Safety, Tolerability, and Pharmacokinetics of PA-824 in Healthy Subjects[▽]

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PA-824 is a novel antibacterial agent that has shown *in vitro* activity against both drug-sensitive and drug-resistant *Mycobacterium tuberculosis*. The compound's MIC is between 0.015 and 0.25 $\mu\text{g/ml}$ for drug-sensitive strains and between 0.03 and 0.53 $\mu\text{g/ml}$ for drug-resistant strains. In addition, it is active against nonreplicating anaerobic *Mycobacterium tuberculosis*. The safety, tolerability, and pharmacokinetics of PA-824 were evaluated in two escalating-dose clinical studies, one a single-dose study and the other a multiple-dose study (up to 7 days of daily dosing). In 58 healthy subjects dosed with PA-824 in these studies, the drug candidate was well tolerated, with no significant or serious adverse events. In both studies, following oral administration PA-824 reached maximal plasma levels in 4 to 5 h independently of the dose. Maximal blood levels averaged approximately 3 $\mu\text{g/ml}$ (1,500-mg dose) in the single-dose study and 3.8 $\mu\text{g/ml}$ (600-mg dose) in the multiple-dose study. Steady state was achieved after 5 to 6 days of daily dosing, with an accumulation ratio of approximately 2. The elimination half-life averaged 16 to 20 h. Overall, PA-824 was well tolerated following oral doses once daily for up to 7 days, and pharmacokinetic parameters were consistent with a once-a-day regimen. The results of these studies, combined with the demonstrated activity of PA-824 against drug-sensitive and multidrug-resistant *Mycobacterium tuberculosis*, support the investigation of this novel compound for the treatment of tuberculosis.

According to the World Health Organization, there were 9.27 million new tuberculosis (TB) cases worldwide in 2007, which claimed the lives of approximately 1.77 million people, including 456,000 patients coinfecting with human immunodeficiency virus (11). In addition, global increases in cases of multidrug-resistant TB and, more recently, extensively drug-resistant TB pose serious treatment challenges (12). New anti-TB drugs are needed that can shorten the duration of treatment, improve the treatment of resistant disease, facilitate the treatment of TB patients coinfecting with human immunodeficiency virus, and shorten the treatment of latent TB infection.

The 4-nitroimidazo-oxazoles (a subclass of nitroimidazoles) have potent sterilizing activity against *Mycobacterium tuberculosis*, as first demonstrated in 1993 (1). The further investigation of nitroimidazoles in an anaerobic model of *M. tuberculosis* dormancy demonstrated that metronidazole is active against slow-growing *M. tuberculosis*, suggesting the potential for the treatment of latent TB infection and for shortening the treatment of active TB disease (10). The further development of the nitroimidazole class by Pathogenesis, Inc., led to the discovery of another subclass, 4-nitroimidazo-oxazines, with promising activity against *M. tuberculosis*. PA-824, with the full chemical name (*S*)-2-nitro-6-[4-(trifluoromethoxy)benzyloxy]-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine, was identified as the lead 4-nitroimidazo-oxazine. Stover et al. (8) reported that the MIC of PA-824 under aerobic conditions against a variety of drug-sensitive clinical isolates was similar to the MIC of isoniazid (MIC of PA-824, 0.015 to 0.25 $\mu\text{g/ml}$; MIC of isoni-

azid, 0.03 to 0.06 $\mu\text{g/ml}$). PA-824 also was found to be active against all single-drug- and multidrug-resistant clinical isolates of *M. tuberculosis* tested, with MICs of 0.03 to 0.53 $\mu\text{g/ml}$. Additional studies using microaerophilic and anaerobic culture models indicated that PA-824 also is active against both replicating and nonreplicating or infrequently replicating *M. tuberculosis* isolates (3, 8).

Like metronidazole, PA-824 requires metabolic activation by *M. tuberculosis* through an F420-dependent nitroreduction (4, 5, 8). Although not thoroughly elucidated at this time, PA-824's novel mechanism of action involves the inhibition of the synthesis of both protein and lipids but not nucleic acid. Studies by Stover et al. (8) demonstrated that PA-824 inhibits the oxidation of hydroxymycolate to ketomycolate, an essential lipid for *M. tuberculosis* cell wall function. Recent work by Singh et al. (7) indicates that the reduction of PA-824 to its des-nitroimidazole metabolite by a deazaflavin (F420)-dependent nitroreductase is associated with the generation of reactive nitrogen species, including nitric oxide, which may represent important effectors of PA-824 killing of *M. tuberculosis* under anaerobic conditions. In an experimental mouse model of infection, Tyagi et al. (9) demonstrated that, at a dose of 100 mg/kg of body weight, PA-824 has substantial bactericidal activity during both the initial and continuation phases of TB treatment. Using a short-course mouse infection model that employs 9 days of the drug treatment of gamma-interferon knockout mice infected with *M. tuberculosis* 14 days before treatment initiation, Lenaerts et al. (3) found that at 100 mg/kg PA-824 was as active as isoniazid at 25 mg/kg, rifampin (rifampicin) at 10 mg/kg, and moxifloxacin at 100 mg/kg. Additional studies of a mouse model of TB examined the activity of PA-824 administered in combination with current TB drugs. When substituted for isoniazid in standard therapy, PA-824

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resulted in significantly fewer CFU after 2 months of therapy and a higher rate of conversion to culture negativity than that of the standard drug combination. Relapse rates after 6 months of treatment were not different in the experimental and control treatment arms in this study, but the study design was such that an improved relapse rate relative to the control could not have been demonstrated (6). Pharmacokinetic analyses reported by NuerMBERGER et al. (6) demonstrated in mice that the standard rifampin-isoniazid-pyrazinamide regimen does not affect core PA-824 pharmacokinetic parameters, such as $C_{\max}$ (maximum concentration observed), AUC_{0-24} (total area under concentration-time curve from 0 to 24 h), or $t_{1/2}$ (half-life). Further nonclinical studies are under way to characterize PA-824's activity and interactions in novel drug combinations.

MATERIALS AND METHODS

This report examines the data from two phase I clinical studies designed to assess the safety, tolerability, and pharmacokinetics of PA-824: an ascending single-dose study (CL-001) and an ascending multiple-dose study (CL-002). The studies were conducted at MDS Pharma Services facilities in Lincoln, NB (CL-001), and Neptune, NJ (CL-002). For each study, the ascending doses were administered to separate groups of PA-824-naïve subjects enrolled serially during the study.

Study design. Study CL-001 was a double-blind, placebo-controlled, single-dose, dose-escalating, pharmacokinetic, tolerability, and safety study of healthy adult male volunteers. Single oral doses (50, 250, 500, 750, 1,000, 1,250, or 1,500 mg) or placebo in a tablet formulation were administered to seven groups of healthy subjects after an overnight fast. Six groups consisted of eight subjects each, with six subjects in each group receiving PA-824 and two receiving placebo. The 50-mg-dose group had five subjects (four received PA-824 and one received placebo).

Study CL-002 was a double-blind, placebo-controlled, multiple-dose, dose-escalating, pharmacokinetic, tolerability, and safety study of healthy adult male and female volunteers. The study design included four dose groups of eight subjects each (six received PA-824 and two received placebo) receiving doses of 200, 600, 1,000, and 1,400 mg of PA-824 or placebo in tablet form each day for 7 days after an overnight fast. Because of an observed increase in serum creatinine levels in the 1,000-mg-dose group, dosing of that cohort was halted on day 5 and the 1,400-mg-dose cohort was not enrolled. Dose groups were enrolled sequentially for both studies, and safety was assessed prior to enrolling the next group.

Subjects. Healthy male volunteers were recruited for study CL-001; healthy male and female volunteers were recruited for study CL-002. Inclusion and exclusion criteria were identical for both studies, with the exception that study CL-002 specified criteria reflecting the inclusion of women. All subjects were aged 19 to 50, and none had any clinically significant findings in their medical history, clinical laboratory results, 12-lead electrocardiograms, or physical examination. Subjects were excluded if they had taken any systemic or topical prescription medication, with the exception of hormonal contraceptives for women, in the 14 days prior to dosing or during the study. Subjects who had taken over-the-counter medications (including vitamins, herbal preparations, antacids, cough medications, and cold medications) for 7 days prior to dosing or during the study also were excluded, as were subjects who within 30 days of dosing or during the study had taken any drugs of abuse or therapeutic drugs known to (i) be strong inhibitors or inducers of cytochrome P450 enzymes, (ii) prolong the electrocardiogram QT interval, or (iii) alter any major organ function. All study protocols and consent forms were reviewed and approved by institutional review boards constituted and operating per the U.S. Code of Federal Regulations. All subjects provided written informed consent prior to the initiation of the study of which they were participating. Subject safety was ensured during the study by means of urinalysis; clinical chemistry, hematology, and coagulation testing; 12-lead electrocardiograms; physical exams and vital signs measurement; and the self-reporting of adverse events and regular direct adverse event queries.

Sampling. In the single-dose study (CL-001), blood samples were collected prior to dosing and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20, 24, 30, and 36 h postdose, as well as 7 days postdose. For the 1,250- and 1,500-mg-dose groups, urine was collected at 4-h intervals starting from the time of dosing through 36 h postdose.

In the multiple-dose study (CL-002), blood samples were collected at the

following time points: before dosing each day during the treatment period; 1, 2, 3, 4, 5, 6, 7, 8, 12, and 16 h after dosing on days 1 and 7; 24, 30, and 36 h after the day 7 dosing (i.e., during day 8); daily during washout on days 9 to 13, at the time daily dosing would have occurred otherwise; and during checkout on day 14. A full urinalysis panel was performed at screening, check-in (day 0), on day 4, 24 h after the last dose (day 8), and at study completion (day 14) or on early withdrawal from the study. In addition, creatinine clearance and total urinary protein excretion were determined with the following steps: (i) the baseline 12-h creatinine clearance and total urinary protein excretion starting at check-in on day 0 and concluding before dosing on day 1 were measured, and (ii) for the 200- and 600-mg-dose groups, 24-h creatinine clearance and total urinary protein excretion measurements were started at hour 0 of days 2, 5, and 13. In the 1,000-mg-dose group, creatinine clearance and urine protein excretion measurements were taken on days 2, 5, 6, and 11.

Bioanalytical methods. Blood samples were collected and centrifuged, and plasma was separated and stored at -20°C . Urine samples were aliquoted and stored at -20°C . Plasma and urine samples were analyzed for PA-824 using validated liquid chromatography/mass spectrometry methods developed at Covance Laboratories.

PA-824 and the internal standard, triazolam (which was added during sample processing), were extracted from human plasma samples using liquid-liquid extraction. After evaporation under nitrogen, the residue was reconstituted and analyzed using liquid chromatography with tandem mass spectrometric detection. The analytical column used for plasma samples was a Chromolith Speed ROD RP-18e (50 by 4.6 mm; Merck). Mass spectrometer analysis was conducted with a Sciex API 3000 with ionization using positive ion electrospray. The standard curve range was from 10 to 10,000 ng/ml for PA-824 using a sample volume of 0.0500 ml. The limit of quantitation was 10.0 ng/ml. The accuracy of the curve ranged from 92.2 to 105%, and the relative standard deviation was $<4.8\%$ for the 12 analytical runs employed. Overall recovery efficiency for PA-824 in human plasma was 71.7% for PA-824 and 72.9% for the internal standard.

Pharmacokinetic analysis. Pharmacokinetics were assessed by measuring serial plasma concentrations of PA-824. The pharmacokinetic parameters determined in these two studies include the following: $t_{1/2}$ (elimination half-life), $C_{\max}$, $T_{\max}$ (time at which $C_{\max}$ occurs), and CL_F (oral clearance) on days 1 and 7; AUC_{0-24} and $AUC_{0-\infty}$ (area under concentration-time curve extrapolated to infinity) on day 1; and $C_{\min}$ (the steady-state trough concentration) and $AUC_{0-\tau}$ (area under the concentration-time curve during the dosing interval) on day 7. These parameters were calculated by applying a noncompartmental approach using WinNonlin Professional Version 4.0 (Pharsight Corporation, Mountain View, CA).

AUCs were calculated using linear trapezoidal summation from time zero to the specified time point (24 h, 36 h, the last available time point, or infinity). $t_{1/2}$ values were estimated by fitting a line to the last portion of the plasma concentration profile using a least-squares approach.

The parameter values were read into SAS data sets, and all descriptive and plasma inferential statistics were calculated in SAS, version 8.2 (SAS Institute, Inc., Cary, NC). Plasma concentrations and pharmacokinetic parameters of PA-824 were listed and summarized with descriptive statistics (number of subjects, mean, median, standard deviations, standard errors of the means, coefficient of variation, minimum, and maximum). For $C_{\max}$, $C_{\min}$, and AUC_{0-24} , the geometric mean and geometric coefficient of variation also were calculated. Descriptive statistics for log-transformed pharmacokinetic parameters of AUC_{0-24} (day 1), $AUC_{0-\tau}$ (day 7), $C_{\min}$ (day 7), and $C_{\max}$ (days 1 and 7) for PA-824 were calculated for each group.

RESULTS

A total of 77 healthy male and female subjects participated in the two clinical studies addressing PA-824 safety, tolerability, and pharmacokinetics, with 58 subjects receiving PA-824 and 19 receiving placebo. These 77 participants represented a racially diverse sample population. The multiple-dose study (CL-002) was ended early because of an observed increase in serum creatinine, which later was determined to be reversible and not caused by a decrease in the glomerular filtration rate (reported in detail in reference 2).

Pharmacokinetics. Plasma concentrations for the single-dose study (CL-001) are shown in Fig. 1 and for the multiple-dose in Fig. 2. Key pharmacokinetic parameters across the

FIG. 1. Mean plasma concentrations of PA-824 for study CL-001 (linear data). Symbols indicate the dose of PA-824.

single-dose and multiple-dose studies are provided in Tables 1 and 2, respectively. PA-824 was moderately rapidly absorbed in both studies. As seen in Tables 1 and 2, T_{max} values across groups within studies and across the single-dose and multiple-dose studies were 4 to 5 h, with no apparent dose dependency.

As indicated in Table 1, after a single oral dose of PA-824 in study CL-001, the C_{max} observed ranged from $0.3 \pm 0.1 \mu\text{g/ml}$ (50-mg-dose group) to $2.9 \pm 0.5 \mu\text{g/ml}$ (1,500-mg-dose group),

and the mean total exposure ($AUC_{0-\infty}$) ranged from $7.5 \pm 3.9 \mu\text{g} \cdot \text{h/ml}$ (50-mg-dose group) to $101.8 \pm 25.3 \mu\text{g} \cdot \text{h/ml}$ (1,000-mg-dose group). Similarly, AUC_{0-36} values ranged from $5.5 \pm 2.4 \mu\text{g} \cdot \text{h/ml}$ (50-mg-dose group) to $73.7 \pm 16.5 \mu\text{g} \cdot \text{h/ml}$ (1,500-mg-dose group).

In both the single-dose and multiple-dose studies, plasma PA-824 levels increased less than dose proportionally, with an apparent plateauing of bioavailability seen at higher dose lev-

* Dosing stopped for 1000-mg dose level after dosing on Day 5

FIG. 2. Mean plasma concentrations of PA-824 for study CL-002 (linear data). Symbols indicate the dose of PA-824.

TABLE 1. Pharmacokinetic parameters for the single-dose study (CL-001)^a

Dose (mg)	$C_{\max}$ ($\mu\text{g/ml}$)	$T_{\max}$ (h)	$t_{1/2}$ (h)	AUC_{0-36} ($\mu\text{g} \cdot \text{h/ml}$)	$\text{AUC}_{0-\infty}$ ($\mu\text{g} \cdot \text{h/ml}$)	CL_F (liters/h)
50 ($n = 5$)	0.3 (0.1)	5.0 (5.0, 12.0)	15.2 (3.04)	5.5 (2.4)	7.5 (3.9)	8.47 (4.67)
250 ($n = 8$)	1.2 (0.3)	4.0 (2.0, 5.0)	14.0 (2.68)	22.0 (4.1)	27.0 (4.3)	9.5 (1.87)
500 ($n = 8$)	1.6 (0.3)	5.0 (3.0, 6.0)	13.5 (1.58)	33.2 (6.6)	41.5 (9.4)	12.6 (3.17)
750 ($n = 8$)	2.0 (0.5)	5.0 (2.0, 8.0)	16.1 (4.45)	45.3 (12.8)	61.4 (19.5)	13.2 (3.9)
1,000 ($n = 8$)	2.8 (0.6)	5.0 (2.0, 16.0)	20.0 (3.32)	67.5 (14.4)	101.8 (25.3)	10.4 (2.59)
1,250 ($n = 8$)	2.9 (0.4)	4.0 (3.0, 12.0)	18.1 (3.53)	69.4 (11.9)	99.2 (21.0)	13.1 (3.18)
1,500 ($n = 8$)	2.9 (0.5)	4.5 (3.0, 12.0)	15.6 (3.07)	73.7 (16.5)	101.2 (28.2)	15.9 (4.78)

^a Arithmetic mean values are given, with standard deviations in parentheses. For $T_{\max}$, medians and ranges (minimum, maximum) are presented.

els. In the single-dose study, dose levels above 1,000 mg achieved minimal additional PA-824 exposure for both the $C_{\max}$ and AUC. Mean $C_{\max}$, AUC_{0-36} , and $\text{AUC}_{0-\infty}$ values for each of the three highest-dose groups (1,000, 1,250, and 1,500 mg) were approximately 2.9 $\mu\text{g/ml}$, 70 $\mu\text{g} \cdot \text{h/ml}$, and 100 $\mu\text{g} \cdot \text{h/ml}$, respectively. Similarly, in the multiple-dose study, mean $C_{\max}$ and AUC values after the first doses at 600 and 1,000 mg were nearly identical (1.8 versus 1.9 $\mu\text{g/ml}$ and 31.6 versus 34.2 $\mu\text{g} \cdot \text{h/ml}$ for the 600- and 1,000-mg-dose groups, respectively).

In the 200- and 600-mg-dose groups examined in the multiple-dose study, the steady state was achieved after 5 to 6 days of dosing, and daily dosing for up to 7 days was associated with an approximate PA-824 accumulation ratio of 2. After 7 daily doses, the mean steady-state trough ($C_{\min}$), $C_{\max}$, and $\text{AUC}_{0-\tau}$ values for the 600-mg-dose group were 2.1 $\mu\text{g/ml}$, 3.8 $\mu\text{g/ml}$, and 70.4 $\mu\text{g} \cdot \text{h/ml}$, respectively. Since dosing in the 1,000-mg-dose group was halted on day 5 because of observed increases in serum creatinine, reliable steady-state data are not available for this dose level.

The $t_{1/2}$ of PA-824 ranged from 11 to 31 h among the 58 subjects. In study CL-001, the mean $t_{1/2}$ ranged from 13.5 to 20 h across dose groups. The mean $t_{1/2}$ for the CL-001 study population was approximately 18 h. In study CL-002, the mean $t_{1/2}$ after 7 days of dosing was 16.0 and 15.5 h in the 200- and 600-mg-dose groups, respectively. At both an individual and group mean level, $t_{1/2}$ values were not related to dose. These elimination kinetics data suggest that PA-824 can be administered once daily.

Safety and tolerability. PA-824 was well tolerated at all doses studied, with no serious adverse events occurring in study CL-001 or study CL-002. No systematic or dose group-

related effects on 2-lead cardiac profiles or 12-lead electrocardiogram parameters (e.g., heart rate, QT, and corrected QT) were noted. In addition, no effects were observed on vital signs, such as heart rate, blood pressure, temperature, or respiration. Overall, headache was the most common adverse event, followed by elevated serum creatinine levels, stomach discomfort (nausea, vomiting, flatulence, and/or diarrhea), and back pain. Generally, these adverse events were not noted or occurred at lower rates among placebo subjects.

PA-824 administration was associated with a reversible elevation in serum creatinine levels. The magnitude of creatinine change from predose values was correlated from subject to subject with the amount of drug exposure ($C_{\max}$ and AUC) experienced by the subjects. As drug levels declined after dosing was completed, creatinine levels returned to predose values. In the multiple-dose study, minimal to moderate elevations in serum creatinine were observed in the PA-824-treated subjects in the 200- and 600-mg-dose groups. No individual value exceeded 1.3 (200-mg-dose group) or 1.4 mg/dl (600-mg-dose group), and no absolute value or predosing-to-dosing period change was considered clinically significant. The study site's clinical laboratory normal range for serum creatinine was 0.8 to 1.3 mg/dl for males and 0.6 to 1.0 mg/dl for females. In the 1,000-mg-dose group (eight males and no females), by day 5 of dosing serum creatinine levels had risen in five of six PA-824 subjects by an average of 0.28 mg/dl relative to the baseline level; the highest recorded absolute value was 1.6 mg/dl. Several other individual serum creatinine values also were beyond the upper limit of the normal range. Consequently, dosing was stopped on day 5. All serum creatinine levels returned to clinically normal levels during the ensuing 7-day washout period in all subjects.

TABLE 2. Pharmacokinetic parameters for the multiple-dose study (CL-002)^a

Dose (mg) and day	$C_{\max}$ ($\mu\text{g/ml}$)	$T_{\max}$ (h)	$t_{1/2}$ (h)	AUC_{0-24} ($\mu\text{g} \cdot \text{h/ml}$)	$\text{AUC}_{0-\tau}$ ($\mu\text{g} \cdot \text{h/ml}$)	CL_F (liters/h)
200 ($n = 8$)						
Day 1	1.0 (0.3)	4.1 (2.0, 5.1)	18.9 (2.9)	15.6 (3.8)		7.7 (2.3)
Day 7	1.7 (0.3)	4.5 (2.0, 8.0)	16.0 (1.6)		30.2 (3.7)	6.7 (0.76)
600 ($n = 8$)						
Day 1	1.8 (0.4)	5.0 (3.0, 5.0)	23.4 (3.9)	31.6 (7.9)		9.9 (3.6)
Day 7	3.8 (0.8)	4.0 (3.0, 4.0)	15.5 (2.1)		70.4 (14.3)	8.8 (1.8)
1,000 ($n = 8$)						
Day 1	1.9 (0.9)	5.0 (3.0, 8.0)	21.2 (5.6)	34.2 (13.8)		16.6 (6.7)
Day 7	ND	ND	ND		ND	ND

^a Other values are arithmetic means, which standard deviations in parentheses. ND, not determined. No day 7 data are available for the 1,000 mg/day dose group, because dosing was halted after day 5. For $T_{\max}$, medians and ranges (minimum, maximum) are presented.

FIG. 3. Change in serum creatinine from the baseline compared to that at day 6 (C_{min}) for study CL-002.

Figure 3 shows the relationship in study CL-002 between day 6 trough (C_{min}) PA-824 levels and the corresponding predose to day 6 changes in creatinine levels for each PA-824 subject. Day 6 was approximately the steady state in this study. The majority of PA-824-dosed subjects with PA-824 concentrations higher than approximately 1,500 ng/ml demonstrated creatinine increases beyond the range seen in placebo subjects. Daily drug and creatinine measurements revealed that creatinine levels progressively rose as PA-824 accumulated during the 5- to 7-day treatment period and then declined in the postdose monitoring period (not shown). No consistent pattern of change was observed in blood urea nitrogen levels with treatment or across dose groups. Moreover, subjects with the greatest changes in blood urea nitrogen were not among those with the greatest changes in serum creatinine; the converse also was true.

DISCUSSION

Nonclinical studies of the efficacy of PA-824 indicate its potential for shortening the treatment of active TB and providing a novel drug for the treatment of multidrug-resistant and extensively drug-resistant TB. Single- and multiple-dose studies of PA-824 in healthy human subjects indicate that PA-824 is readily absorbed, bioavailable (subdose proportionally), and well tolerated.

Pharmacokinetic parameters for PA-824 demonstrate oral bioavailability and a half-life consistent with a once-per-day (or less frequent) dosing regimen. In single- and multiple-dose studies, the mean T_{max} across studies was 4 to 5 h and the $t_{1/2}$ averaged 16 to 20 h, with the steady state being reached at 5 to 6 days. Plasma PA-824 levels increased subdose proportionally with increasing doses up to 1,000 mg. Dose levels above 600 mg achieved minimal additional PA-824 absorption with respect to C_{max} and AUC. The reason(s) behind the subdose proportionality in pharmacokinetics remain to be elucidated definitively but could be due, for example, either to reduced dissolution at relatively high doses of this lipophilic compound or to the saturation of absorption mechanisms.

The PA-824 maximal blood levels observed in these studies after a single dose are approximately 6- to 200-fold higher than MICs determined in vitro for both drug-sensitive and drug-resistant strains of *M. tuberculosis*. These findings suggest that PA-824 tablets may demonstrate efficacy in vivo, although efficacy ultimately may be influenced by in vivo protein binding, which has been determined to be on the order of 95% in vitro (data not shown). The avidity of this binding, however, has not been determined.

In the two clinical studies reported here, no significant or serious adverse events were observed in the 58 subjects dosed with up to 1,000 mg PA-824 for up to 7 days (the multidosing was halted at 5 days at 1,000 mg due to increases in serum creatinine; see below). In general, the common adverse events detected to date can be monitored and managed easily and are not likely to preclude patient tolerance of PA-824 for the treatment of TB, should it ultimately be shown to be safe and effective in pivotal clinical trials. Furthermore, serum creatinine elevation, the one common adverse event that is not monitored as easily in the field, has been shown to be unrelated to human safety when directly examined in a renal effects study (2).

Overall, PA-824 was well tolerated following oral doses of up to 1,000 mg once daily for up to 5 days and up to 600 mg once daily for up to 7 days. Additionally, it demonstrated oral bioavailability and pharmacokinetic parameters consistent with a once-a-day regimen.

The results of these studies, combined with the activity PA-824 demonstrated in vitro against drug-sensitive and drug-resistant *M. tuberculosis* and in a mouse model against drug-sensitive *M. tuberculosis*, support the further investigation of this novel compound for the treatment of TB. Additional clinical trials planned for PA-824's clinical development program include drug-drug interaction studies with other anti-TB drugs and antiretroviral agents and a suite of efficacy studies of TB patients, including a proof-of-concept and dose-finding study to assess the extended early bactericidal activity of PA-824 in TB patients. Further studies of the mouse model of TB are planned to explore the activity of PA-824 when it is combined with other current and investigational drugs in novel regimens.

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