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PA-824 Exhibits Time-Dependent Activity in a Murine Model of Tuberculosis▽

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PA-824 is one of two nitroimidazoles in phase II clinical trials to treat tuberculosis. In mice, it has dose-dependent early bactericidal and sterilizing activity. In humans with tuberculosis, PA-824 demonstrated early bactericidal activity (EBA) at doses ranging from 200 to 1,200 mg per day, but no dose-response effect was observed. To better understand the relationship between drug exposure and effect, we performed a dose fractionation study in mice. Dose-ranging pharmacokinetic data were used to simulate drug exposure profiles. Beginning 2 weeks after aerosol infection with *Mycobacterium tuberculosis*, total PA-824 doses from 144 to 4,608 mg/kg were administered as 3, 4, 8, 12, 24, or 48 divided doses over 24 days. Lung CFU counts after treatment were strongly correlated with the free drug $T_{>MIC}$ ($R^2 = 0.87$) and correlated with the free drug AUC/MIC ($R^2 = 0.60$), but not with the free drug C_{max}/MIC ($R^2 = 0.17$), where $T_{>MIC}$ is the cumulative percentage of the dosing interval that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions and AUC is the area under the concentration-time curve. When the data set was limited to regimens with dosing intervals of ≤72 h, both the $T_{>MIC}$ and the AUC/MIC values fit the data well. Free drug $T_{>MIC}$ of 22, 48, and 77% were associated with bacteriostasis, a 1-log kill, and a 1.59-log kill (or 80% of the maximum observed effect), respectively. Human pharmacodynamic simulations based on phase I data predict 200 mg/day produces free drug $T_{>MIC}$ values near the target for maximal observed bactericidal effect. The results support the recently demonstrated an EBA of 200 mg/day and the lack of a dose-response between 200 and 1,200 mg/day. $T_{>MIC}$, in conjunction with AUC/MIC, is the parameter on which dose optimization of PA-824 should be based.

The development of new chemotherapeutic regimens capable of shortening the duration of tuberculosis (TB) treatment and/or improving the treatment of multidrug-resistant (MDR) TB is a major research objective (2). PA-824 is one of two novel bicyclic nitroimidazoles in phase II clinical trials for TB treatment, the other being OPC-67683 (20). PA-824 has potent *in vitro* activity against *Mycobacterium tuberculosis*, as evidenced by an MIC range of 0.015 to 0.25 μg/ml, and retains this activity against isolates resistant to a variety of commonly used anti-TB drugs (30). PA-824 is bactericidal against actively replicating bacilli, as well as nonreplicating bacilli under hypoxic or prolonged culture conditions (12, 17, 30). However, the mechanism(s) by which it exerts its bactericidal effect have yet to be fully defined. Two potential mechanisms of action have been elucidated thus far. PA-824 inhibits synthesis of ketomycolates, an essential component of the mycobacterial cell wall (19, 30). By analogy with other cell wall synthesis inhibitors, including isoniazid and ethionamide, this mechanism may explain bactericidal activity against actively multiplying bacilli but seems unlikely to explain activity against nonreplicating bacilli. The activity of PA-824 against slowly or nonreplicating bacilli was recently attributed to its capacity to donate nitric oxide during enzymatic nitroreduction within the tubercle bacillus and thereby poison the respiratory apparatus (19, 29).

In murine models of TB, PA-824 has shown substantial dose-dependent bactericidal activity during both the initial phase of treatment and the continuation phase after 2 months of treatment with isoniazid, rifampin, and pyrazinamide (17, 32). When PA-824 is administered alone as single daily doses, the minimal effective dose necessary for bacteriostatic activity is 12.5 mg/kg, and the minimal bactericidal dose (MBD) is 100 mg/kg (32). When dosed daily at the MBD in combination with moxifloxacin and pyrazinamide, PA-824 contributes a sterilizing effect that is at least as strong as that of rifampin (24). Furthermore, replacement of isoniazid with PA-824 in the standard first-line regimen of isoniazid, rifampin, and pyrazinamide results in more rapid culture conversion (23, 31). These results in mice suggest PA-824 may have the potential to shorten the duration of treatment for drug-susceptible, as well as MDR, TB in humans. However, it became apparent during phase I testing that human dosing with the existing formulation would not attain the maximal serum concentration (C_{max}) or area under the serum concentration-time curve (AUC) observed in mice receiving 100 mg/kg, or even 50 mg/kg, once daily. Among healthy subjects receiving escalating daily doses of PA-824, near-maximal absorption was observed at the 600-mg dose, where the mean steady-state C_{max} and $AUC_{0-\tau}$ were 3.8 μg/ml and 70.4 μg · h/ml, respectively, compared to values of 21.4 μg/ml and 327.6 μg · h/ml in mice receiving 100 mg/kg/day (8, 23).

To better understand the relationship between drug expo-

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sure and effect, we undertook a dose fractionation study to determine the pharmacodynamic parameter most closely correlated with the anti-TB activity of PA-824 in mice and the magnitude of that parameter associated with bacteriostatic and bactericidal effects. In the meantime, the results of a dose-ranging, extended early bactericidal activity (EBA) trial in patients with smear-positive pulmonary TB have been reported (5). In subjects receiving PA-824 once daily at doses of 200, 600, 1,000, and 1,200 mg for 14 consecutive days, substantial reduction of sputum CFU counts at a rate of ~0.1 $\log_{10}$ CFU/ml/day was observed at all doses tested (5). In contrast to the dose-dependent activity of PA-824 observed in mice (32), no dose-response was observed in the EBA trial (5). The present pharmacodynamic study provides evidence that the favorable EBA of PA-824 and the lack of a dose-response effect in the EBA trial, when it may have been expected from the preceding experiments in mice, are due to the time-dependent nature of the relationship between drug exposure and its anti-TB effect.

MATERIALS AND METHODS

Bacterial strain. *M. tuberculosis* H37Rv was passaged in mice and frozen in aliquots at −80°C. After thawing, an aliquot was subcultured in Middlebrook 7H9 broth (Fisher, Pittsburgh, PA) with 10% oleic acid-albumin-dextrose-catalase (OADC; Difco, Detroit, MI) and 0.1% Tween 80 (Sigma, St. Louis, MO).

Antimicrobials. PA-824 was provided by the Global Alliance for TB Drug Development through RTI International (Research Triangle Park, NC). For administration to mice, PA-824 was suspended in a cyclodextrin micelle formulation (CM-2) as previously described (32). A 50-mg/ml stock suspension was prepared and stored at 4°C. Aliquots were diluted with distilled water weekly to give the desired concentrations for dosing suspensions. Suspensions were shaken between doses to ensure uniform dosing.

Determination of the MIC. The MIC was determined by the agar proportion method (33). Middlebrook 7H11 agar supplemented with 10% OADC and containing serial 2-fold concentrations of PA-824 ranging from 0.007 to 2.0 μg/ml were inoculated with 0.5 ml of serial 100-fold dilutions of a log-phase broth culture of *M. tuberculosis* H37Rv with an optical density at 600 nm corresponding to ~10^8 CFU/ml. Drug-free and isoniazid-containing plates served as negative and positive controls, respectively. CFU were counted after 21 days incubation at 37°C with 5% ambient CO_2. The MIC was defined as the lowest concentration at which the CFU count on drug-containing plates was <1% of the CFU count on drug-free plates.

Dose-ranging PK of PA-824. All procedures involving animals were approved by the institutional animal care and use committee. Single-dose pharmacokinetics (PK) of PA-824 in serum were evaluated in uninfected 6-week-old female BALB/c mice (Charles River, Wilmington, MA) after oral administration of 3, 10, 18, 30, 54, 96, 162, 243, 486, 729, and 1,458 mg/kg doses. Multidose PK were also determined for 6-, 9.6-, 28.8-, 96-, and 192-mg/kg doses administered once daily for 5 days, with serum sampling performed after the fifth dose. In a second multidose PK study, mice received PA-824 at 192 mg/kg every 6 days, with serum sampling performed after the third dose. Mice had access to food and water *ad libitum*. PA-824 was administered by esophageal gavage. Three mice from each group were sacrificed at 0.5, 1, 2, 4, 8, 16, 24, 36, 48, 72, 96, and 120 h after the last dose. Mice were anesthetized with isoflurane and exsanguinated by cardiac puncture. Blood was collected in microcentrifuge tubes and left at room temperature for 30 min before being centrifuged to harvest the serum. Serum samples were frozen at −80°C before the concentration of PA-824 was determined by a validated high-pressure liquid chromatography (HPLC) method (23). Briefly, the concentration of PA-824 was determined with a system consisting of a ThermoFinnegan P4000 HPLC pump (San Jose, CA) with a model AS1000 fixed-volume autosampler, a model UV2000 UV detector, a Gateway series e computer (Poway, CA), and the Chromquest HPLC data management system. The plasma standard concentration curve for PA-824 ranged from 0.20 to 50 μg/ml. The absolute recovery of PA-824 from plasma was 88.2%. The overall precision of the validation assay across all standards was 0.67 to 5.38%.

Serum concentration data were entered into a WinNonlin worksheet (WinNonlin version 4.0, 2002; Pharsight, Mountain View, CA) and analyzed by using standard noncompartmental and compartmental techniques in order to determine the relevant PK parameters for simulations. The serum concentration-time profile of each dosing regimen described in the dose fractionation protocol (Table 1) was modeled over a 6-day period to estimate the Cmax, the AUC_{0-144}, and the $T_{>MIC(0-144\ h)}$ for each regimen for free PA-824 concentrations, assuming 92.5% serum protein binding based on 93% protein binding in serum obtained from mice at the T_{max} after a 25-mg/kg dose and up to 90% protein binding at a concentration of 3.6 μg/ml in spiked mouse serum, as determined by equilibrium dialysis (TB Alliance, data on file). The AUC_{0-24} was calculated by dividing the AUC_{0-144} by 6.

TABLE 1. Dose fractionation scheme indicating the individual dose amount and the number of doses administered to achieve the desired total dosage over 24 days

No. of doses in 24 days	Dose amt (mg/kg) used to achieve a total dosage in mg/kg over 24 days of:										
	0	144	288	576	768	864	1,152	1,536	1,728	2,304	4,608
48	0		6	12			24			48	96
24			12	24		36	48			96	192
12		12	24	48		72	96		144	192	
8			36	72			144	192		288	
4			72	144	192		288	384			
3				192	256						

Dose fractionation study. Six-week-old female BALB/c mice were infected by the aerosol route in the Inhalation Exposure System (Glas-Col, Inc., Terre Haute, IN) using a log-phase broth culture (optical density at 600 nm of ~0.8) with the expectation of implanting 3.5 $\log_{10}$ CFU of *M. tuberculosis* H37Rv. Five mice were sacrificed the following day (day −13) to determine the number of CFU implanted in the lungs. Fourteen days after infection (day 0), five additional mice were sacrificed to determine the bacterial load at the start of treatment. The remaining animals were randomly distributed (three mice each) into 31 treatment groups. Treatment was administered by gavage for 24 consecutive days. During this time period, a total dosage of 0, 144, 288, 576, 768, 864, 1,152, 1,536, 1,728, 2,304, or 4,608 mg/kg was administered as 3, 4, 8, 12, 24, or 48 equally divided doses, as indicated in Table 1. With the exception of 384 mg/kg administered every 6 days, individual doses did not exceed 288 mg/kg since the serum AUC did not increase in a dose-proportional fashion between 243 and ≥486 mg/kg. Mice were sacrificed 4 days after the final day of dosing to reduce the potential for drug carryover. Quantitative cultures were performed from lung homogenates using OADC-enriched 7H11 agar medium with selective antibiotics, as previously described (1, 23). Lung CFU counts (x) were log transformed as $\log_{10}(x+1)$ before analysis.

PD analyses. The data were analyzed with WinNonlin, Excel, and JMP statistical software (v.7.0.2; SAS Institute, Cary, NC). The data were corrected for a range of potential protein binding values (85 to 95%), and the resultant free drug concentrations were used to calculate pharmacodynamic (PD) parameters over 144 h (6 days). The relationships of these parameters to a range of potential MIC values (0.03125 to 0.25 μg/ml) (30) were calculated. A series of exploratory models were analyzed in JMP statistical software. Finally, data were analyzed with four inhibitory E_{max} and inhibitory sigmoid E_{max} models (103, 104, 107, and 108) in WinNonLin, where E_{max} is the maximum CFU count observed in the absence of drug (unabated growth). Model fit to the data was assessed using visual inspection of the y by x plots and by evaluating the variability (i.e., the percent coefficient of variation) in the parameter estimates, correlation analysis, and Akaike information criterion (AIC).

Human PK/PD simulations. Human phase I PK data were analyzed with noncompartmental methods and with a one-compartment open model with first order elimination, as well as a two-compartment model. On the basis of the AIC, the one-compartmental model resulted in a better fit of the data for each patient, as was the case in mice. Therefore, a one-compartment PK model was used to estimate the median PK parameter values and simulate the total and free PA-824 (assuming 5, 7, 10, or 15% unbound drug) concentrations at steady state for doses of 100, 200, and 400 mg of PA-824 daily. The free drug estimates were compared to MIC values ranging from 0.03125 to 0.25 μg/ml (30).

RESULTS

Determination of MIC. The MIC of PA-824 against *M. tuberculosis* H37Rv was 0.06 μg/ml.

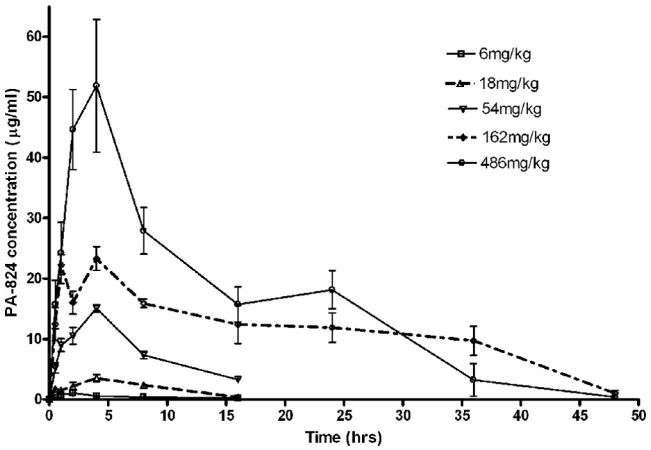

FIG. 1. Serum profile of PA-824 in mice after oral administration of selected doses.

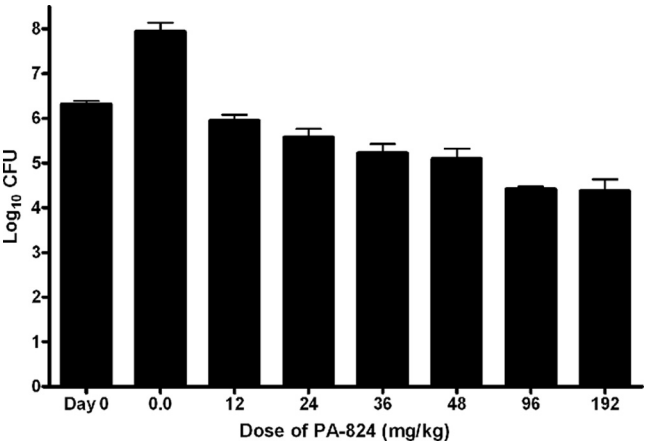

FIG. 2. Dose-dependent bactericidal effect of PA-824 administered once daily.

Dose-ranging PK of PA-824 in mice. Single doses of PA-824 up to 1,456 mg/kg were well tolerated, with no adverse effects observed. Similarly, no untoward effect was observed in the multidose PK studies. The time to reach C_{max} (T_{max}) in serum was 4.0 h. The elimination half-life was 4 to 6 h. PA-824 concentrations increased in a dose-proportional fashion over the dose range from 10 to 243 mg/kg (Fig. 1). At doses of >486 mg/kg, the serum concentration-time profile suggested more complex PK behavior, possibly due to saturation of oral absorption. Also, late secondary and tertiary peaks at 24 and 48 h suggested precipitation and subsequent redissolution of PA-824 in the gastrointestinal tract, with diurnal variation. Hence, with the exception of 384 mg/kg administered every 6 days, individual dosing regimens in the dose fractionation study did not exceed 288 mg/kg. This dose range easily encompasses the achievable range of serum concentrations in humans with current oral formulations (8).

Dose-response results. The mean lung CFU count on day −13 was 3.34 ± 0.14 $\log_{10}$. At the start of the treatment on day 0, the mean lung CFU count was 6.30 ± 0.10 $\log_{10}$. The final mean lung CFU count in untreated mice was 7.94 ± 0.19 $\log_{10}$. Dose-dependent activity was observed with single daily doses ranging from 12.5 to 96 mg/kg (Fig. 2). As observed in a previous experiment in which PA-824 was administered 5 days per week, the 12.5-mg/kg daily dose was associated with bacteriostasis, whereas 96 mg/kg was associated with a nearly 2-$\log_{10}$ reduction in CFU counts compared to the day 0 count. No further significant reduction in CFU counts was observed with the dose increase from 96 to 192 mg/kg once daily.

Dose fractionation results. The maximum observed effect, a CFU reduction of 2.4 $\log_{10}$ (or 0.1 $\log_{10}$ CFU/day), was observed with twice daily administration of 96 mg/kg. Figure 3 shows the relationships between the $\log_{10}$ CFU per lung at the end of treatment and the three primary descriptive PD parameters, assuming 7.5% free drug. Several models were tested, and we selected model 108, an inhibitory effect sigmoid E_{max} model with a nonzero minimum for the lowest achievable $\log_{10}$ CFU count. When modeled over a 6-day period, free drug $T_{>MIC}$ showed the best fit of the data (R^2 = 0.87), where $T_{>MIC}$ is the cumulative percentage of the dosing interval that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions. A modest correlation was observed for *f*AUC/MIC (R^2 = 0.60) and fC_{max}/MIC showed little correlation (R^2 = 0.17), where *f*AUC is the area under the concentration-time curve for the free, unbound fraction of a drug. The model 108 parameter estimates were as folows: E_{max}, 7.94 $\log_{10}$ CFU; 50% effective concentration (EC_{50}), 41.08% $T_{>MIC}$; E_0, 3.06 $\log_{10}$ CFU; and gamma, 1.06. Because it is an inhibitory model, E_{max} is the maximum CFU count observed in the absence of drug (unabated growth), whereas E_0 is the model-predicted lowest achievable value for $\log_{10}$ CFU count at the end of treatment. It should be noted that the model assumes that *x* is a continuous variable that could extend to infinity whereas, in this instance, $T_{>MIC}$ is a finite value capped at 100%. This limitation is not encountered with C_{max}/MIC or AUC/MIC. The EC_{50} (41.08%) is the $T_{>MIC}$ value associated with 50% of the maximum predicted effect, and gamma is the sigmoidicity factor describing the steepness of the curve.

At $T_{>MIC}$ of 100%, the model predicted 4.43 $\log_{10}$ CFU remaining, which is 1.87 $\log_{10}$ lower than the CFU count at the start of treatment (i.e., 6.30) and 3.51 $\log_{10}$ lower than the CFU count in untreated mice (i.e., 7.94). The model predicted that 22% $T_{>MIC}$ is required for a bacteriostatic effect, 48% $T_{>MIC}$ is required for a 1-$\log_{10}$ kill (compared to the CFU count at the start of treatment), and 77% $T_{>MIC}$ is required for a 1.59-$\log_{10}$ kill (representing 80% of the maximum observed effect), respectively.

Within each dosing frequency, higher values for all three parameters were associated with greater kill. All regimens dosed every 144 h resulted in an increase in CFU counts, or in one case, bacteriostasis. When the regimens dosed every 144 h and every 72 h were successively removed from the model, the model fit of the parameters converged, and *f*AUC/MIC and $T_{>MIC}$ fit the data equally well: (i) for 12 h to 72 h, $T_{>MIC}$ (R^2 = 0.81), *f*AUC/MIC (R^2 = 0.76), and fC_{max}/MIC (R^2 = 0.40), and for 12 h to 48 h, $T_{>MIC}$ (R^2 = 0.82); *f*AUC/MIC (R^2 = 0.83); fC_{max}/MIC (R^2 = 0.58).

All regimens resulting in ≥2-$\log_{10}$ reductions in CFU counts had $fAUC_{0\text{-}144}$/MIC values greater than 1,000, corresponding to average $fAUC_{0\text{-}24}$/MIC values greater than 167.

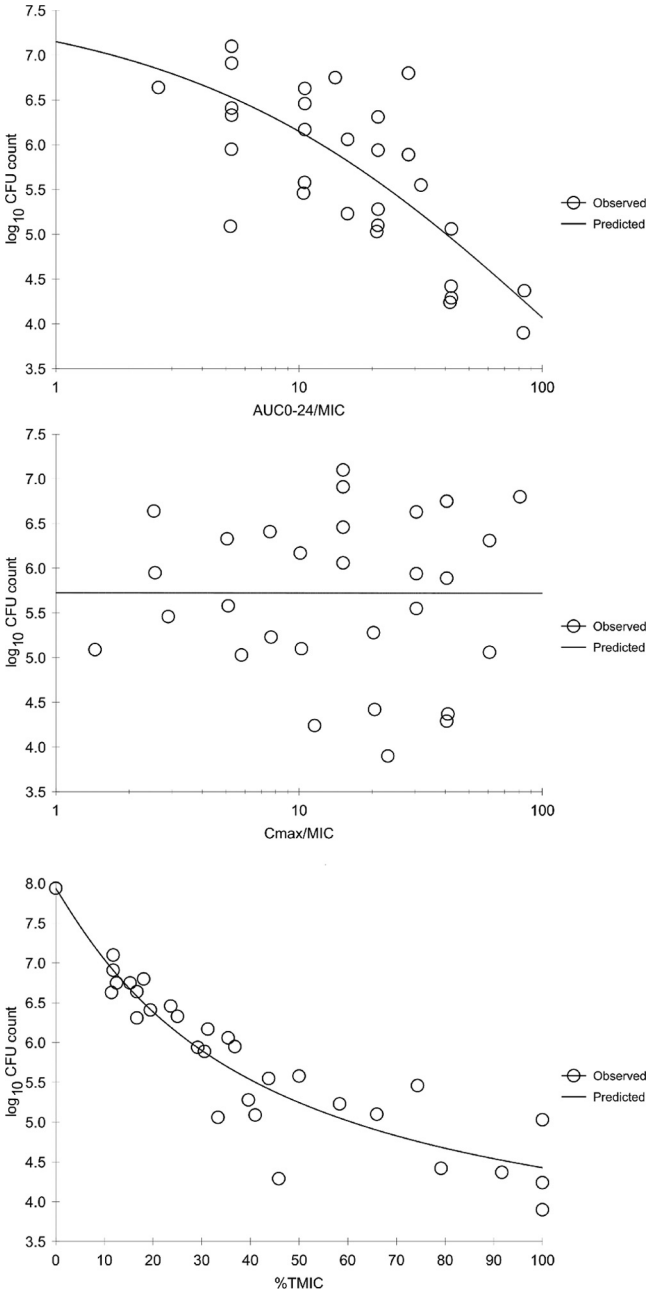

FIG. 3. Correlation of PA-824 activity with primary descriptive PD parameters.

Human pharmacodynamic simulations. PA-824 displayed favorable $T_{>MIC}$ values with doses as low as 100 mg once daily, depending on the assumptions for free drug and MIC (Table 2). $T_{>MIC}$ values predictive of a bactericidal effect (i.e., ≥1-log reduction in CFU counts) were achieved with a 100-mg dose when the MIC was 0.03125 μg/ml and were achieved when the MIC was 0.0625 μg/ml as long as the percentage of free drug was ≥10%. A 200-mg dose produced better results, with bactericidal effects predicted against isolates for which the MIC was <0.1 μg/ml (as in the recent EBA study [5]), regardless of the free drug fraction, including 100% $T_{>MIC}$ observed with MIC values of 0.03125 μg/ml and with an MIC of 0.0625 μg/ml provided the percentage of free drug was ≥10%. A 400-mg dose reached 100% $T_{>MIC}$ with MIC values of <0.1 μg/ml at any level of protein binding assessed.

DISCUSSION

PD analyses have played an increasingly important role in the development of new anti-infective drugs (3, 4, 22, 25). Ideally, elucidation of the PD parameter most closely linked to a drug's antimicrobial effect and the magnitude of that parameter necessary for a given therapeutic effect enables the design of dosing regimens that optimize the odds of success without exposing patients to unnecessary toxicity (4, 22). Animal models facilitate PD studies because drug exposures may incorporate a large range of drug exposures which are not suitable for clinical use (3). Provided the animal model is relevant to the clinical condition, the results of PD analyses in animal models should inform the design and analysis of clinical trials, as well as clinical practice (3, 4, 11, 13, 22, 27). Moreover, because the PD drivers are likely to be similar among members of a particular drug class, the information gained from the study of a single compound may be extrapolated to other compounds in development.

Since virtually all anti-TB drugs in regular clinical use were developed more than 40 years ago, there are few examples on which to assess the potential of PD studies in murine models to inform the clinical experience. However, the results with the two principal first-line agents, isoniazid and rifampin, have been promising. Jayaram et al. established AUC/MIC as the parameter most predictive of isoniazid's bactericidal effect (15). A subsequent analysis of isoniazid PD demonstrated that the AUC/MIC value associated with 50% of the maximal effect was remarkably similar between the murine model, an *in vitro* hollow fiber model, and human EBA studies (7, 10). Moreover, the EBA of isoniazid has been shown to plateau as the dose exceeds 300 mg in slow acetylators of the drug, for whom the

TABLE 2. Simulated human $T_{>MIC}$ values based on healthy volunteer PK data and various free drug and MIC assumptions

MIC (μg/ml)	PA-824 (% unbound drug)	$\%T_{>MIC}$ at:		
		100 mg	200 mg	400 mg
0.03125	5	46	100	100
	7	67	100	100
	10	100	100	100
	15	100	100	100
0.06250	5	0	46	100
	7	0	67	100
	10	46	100	100
	15	67	100	100
0.12500	5	0	0	46
	7	0	4	67
	10	0	46	100
	15	6	67	100
0.25000	5	0	0	0
	7	0	0	4
	10	0	0	46
	15	0	6	67

AUC is expected to be approximately 20 to 25 μg · h/ml, whereas doses greater than 600 mg may be necessary for maximal effect in rapid acetylators for whom the AUC after the 300-mg dose is approximately 6 to 8 μg · h/ml (7, 27). Similarly, Jayaram et al. found the AUC necessary for maximal isoniazid effect in mice to be 25 μg · h/ml (15). Thus, results in the murine model correlate well with isoniazid's effect in humans and define the exposure above which no additional therapeutic benefit is observed. Jayaram et al. also established AUC/MIC as the principal PD correlate of rifampin's bactericidal activity in mice (14). Unlike the findings with isoniazid, the findings with rifampin clearly demonstrated that the currently recommended 600-mg human dose of rifampin produces an AUC that is at the low end of a steep dose-response curve and that increased rifampin doses beyond 600 mg should result in increased activity (14). This observation is affirmed by results of EBA trials (6, 16) and provides support for exploring the efficacy, safety, and tolerability of higher rifamycin exposures (26, 28).

In the present study, we performed a dose fractionation study in the murine model to describe the PD of PA-824, a promising new agent in phase II clinical trials for TB treatment. The results define free drug $T_{>MIC}$ as the parameter that correlates best with bactericidal activity and establish target values for bacteriostatic, bactericidal, and near-maximal bactericidal effects. The findings that (i) the regimens associated with the greatest reductions in $\log_{10}$ CFU counts also had $f\text{AUC}_{0\text{-}144}$/MIC values greater than 1,000 (or $f\text{AUC}_{0\text{-}24}$/MIC values greater than 167) and (ii) the explanatory power of AUC/MIC was equal to that of $T_{>MIC}$ when the analysis was limited to regimens dosed every 48 h or less suggests that the magnitude with which free drug concentrations exceed the MIC also influences the bactericidal effect.

Importantly, the $T_{>MIC}$ target necessary for near-maximal bactericidal effects appears readily achievable in humans with daily PA-824 doses which have been safe and well tolerated in phase I studies (8, 9). This conclusion is strengthened by the results of a recent dose-ranging EBA trial in which PA-824 was found to have a substantial EBA of roughly 0.1 $\log_{10}$ CFU/ml of sputum per day, but no difference in EBA was observed between the 200-, 600-, 1,000-, and 1,200-mg dose groups (5). Although this finding may have been unexpected due to the dose-dependent nature of PA-824 with single daily doses in mice, the simplest explanation is that, due to the longer half-life of PA-824 in humans, the $T_{>MIC}$ target for near-maximal bactericidal effects was approached or attained in all human dose groups, whereas similar target attainment was not achieved in mice when single daily doses had similar AUC values but lower $T_{>MIC}$ values. Our simulations based on existing phase I PK data and MIC values from the EBA study suggest that this was indeed the case, since the $T_{>MIC}$ for the 200-mg dose is predicted to approach or attain the $T_{>MIC}$ target for near-maximal effect even if the free-drug fraction is assumed to be as low as 7% and to have substantial bactericidal effect even if the free drug fraction is as low as 5%. Interestingly, the average daily log CFU/ml reduction in the sputum of subjects in the EBA trial was 0.1, which is identical to the maximum observed effect in mice.

If the clinical results continue to follow the results in the murine model, our simulations suggest that a more typical dose-response relationship will be observed in an ongoing PA-824 EBA trial evaluating 50-, 100-, 150-, and 200-mg doses (A. Ginsberg, unpublished data). For example, lowering the dose from 200 to 100 mg may reduce the bactericidal effect, since 100 mg did not produce a meaningful $T_{>MIC}$ when the simulations were performed with our baseline assumptions of 5% free drug and MIC of 0.0625 μg/ml (Table 2). However, this analysis is very sensitive to the protein binding assumption. When the protein binding was assumed to be ≤90%, this dose produced a bactericidal $T_{>MIC}$ with MIC values of ≤0.0625 μg/ml. Using the same set of assumptions, the 50-mg dose would not be expected to have significant bactericidal activity. Therefore, bacteriostatic or submaximal bactericidal effects may be expected at or below 100 mg. Regardless of its outcome, the above described, "low-dose" EBA trial provides an opportunity to reevaluate the correspondence between results in the murine model and those in human subjects.

The results and discussion presented here must be interpreted with several caveats in mind. First, our study assessed the PD of PA-824 against an acute infection in mice. As evidenced by a >1.5-$\log_{10}$ increase in CFU counts among untreated mice over the duration of the treatment phase, the predominant bacterial population was in the logarithmic growth phase at the initiation of treatment. Thus, while the results and conclusions of the present study may correspond well with the outcomes of an EBA study, they may not apply to the activity of PA-824 against nonreplicating or slowly replicating bacterial subpopulations. Additional studies in animal models enriched for such "persisters" are under way to determine whether a similar PD relationship holds for bactericidal effects against these subpopulations. This question is of particular interest for PA-824 because at least two different mechanisms of action have been described (19, 29, 30). For example, it is tempting to hypothesize that the time-dependent killing of actively multiplying bacilli observed in the present study results from inhibition of mycolic acid synthesis by PA-824 but that another mechanism, such as respiratory toxicity caused by reactive nitrogen intermediates, may drive the bactericidal activity against nonreplicating or slowly replicating bacilli according to a different PD relationship.

A second limitation of the present study relates to challenges inherent in estimating the impact of protein binding on the PD of highly bound drugs for which small changes in the estimate of the protein-bound fraction results in large changes in the free, biologically active fraction. Estimates of the $T_{>MIC}$ are particularly sensitive to such changes. Moreover, applying a single constant protein binding value across the range of drug concentrations and *in vivo* conditions, as we have done here, is certain to be overly simplistic. Other unmeasured factors, such as the kinetics of drug-protein interactions and the proportion of free drug in various tissues and the intracellular environment, may also contribute to the impact of protein binding in ways that cannot be modeled effectively. We have relied on plasma protein binding data from mice and from humans and presented analyses with differing protein binding constants in order to clearly represent the sensitivity of the model to changes in protein binding assumptions.

Our human PD simulations were based on data from healthy volunteers and not the subjects of the EBA trial. Although the

MIC values were taken from the EBA trial, the precise MICs of PA-824 are unknown, except that all were <0.1 μg/ml. Further, debate continues regarding the most relevant measure of *in vitro* activity to include in PD analyses. MIC is the traditional measure, but has many limitations. Time-kill data may be preferred (21). Moreover, our simulations were deterministic in nature and did not specifically account for intersubject variation in the PK of PA-824 or the susceptibility of different bacterial isolates to PA-824. Future evaluations may include population PK data from TB patients and probabilistic PD models to further define the optimal doses of PA-824 for future clinical studies.

Finally, we used a single *M. tuberculosis* strain in the present study. MacGowan et al. recently presented evidence that, among *Staphylococcus aureus* isolates exposed to moxifloxacin *in vitro*, the magnitude of the AUC/MIC associated with a targeted antimicrobial effect varies significantly and may affect target attainment rates and breakpoint selection (18). Since *M. tuberculosis* is more highly conserved genetically than *S. aureus*, the variability may not be as significant. However, this issue warrants consideration in future studies.

In conclusion, PA-824 exhibited time-dependent bactericidal activity in a murine model of active TB with a maximal observed bactericidal effect of 0.1 log CFU/day over 24 days. *f*AUC/MIC also was a useful measure of PA-824 activity. This analysis supports, and is supported by, the findings of a recent dose-ranging EBA study in humans in which PA-824 resulted in a 0.1 log CFU/ml/day reduction in sputum over 14 days, with no dose-response relationship observed between 200 and 1200 mg, a range over which our PD simulations predict all dose groups would have approached or met the target $T_{>\mathrm{MIC}}$ for maximal observed bactericidal effect. A dose-response effect and reduction in bactericidal activity is predicted for lower doses, beginning at 50 to 100 mg, depending on the protein binding assumption. If confirmed in the ongoing low-dose EBA trial, these results indicate that $T_{>\mathrm{MIC}}$, in conjunction with *f*AUC/MIC, is the parameter on which dose optimization of PA-824 should be based. Similar time-dependent PD are likely to be observed for OPC-67683 and other nitroimidazole derivatives with similar mechanisms of action. PD studies should be performed for new anti-TB drugs in development to aid compound and dose selection for preclinical studies and dose selection for clinical trials.

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