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 $C_{\text{max}}$ and $\text{AUC}_{0-\text{t}}$ 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 $t_{\text{max}}$ 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 $t_{\text{max}}$ 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.)

**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 Cerillion, 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 (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.

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.
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 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 Cmax (maximum observed concentration), Tmax (time at which Cmax occurs), kel (terminal elimination rate constant), t1/2 (elimination half-life), AUC(0–t) (area under the concentration-time curve up to the last observed plasma concentration), and AUC(0–∞) (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 CLu (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). k1 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 (t1/2) was calculated from k1, using the formula ln(2)/k1. The amount excreted in urine (Ae(0–t)) was calculated from the sum of the products of the analytic concentrations in urine and the urine volumes for all collection intervals. CLu was calculated by dividing Ae(0–t) by AUC(0–∞).

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–∞), and Cmax 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–∞), and Cmax 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 Cmax 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 Cmax, AUC(0–t), and AUC(0–∞) differed between the fed and fasted state. The percent geometric mean ratios of Cmax, AUC(0–t), and AUC(0–∞) were 140, 147, and 145%, respectively (Table 3). Tmax did not differ between the fed and fasted state. t1/2 in the fasted 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 Cmax, AUC(0–t), and AUC(0–∞) between the fed and fasted state. The percent geometric mean ratios of Cmax, AUC(0–t), and AUC(0–∞) (see
Table 3) were 176, 188, and 188%, respectively. As noted in Table 2, \( T_{\text{max}} \) 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; \( P = 0.2979 \)). CLR 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 \( \leq 0.2\% \) 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 \( C_{\text{max}}, \) \( \text{AUC}_{0–t} \), and \( \text{AUC}_{0–\infty} \) were higher in the fed compared to the fasted state. The percent geometric mean ratios of \( C_{\text{max}}, \text{AUC}_{0–t} \), and \( \text{AUC}_{0–\infty} \) fed versus fasted were 450, 374, and 373%, respectively. \( T_{\text{max}} \) 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) \((P = 0.0042)\).

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 \((P = <0.0001, \text{Table 2})\). Comparisons of PA-824 PK parameters for urine showed that the LSM ratios of \( \text{Ae}_{0–t} \) and CLR following dosing of 1,000 mg in the fed state were 52 and 14%, respectively, of those in the fasted state. CLR reflects the \( \sim 2\)-fold difference between the two treatment conditions for \( \text{Ae}_{0–t} \) and the \( \sim 3.5\)-fold difference between the two treatment conditions for \( \text{AUC}_{0–\infty} \). 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 \( T_{\text{max}} \) 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 \((C_{\text{max}}, \text{AUC}_{0–t} \text{, and AUC}_{0–\infty})\) 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...
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.

**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 $C_{\text{max}}$ and AUC increased less than dose proportionally in the fasted state. The highest dose of PA-824 had the greatest relative increase in $C_{\text{max}}$ and AUC after the high-fat, high-calorie meal, with more modest increases in $C_{\text{max}}$ 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 $T_{\text{max}}$ 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 $T_{\text{max}}$ 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.

**ACKNOWLEDGMENTS**

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

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>CL-009</th>
<th>CL-003</th>
<th>Fed state (mean ± SD)</th>
<th>CL-009</th>
<th>CL-003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 mg (n = 16)</td>
<td>200 mg (n = 16)</td>
<td>1,000 mg (n = 16)</td>
<td>50 mg (n = 16)</td>
<td>200 mg (n = 16)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>392 ± 84.0</td>
<td>1,130 ± 213</td>
<td>1,630 ± 367</td>
<td>541 ± 55.9</td>
<td>1,970 ± 297</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>4.00 (2.00, 8.00)</td>
<td>4.00 (2.00, 6.00)</td>
<td>6.50 (4.00, 16.0)</td>
<td>4.00 (2.00, 8.00)</td>
<td>5.00 (3.00, 8.00)</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>18.9 ± 4.41</td>
<td>16.9 ± 3.08</td>
<td>18.94 ± 4.12</td>
<td>19.2 ± 3.76</td>
<td>17.4 ± 2.76</td>
</tr>
<tr>
<td>AUC$_{0-\text{t}}$ (ng · h/ml)</td>
<td>9.51 ± 3.14</td>
<td>28.76 ± 7.99</td>
<td>68.84 ± 23.40</td>
<td>14,032 ± 2,897</td>
<td>51,643 ± 10.102</td>
</tr>
<tr>
<td>AUC$_{\text{max}}$ (ng · h/ml)</td>
<td>10.351 ± 3.373</td>
<td>28.769 ± 8.258</td>
<td>68.445 ± 23.706</td>
<td>14,161 ± 3,144</td>
<td>52,967 ± 10.630</td>
</tr>
<tr>
<td>AEC$_{\text{max}}$ (mg)</td>
<td>0.049 ± 0.017</td>
<td>0.166 ± 0.092</td>
<td>3.792 ± 2.254</td>
<td>0.082 ± 0.040</td>
<td>0.384 ± 0.266</td>
</tr>
<tr>
<td>CL$_{\text{R}}$ (ml/h)</td>
<td>8.56 ± 3.31</td>
<td>9.72 ± 5.15</td>
<td>56.49 ± 30.98</td>
<td>10.39 ± 5.64</td>
<td>13.12 ± 10.68</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Dose (mg) and PK parameter</th>
<th>Geometric LSM$^c$</th>
<th>% Geometric MR$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasted state</td>
<td>Fed state</td>
</tr>
<tr>
<td>50 mg</td>
<td>383</td>
<td>538</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>3.965</td>
<td>13,774</td>
</tr>
<tr>
<td>AUC$_{0-\text{t}}$ (ng · h/ml)</td>
<td>9,885</td>
<td>14,345</td>
</tr>
<tr>
<td>200 mg</td>
<td>1,107</td>
<td>9,496</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>27,020</td>
<td>50,763</td>
</tr>
<tr>
<td>AUC$_{0-\text{t}}$ (ng · h/ml)</td>
<td>27,656</td>
<td>52,967</td>
</tr>
<tr>
<td>1,000 mg</td>
<td>1,581</td>
<td>7,132</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>64,117</td>
<td>239,968</td>
</tr>
<tr>
<td>AUC$_{0-\text{t}}$ (ng · h/ml)</td>
<td>64,702</td>
<td>241,183</td>
</tr>
</tbody>
</table>

$^a$ $C_{\text{max}}$ maximum observed concentration; AUC$_{0-\text{t}}$, area under the concentration-time curve extrapolated to infinity. The PK parameters were ln transformed prior to analysis.

$^b$ LSM using ANOVA.

$^c$ A geometric least-squared means (LSM) calculated by exponentiating the LSM using ANOVA.

$^d$ MR, mean ratio. The percent geometric MR was calculated as follows: $100 \cdot (\text{fed}/\text{fasted}).$

We thank the staff of Celerion, Inc., and the study participants.

REFERENCES


