		CL-003
PK parameter[a]	50 mg ($n = 16$)	200 mg ($n = 16$)	1,000 mg ($n = 16$)	50 mg ($n = 16$)	200 mg ($n = 16$)	1,000 mg ($n = 16$)
C_{max} (ng/ml)	392 ± 84.0	1,130 ± 213	1,630 ± 367	541 ± 55.9	1,970 ± 297	7,350 ± 2,130
T_{max} (h)	4.00 (2.00, 8.00)	4.00 (2.00, 6.00)	6.50 (4.00, 16.0)	4.00 (2.00, 8.00)	5.00 (3.00, 8.00)	5.00 (4.00, 16.0)
$t_{1/2}$ (h)	18.9 ± 4.41	16.9 ± 3.08	18.94 ± 4.12	19.2 ± 3.76	17.4 ± 2.76	19.74 ± 4.16
AUC_{0-t} (ng · h/ml)	9,811 ± 3,145	28,087 ± 7,999	67,836 ± 23,401	14,052 ± 2,897	51,643 ± 10,102	254,199 ± 97,626
$AUC_{0-\infty}$ (ng · h/ml)	10,351 ± 3,373	28,769 ± 8,258	68,445 ± 23,706	14,661 ± 3,144	52,967 ± 10,630	255,674 ± 98,856
Ae_{0-t} (mg)	0.049 ± 0.017	0.166 ± 0.092	3.792 ± 2.254	0.082 ± 0.040	0.384 ± 0.266	1.987 ± 1.003
CL_R (ml/h)	8.56 ± 3.31	9.72 ± 5.15	56.49 ± 30.98	10.39 ± 5.64	13.12 ± 10.68	8.04 ± 2.64

[a] T_{max} values are presented as "median (minimum, maximum)." The averages noted here were calculated as arithmetic means. C_{max}, maximum observed concentration; T_{max}, time at which C_{max} occurs; $t_{1/2}$, elimination half-life; AUC_{0-t}, area under the concentration-time curve up to the last observed concentration; $AUC_{0-\infty}$, area under the concentration-time curve extrapolated to infinity; Ae_{0-t}, amount excreted in urine; CL_R, renal clearance.

that led to withdrawal of a subject. No systematic or dose-group-related effects on 2-lead cardiac profiles or 12-lead ECG parameters (e.g., heart rate, QT interval, corrected QT interval, etc.) were noted. In addition, no effects on vital signs (e.g., heart rate, blood pressure, temperature, and respiration) were observed. Some subjects who received 1,000 mg of PA-824 had mild, transient increases in serum creatinine, which is expected at higher doses of PA-824 (10). These elevations were slightly above the laboratory reference range, and none were considered clinically significant or to be AEs by the investigators. All other clinical laboratory values (for chemistry, hematology, coagulation, and urinalysis) remained within the laboratory reference range in both studies at time points assessed after drug dosing.

Overall, more AEs were experienced in CL-003 at the PA-824 dose of 1,000 mg than in CL-009 at the doses of 50 or 200 mg, and slightly more AEs were experienced in the fed state than in the fasted state. Although a slightly larger number of AEs occurred following dosing in the fed state, the limited number of subjects participating in these studies did not permit confident distinction between treatments with respect to the frequency of any particular AE or in total AEs. Overall, headache was the most common AE, followed by gastrointestinal AEs (e.g., nausea, diarrhea, and intestinal pain) and dizziness.

TABLE 3 Summary of statistical comparisons of PA-824 pharmacokinetic parameters following fed and fasted states

	Geometric LSM[b]			
Dose (mg) and PK parameter[a]	Fasted state	Fed state	90% CI[c]	% Geometric MR[d]
50				
C_{max} (ng/ml)	383	538	128.87–152.73	140.29
AUC_{0-t} (ng · h/ml)	9,365	13,774	135.65–159.46	147.07
$AUC_{0-\infty}$ (ng · h/ml)	9,885	14,345	134.42–156.68	145.13
200				
C_{max} (ng/ml)	1,107	1,946	164.71–187.49	175.73
AUC_{0-t} (ng · h/ml)	27,020	50,763	168.22–209.81	187.87
$AUC_{0-\infty}$ (ng · h/ml)	27,656	52,009	168.41–210.00	188.05
1,000				
C_{max} (ng/ml)	1,585	7,132	401.20–504.62	449.93
AUC_{0-t} (ng · h/ml)	64,117	239,968	337.40–415.12	374.26
$AUC_{0-\infty}$ (ng · h/ml)	64,702	241,183	336.20–413.29	372.76

[a] C_{max}, maximum observed concentration; AUC_{0-t}, area under the concentration-time curve during the dosing interval; $AUC_{0-\infty}$. area under the concentration-time curve extrapolated to infinity. The PK parameters were ln transformed prior to analysis.
[b] That is, the geometric least-squared means (LSM) calculated by exponentiating the LSM using ANOVA.
[c] 90% CI, 90% confidence interval.
[d] MR, mean ratio. The percent geometric MR was calculated as follows: 100 · (fed/fasted).

DISCUSSION

The effect of a high-fat, high-calorie meal on PA-824 exposure after oral administration was assessed across a wide range of doses (50, 200, and 1,000 mg). The PA-824 C_{max} and AUC increased less than dose proportionally in the fasted state. The highest dose of PA-824 had the greatest relative increase in C_{max} and AUC after the high-fat, high-calorie meal, with more modest increases in C_{max} and AUC being observed for the 50- and 200-mg doses compared to the fasted state. After a high-fat, high calorie meal, the median T_{max} occurred ~1.5 h sooner after administration of 1,000 mg PA-824 and ~1 h later after administration of 200 mg compared to the fasted state. No significant difference in T_{max} was observed at the 50- or 1,000-mg doses. The mean $t_{1/2}$ of PA-824 at all doses was marginally longer in the fed state than in the fasted state. No significant difference in $t_{1/2}$ was observed at the 50- or 200-mg doses.

Overall, PA-824 was moderately rapidly absorbed in both fed and fasted states. These data suggest that the less-than-dose-proportional increase in PA-824 exposure with increasing dose in the fasted state is due to decreased oral bioavailability at higher doses. The presence of food appears to increase the overall solubility of PA-824, and/or dissolution of PA-824 tablets, in the gastrointestinal tract and thereby enhances drug absorption.

Administration of PA-824 as a single dose of 50, 200, or 1,000 mg in the fed state and fasted state was safe and generally well tolerated by the healthy male and female subjects in these studies. No SAEs or AEs of significant clinical concern were observed in the subjects at any dose. Although the PA-824 exposure in the fed state was higher than that observed in the fasted state, based on all previously collected clinical and nonclinical data, doses of PA-824 up to 200 mg given once daily in the fed state or fasted state are considered to be generally well tolerated and acceptable for continued use in long-term clinical studies in TB patients.

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