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Phase I Safety, Pharmacokinetics, and Pharmacogenetics Study of the Antituberculosis Drug PA-824 with Concomitant Lopinavir-Ritonavir, Efavirenz, or Rifampin

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There is an urgent need for new antituberculosis (anti-TB) drugs, including agents that are safe and effective with concomitant antiretrovirals (ARV) and first-line TB drugs. PA-824 is a novel antituberculosis nitroimidazole in late-phase clinical development. Cytochrome P450 (CYP) 3A, which can be induced or inhibited by ARV and antituberculosis drugs, is a minor (~20%) metabolic pathway for PA-824. In a phase I clinical trial, we characterized interactions between PA-824 and efavirenz (arm 1), lopinavir/ritonavir (arm 2), and rifampin (arm 3) in healthy, HIV-uninfected volunteers without TB disease. Participants in arms 1 and 2 were randomized to receive drugs via sequence 1 (PA-824 alone, washout, ARV, and ARV plus PA-824) or sequence 2 (ARV, ARV with PA-824, washout, and PA-824 alone). In arm 3, participants received PA-824 and then rifampin and then both. Pharmacokinetic sampling occurred at the end of each dosing period. Fifty-two individuals participated. Compared to PA-824 alone, plasma PA-824 values (based on geometric mean ratios) for maximum concentration (C_{max}), area under the concentration-time curve from 0 to 24 h (AUC_{0-24}), and trough concentration (C_{min}) were reduced 28%, 35%, and 46% with efavirenz, 13%, 17%, and 21% with lopinavir-ritonavir (lopinavir/r) and 53%, 66%, and 85% with rifampin, respectively. Medications were well tolerated. In conclusion, lopinavir/r had minimal effect on PA-824 exposures, supporting PA-824 use with lopinavir/r without dose adjustment. PA-824 exposures, though, were reduced more than expected when given with efavirenz or rifampin. The clinical implications of these reductions will depend upon data from current clinical trials defining PA-824 concentration-effect relationships. (This study has been registered at ClinicalTrials.gov under registration no. NCT01571414.)

In 2012 there were 8.6 million cases of tuberculosis (TB) and 1.3 million tuberculosis-related deaths (1). Short-course treatment of drug-sensitive tuberculosis requires 6 months of therapy. Multidrug-resistant (MDR) tuberculosis (i.e., resistant to isoniazid and rifampin) is a growing public health threat, with therapeutic options limited by drug availability, acceptability, and efficacy (2). Current MDR-tuberculosis treatment requires ≥18 months of multidrug therapy with at least 6 months of an injectable agent (3), is poorly tolerated, and is successful in only 48% of patients (2). Almost one-third of tuberculosis-related deaths globally are in patients with HIV coinfection (1). There is an urgent need for novel antituberculosis regimens to shorten treatment duration for drug-sensitive tuberculosis and to improve the efficacy and safety for MDR tuberculosis. The utility of novel drugs will be significantly enhanced if they are safe and effective among patients who require anti-HIV therapy.

The investigational nitroimidazole PA-824 has potent *in vitro* activity against *Mycobacterium tuberculosis* and no cross-resistance with marketed antituberculosis drugs (4, 5). Its activity against metabolically active and nonreplicating *M. tuberculosis* (5) suggests likely bactericidal and sterilizing activity. The latter is critical for treatment shortening. In mouse models of tuberculosis, PA-824 given with rifampin and pyrazinamide reduced curative treatment duration from 6 months to 4 months (6). In mice, PA-824 with moxifloxacin and pyrazinamide was similarly potent; in humans, this same three-drug combination reduced sputum mycobacterial colony counts more effectively than standard treatment over 2 weeks' time when PA-824 was given at a dose of 200 mg once daily (7, 8). A phase 2B trial of PA-824 over 8 weeks at doses of 100 mg and 200 mg with moxifloxacin and pyrazinamide finished recently (results pending). PA-824 has not been tested clinically in a combination with rifampin plus pyrazinamide, the key sterilizing drugs in antituberculosis therapy. It is not known whether PA-824 can reduce treatment duration when it is given with first-line or second-line antituberculosis drugs.

To include PA-824 in antituberculosis regimens in HIV-infected patients, the safety and pharmacokinetics (PK) of drug combinations must be assessed. PA-824 is extensively metabolized via a combination of reductive metabolism and oxidative metabolism with no one single metabolic path that can be considered major. *In vitro* studies suggest that cytochrome P450 (CYP) 3A

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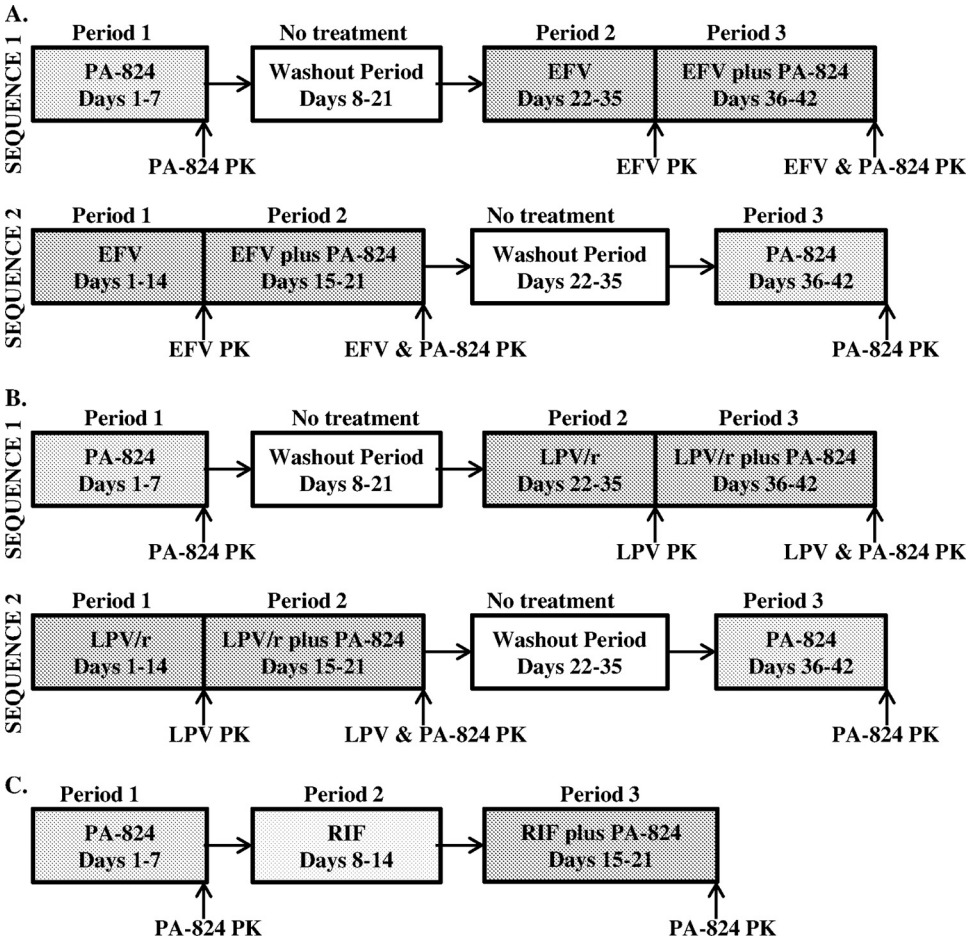

FIG 1 Schematic of the dosing regimen and pharmacokinetic sample collection: arm 1, PA-824 with efavirenz (EFV) (A); arm 2, PA-824 with lopinavir/ritonavir (LPV/r) (B); arm 3, PA-824 with rifampin (RIF) (C).

contributes up to 20% to overall metabolism; PA-824 is not a substrate of CYP2C9, -2C19, or -2D6 metabolizing enzymes (Stephen Murray, TB Alliance, personal communication). Rifampin induces many metabolizing enzymes including CYP3A (9). Efavirenz (EFV) is included in first-line regimens for HIV in many settings, and lopinavir (LPV; with ritonavir, lopinavir/r) is the most widely prescribed HIV-1 protease inhibitor globally. Efavirenz induces CYP3A enzymes, while lopinavir and ritonavir can inhibit or induce CYP3A (10). In addition, the *CYP2B6* genotype is a key determinant of efavirenz concentrations (11–15), while *SLCO1B1* polymorphisms impact lopinavir exposures (16–18). In this phase I trial, we investigated the safety and PK interactions of PA-824 with efavirenz, lopinavir/r, and rifampin, taking into account pharmacogenetics.

MATERIALS AND METHODS

Study population. Healthy adults 18 to 65 years were recruited at AIDS Clinical Trials Group (ACTG) sites in the United States. Eligible participants had negative HIV and hepatitis C antibody tests, normal alanine aminotransferase (ALT) levels, and creatinine clearance values of >50 ml/min. Volunteers were excluded for hemoglobin of ≤12.0 g/dl (male) or ≤11.0 g/dl (female), absolute neutrophil count of <1,250 cells/mm^3, platelet counts of <125,000 cells/mm^3, electrocardiogram (ECG) with a corrected QT (QTc) of >450 or PR of >200 ms, or active tuberculosis. Frequent headaches was another exclusion criterion. The study was approved by institutional review boards of the participating sites. All participants provided written informed consent. ACTG study A5306 was registered at ClinicalTrials.gov under registration number NCT01571414.

Experimental protocol. (i) Study design of the phase I, open-label PK and safety study. PA-824 was dosed 200 mg once daily, efavirenz was given at 600 mg once daily, rifampin was dosed at 600 mg once daily, and lopinavir/r was given at 400/100 mg every 12 h. Efavirenz was taken in the evenings. PA-824 and rifampin were taken in the mornings. All medications were taken on an empty stomach. Participants were sequentially assigned to arm 1 (efavirenz), arm 2 (lopinavir/r), or arm 3 (rifampin) (Fig. 1). In arm 1, participants were randomized to sequence 1, consisting of PA-824 for 7 days, a 2-week washout period, efavirenz for 14 days, and then efavirenz with PA-824 for 7 days, or sequence 2, consisting of efavirenz for 14 days, efavirenz with PA-824 for 7 days, a 2-week washout period, and then PA-824 alone for 7 days. Arm 2 participants were randomized to two sequences as follows: sequence 1, consisting of PA-824 for 7 days, a 2-week washout period, lopinavir/r for 14 days, and then lopinavir/r with PA-824 for 7 days, or sequence 2, consisting of lopinavir/r for 14 days, lopinavir/r with PA-824 for 7 days, a two-week washout period, and PA-824 alone for 7 days. In arm 3, participants received PA-824 for 7 days, rifampin for 7 days, and then PA-824 with rifampin for 7 days. Adherence was assessed by pill counts and medication diaries. All doses prior to PK sampling were observed by study staff. Serial plasma sampling for PK was performed at the end of each dosing period for PA-824, efa-

virenz, and lopinavir. For PA-824, plasma was obtained predose and at 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h postdose. For efavirenz, plasma was obtained predose and at 1, 2, 3, 4, 8, 12, and 24 h postdose. For lopinavir, plasma was obtained predose and at 1, 2, 3, 4, 5, 6, 8, 10, and 12 h postdose. Rifampin concentrations were not measured in this small PK study because the effects of PA-824 on rifampin PK were expected to be small. In addition, since rifampin concentrations are highly variable and since that variability is not well explained by known genetic polymorphisms, even large changes would be unlikely to be detected.

(ii) Safety monitoring. Participants underwent weekly safety evaluations. ECG evaluations were performed at baseline and on the final day of PA-824 dosing periods, given that some nitroimidazole antibiotics can cause QT prolongation. Adverse events were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0 (19).

Drug concentration analysis. (i) Plasma assay for PA-824. PA-824 and the internal standard, triazolam, were isolated from EDTA-plasma by liquid-liquid extraction. The organic phase was removed, transferred to a clean test tube, and evaporated under nitrogen. The residue was reconstituted in MeOH-water (1:1), transferred to autosampler vials for injection onto a Chromolith SpeedROD-18 high-performance liquid chromatograph (HPLC) column, and eluted with a linear gradient of 10 mM ammonium acetate and methanol (30:70). The ion pairs 359.8/174.7 for PA-824 and 342.8/307.7 for triazolam were selected for tandem mass detection in multiple reaction monitoring (MRM) mode. Quantification of PA-824 was performed with a liquid chromatography-tandem mass spectrometer (LC-MS/MS) system comprising two PerkinElmer series 200 micro-LC pumps and a series 200 autosampler coupled with an AB Sciex API 2000 tandem mass spectrometer. For calibration curves, spiked concentrations and peak area ratios of PA-824 and the internal standard were fitted by linear least-squares regression, weighted by $1/x$. The method was validated over a linear range of 10 to 10,000 ng/ml with a correlation value, R, of 0.9984. For the measurements of PA-824, the interassay precision (percent coefficient of variation [CV]) ranged from 2.65 to 4.71% and the percent deviation ranged from 0.8 to 5.2% of the nominal values of the control concentrations. The intra-assay precision (percent CV) ranged from 1.58 to 6.29%, and the percent deviation ranged from −0.40 to 11.19% of the nominal values of the control concentrations. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were 10 and 10,000 ng/ml, respectively. Plasma aliquots of 50 μl were sufficient for analysis.

(ii) Plasma assays for efavirenz and lopinavir. Efavirenz was quantified by reversed-phase HPLC following extraction from human plasma by simple protein precipitation. Detection involved a photodiode array detector, scanning at a wavelength of 247 nm, and reserpine as the internal standard. The calibration curve concentration range was 100 ng/ml to 6,000 ng/ml. For calibration curves, spiked concentrations and peak height ratios of efavirenz and the internal standard were fitted by linear least-squares regression, weighted $1/x$. Efavirenz concentrations were calculated from regression parameters using peak height ratios. The method was validated over a linear range of 100 to 10,000 ng/ml with a correlation value, R, of 0.9995. For the measurements of EFV, the interassay precision (percent CV) ranged from 2.4 to 4.5%, and the percent deviation ranged from −0.4 to 3.3% of the nominal values of the control concentrations. The intra-assay precision (percent CV) ranged from 0.6 to 5.4%, and the percent deviation ranged from −1.7 to 6.1% of the nominal values of the control concentrations. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were 100 and 10,000 ng/ml, respectively.

Lopinavir and its deuterated internal standards were extracted from 50 μl of EDTA-human plasma by protein precipitation with acetonitrile, followed by centrifugation. The clear supernatant was transferred into autosampler vials for a 10-μl injection onto an Agilent Zorbax XDB-C_8 (5-μm particle size; 2.1- by 50-mm HPLC column). The mobile phase comprised 10 mM ammonium formate buffer (pH 4.0) and acetonitrile containing 0.1% formic acid. Elution was performed using a gradient flow rate of 400 μl/minute with MS/MS detection on an ABSCIEX API 2000 mass spectrometer (MS) using electrospray in positive-ion mode. The ion pairs 629.2/429.0 for lopinavir and 637.2/429.0 for LPV-D^8 were selected for tandem mass detection. For calibration curves, spiked concentrations using peak area ratios of lopinavir were fitted by $1/x$ linear regression. The method was validated over a linear range of 50 to 8,000 ng/ml with a correlation R of 0.9988. For the measurements of LPV, the interassay precision (percent CV) ranged from 4.50 to 5.11%, and the percent deviation ranged from −8.15 to 0.037% of the nominal values of the control concentrations. The intra-assay precision (percent CV) ranged from 2.35 to 6.39%, and the percent deviation ranged from −9.33 to 2.72% of the nominal values of the control concentrations. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were 50 and 8,000 ng/ml, respectively.

Pharmacogenetic testing. Genetic polymorphisms reported to predict plasma PK of efavirenz (11–15), rifampin (20, 21), and lopinavir (17, 18) were genotyped in duplicate. For efavirenz, *CYP2B6* 516 G→T (rs3745274), 983 T→C (rs28399499), and 15582 C→T (rs4803419) were assayed using MassARRAY iPLEX Gold (Sequenom Inc., San Diego, CA, USA). The composite *CYP2B6* genotype was defined as follows (15): an extensive metabolizer contains 516 G/G and 983 T/T, with either 15582 C/C or C/T; an intermediate metabolizer contains 516 G/T or 983 T/C but not both, or homozygosity for 15582 T/T; and a slow metabolizer contains 516 T/T, 983 C/C, or the combination of 516 G/T and 983 T/C. *SLCO1B1* C→T (rs4149032) (for rifampin) and *SLCO1B1* 521 T→C (rs4149056) (for lopinavir/r) were genotyped by TaqMan (Applied Biosystems, Inc., Foster City, CA). The *CYP3A5*3* 6986 A→G variant (rs776746) (22) was genotyped by MassARRAY iPLEX Gold.

Pharmacokinetic and statistical analyses. (i) Sample size. Thirteen volunteers per arm were estimated to provide 80% power to detect a 20% mean difference in the area under the concentration-time curve from 0 to 24 h ($AUC_{0-24\,h}$) for PA-824 when it was coadministered with companion drug versus being given alone, using a two-sided t test at a significance level of 0.05. We targeted enrollment of 16 participants per arm.

(ii) Pharmacokinetic and statistical evaluation. PK parameters for PA-824, efavirenz, and lopinavir, including AUC, maximum plasma concentration (C_{max}), time of maximum plasma concentration (T_{max}), half-life ($t_{1/2}$), and oral clearance (CL/F) were determined using standard noncompartmental methods performed in SAS (SAS Institute, Inc., Cary, NC). Statistical analyses were based on nonparametric tests. The P values evaluating changes in PK of PA-824 coadministered with efavirenz, lopinavir/r, or rifampin to PA-824 alone determined using a Wilcoxon signed-rank test and comparing changes in PK parameters of these drugs among metabolizer groups using a Wilcoxon rank sum test or Kruskal-Wallis test are reported. Calculated geometric means of ratios (GMR) and 90% confidence intervals based on log-transformed PK parameters were also used for PK comparisons. Associations between genotypes and PK parameters were assessed using the Jonckheere-Terpstra trend test and assuming additive genetic models.

RESULTS

Study subjects. Fifty-two participants enrolled. Median age was 34 years (range, 19 to 63 years), median weight was 83 kg (range, 47 to 119 kg), median body mass index was 27 kg/m^2 (range, 18 to 41 kg/m^2), and 30 (58%) were male. Thirty-one (60%) were white, 17 (33%) were African-American or black, and 2 were Asian, and 2 were not reported. Of 52 participants, 48 completed all PK visits. There were two early discontinuations each in arms 1 and 2. In arm 1, one individual had efavirenz-related side effects, and another was a passenger in a motor vehicle accident. In arm 2, two participants self-administered a lower-than-prescribed lopinavir/r dose and so were discontinued. Thus, 48 participants were eligible for PK analyses.

TABLE 1 Pharmacokinetic parameters of PA-824 when PA-824 is administered alone or coadministered with steady-state efavirenz, lopinavir/r, or rifampin

Companion drug (treatment group)[a]	Pharmacokinetic parameter for PA-824	Median (IQR) for the parameter[c]		GMR[b]	90% CI[d]	*P* value[e]
		PA-824 alone	PA-824 and companion drug			
Efavirenz (arm 1)	AUC_{0-24} (ng · h/ml)	36,495 (30,853, 53,857)	24,917 (19,094, 34,257)	0.65	(0.56, 0.76)	<0.001
	C_{max} (ng/ml)	2,035 (1,805, 2,840)	1,510 (1,225, 2,025)	0.72	(0.62, 0.83)	0.001
	C_{min} (ng/ml)	1,110 (892, 1,650)	653 (502, 936)	0.54	(0.45, 0.64)	<0.001
	T_{max} (h)	4.0 (4.0, 5.0)	4.0 (3.5–5.0)	0.88	(0.65, 1.20)	0.383
	$t_{1/2}$ (h)	24.8 (18.9, 27.1)	16.2 (14.9, 21.0)	0.74	(0.68, 0.80)	<0.001
	CL/*F* (liters/h)	5.48 (3.71, 6.48)	8.03 (5.87, 10.6)	1.53	(1.31, 1.78)	<0.001
Lopinavir/r (arm 2)	AUC_{0-24} (ng · h/ml)	39,035 (24,295, 42,187)	29,899 (20,691, 37,949)	0.83	(0.71, 0.98)	0.02
	C_{max} (ng/ml)	2,130 (1,440, 2,425)	1,770 (1,285, 2,245)	0.87	(0.75, 1.0)	0.03
	C_{min} (ng/ml)	1,085 (708, 1,320)	838 (509, 1,155)	0.79	(0.66, 0.93)	0.01
	T_{max} (h)	4.0 (3.5, 5.0)	4.5 (3.5, 5.0)	1.10	(0.88, 1.38)	0.47
	$t_{1/2}$ (h)	21.6 (18.4, 28.7)	16.7 (15.1, 23.7)	0.83	(0.73, 0.94)	0.04
	CL/*F* (liters/h)	5.13 (4.74, 8.23)	6.69 (5.28, 9.67)	1.20	(1.03, 1.41)	0.03
Rifampin (arm 3)	AUC_{0-24} (ng · h/ml)	42, 495 (29,501, 48,661)	13,659 (9,981, 19,070)	0.34	(0.27, 0.42)	<0.001
	C_{max} (ng/ml)	2,490 (1,925, 2,885)	1,165 (769, 1,520)	0.47	(0.39, 0.56)	<0.001
	C_{min} (ng/ml)	1,080 (722, 1,300)	173 (93, 320)	0.15	(0.11, 0.21)	<0.001
	T_{max} (h)	4.0 (3.0, 6.0)	4.0 (3.5, 4.5)	1.00	(0.81, 1.22)	0.58
	$t_{1/2}$ (h)	19.25 (15.66, 20.78)	8.07 (6.28, 9.22)	0.41	(0.36, 0.46)	<0.001
	CL/*F* (liters/h)	4.72 (4.12, 6.78)	14.67 (10.52, 20.31)	2.97	(2.41, 3.67)	<0.001

[a] Dosing was as follows: PA-824, 200 mg once daily; efavirenz, 600 mg once daily; lopinavir/r, 400 mg/100 mg twice daily; rifampin, 600 mg once daily.
[b] Geometric mean of ratios (GMR) of pharmacokinetics of PA-824 coadministered with a companion drug to PA-824 alone.
[c] IQR, interquartile range.
[d] CI, confidence interval.
[e] *P* value of Wilcoxon signed-rank test comparing pharmacokinetics of PA-824 coadministered with the companion drug to PA-824 alone.

Pharmacokinetics of PA-824, efavirenz, and lopinavir. Compared to PA-824 alone, plasma exposures (AUC_{0-24}) of PA-824 (based on GMR) were reduced by 35% with efavirenz, 17% with lopinavir/r, and 66% with rifampin (Table 1). Plasma concentration-time curves for PA-824 alone versus PA-824 with efavirenz, lopinavir/r, and rifampin are shown in Fig. 2 and in color in Fig. S1 in the supplemental material. Plasma efavirenz and lopinavir concentrations were not appreciably affected by PA-824 (Table 2). In a *post hoc* nonlinear mixed-effects modeling analysis of our phase 1 trial data plus raw data from phase 1 and 2 trials of PA-824 supplied by TB Alliance, we found that PA-824 exposures were similar for a 200-mg dose taken together with efavirenz and a 100-mg dose taken without efavirenz. The same was true for PA-824 coadministered with rifampin, except that while overall expo-

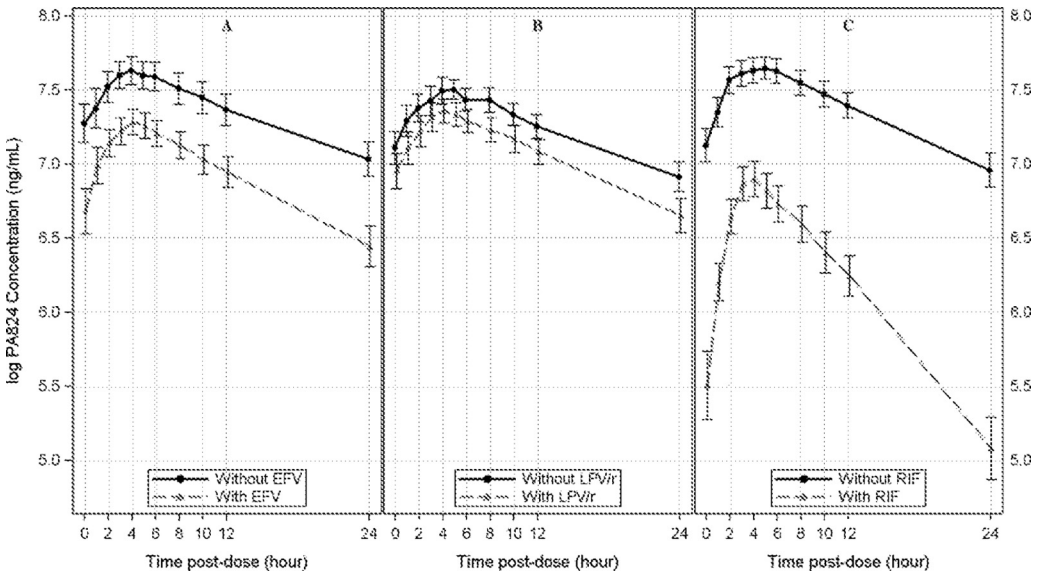

FIG 2 Mean $\log_e$ PA-824 plasma concentration-versus-time curve of PA-824 at 200 mg once daily alone (solid lines) or together (dotted lines) with steady-state efavirenz (EFV) (A), lopinavir/ritonavir (LPV/r) (B), or rifampin (RIF) (C). Values shown represent means with standard errors.

TABLE 2 Pharmacokinetic parameters of efavirenz and lopinavir when administered alone or coadministered with PA-824

Drug (treatment group)[a]	Pharmacokinetic parameter	Median (IQR) for the parameter[c]		GMR[b]	90% CI[d]	*P* value
		Drug alone	Drug with PA-824			
Efavirenz (arm 1)	AUC_{0-24} (ng · h/ml)	62,112 (48,568, 72,301)	55,835 (44,347, 73,023)	0.96	(0.91, 1.02)	0.25
	C_{max} (ng/ml)	4,380 (3,712, 4,773)	3,945 (2,616, 4,897)	0.86	(0.72, 1.02)	0.21
	C_{min} (ng/ml)	1,868 (1,359, 2,275)	1,768 (1,193, 2,270)	0.96	(0.90, 1.04)	0.82
	$t_{1/2}$ (h)	20.2 (15.6, 24.3)	18.5 (16.5, 33.3)	1.14	(0.93, 1.40)	0.38
	CL/*F* (liters/h)	3.23 (2.77, 4.12)	3.61 (2.74, 4.51)	1.04	(0.98, 1.11)	0.19
Lopinavir/r (arm 2)	AUC_{0-24} (ng · h/ml)	95,689 (71,321, 117,600)	87,092 (58,858, 98,231)	0.86	(0.77, 0.96)	0.005
	C_{max} (ng/ml)	11,450 (8,955, 13,400)	10,150 (7,400, 10,850)	0.83	(0.76, 0.92)	0.004
	C_{min} (ng/ml)	4,095 (2,245, 6,255)	2,925 (2,200, 5,110)	1.03	(0.60, 1.80)	0.009
	$t_{1/2}$ (h)	6.71 (4.67, 8.76)	6.54 (4.95, 8.22)	0.96	(0.84, 1.09)	0.63
	CL/*F* (liters/h)	4.18 (3.41, 5.64)	4.59 (4.07, 6.80)	1.17	(1.05, 1.30)	0.005

[a] Efavirenz was given at a dose of 600 mg daily; lopinavir/r was given at a dose of 400 mg/100 mg twice daily.
[b] Geometric mean of ratios (GMR) of pharmacokinetics of PA-824 coadministered with a companion drug to PA-824 alone.
[c] IQR, interquartile range.
[d] CI, confidence interval.

sures were similar, trough concentrations remained modestly reduced with rifampin coadministration (data not shown).

Pharmacogenetic associations. Of 16 subjects evaluable for PK in arm 1 (efavirenz), 6 (38%) were *CYP2B6* extensive metabolizers, 10 (63%) were intermediate metabolizers, and none were slow metabolizers. Changes in PK parameter values for PA-824 (based on geometric means of ratios [GMR]) with concomitant efavirenz did not differ significantly between *CYP2B6* intermediate and extensive metabolizers (e.g., PA-824 C_{min} reduced by 44% and 49%, respectively; $P = 0.692$). Of 16 subjects evaluable for PK in arm 2 (lopinavir/r), 12 (75%) were homozygous for *SLCO1B1* T/T and 4 (25%) were heterozygous for *SLCO1B1* 521 C/T. Regarding *CYP3A5* 36986A→G (rs776746), 5 subjects (31%) had extensive, 3 (19%) had intermediate, and 8 (50%) had slow metabolizer genotypes. Changes in PK parameter values for PA-824 (based on GMR) with concomitant lopinavir/r did not differ consistently between *SLCO1B1* C/T and *SLCO1B1* T/T or by *CYP3A5* genotype. Of 16 subjects evaluable for PK in arm 3 (rifampin), 4 (25%) were homozygous for *SLCO1B1* 38664 (rs4149032), 5 (31%) were heterozygous for *SLCO1B1* 38664 (rs4149032), and 7 (44%) were homozygous for *SLCO1B1* 38664 (rs4149032). Regarding *CYP3A5* 36986A→G (rs776746), 4 subjects (25%) had extensive, 6 (38%) had intermediate, and 6 (38%) had slow metabolizer genotypes. Changes in PK parameter values for PA-824 (based on GMR) with concomitant rifampin did not differ significantly by *SLCO1B1* rs4149032 or by *CYP3A5* genotype. Relationships between polymorphisms and PK parameters of efavirenz and lopinavir as well as relationships between polymorphisms and magnitude of drug-drug interactions when PA-824 was administered with efavirenz, lopinavir/r, or rifampin are described in the supplemental material.

To explore genetic associations, we considered PA-824 PK data without concomitant efavirenz, lopinavir/r, or rifampin in all 48 subjects. We found no apparent association between *CYP2B6*, *SLCO1B1*, and *CYP3A5* polymorphisms and PA-824 PK parameters. The relationship between these polymorphisms and C_{min} is shown in the supplemental material.

Safety and tolerability. PA-824 was well tolerated alone and with concomitant efavirenz, lopinavir/r, and rifampin. There were two adverse events of grade ≥3. In arm 2, one participant had an asymptomatic elevation of aspartate transaminase (AST) following vigorous exercise (PA-824 alone, sequence 2). One subject in arm 3 experienced grade 3 neutropenia on the last day of dosing, likely due to rifampin. Both adverse events resolved quickly after drug discontinuation. There were no QTc events of grade ≥2.

DISCUSSION

For the first time in decades, there is a robust drug development pipeline for tuberculosis. The nitroimidazole PA-824 is poised to enter phase 3 clinical trials. Anticipating the need to treat patients coinfected with HIV-1, we examined the safety, tolerability, and PK of PA-824 given with commonly used antiretrovirals that induce or inhibit P450 metabolizing enzymes (efavirenz and lopinavir/r) and with the essential first-line tuberculosis drug, rifampin. We showed substantial reduction of plasma PA-824 exposure by efavirenz and rifampin but modest changes with lopinavir/r. The combinations were safe and well tolerated, and PA-824 did not affect plasma efavirenz or lopinavir exposures.

Concurrent treatment of HIV and tuberculosis reduces mortality and new AIDS-defining illnesses (23–25). Because cotreatment can be complicated by drug-drug interactions, overlapping drug toxicities, immune reconstitution syndrome, and high pill burden (26), late-phase clinical trials of antituberculosis drugs typically exclude patients requiring antiretroviral therapy. In the present study, coadministration of PA-824 with lopinavir/ritonavir did not increase PA-824 concentrations; rather, exposures were modestly reduced. Ritonavir is a mixed inducer and inhibitor, and with regard to interactions with PA-824, induction apparently dominated. This demonstrates the importance of empirical data when ritonavir is used with other drugs as it is difficult to make *a priori* predictions about its likely effects on companion drugs (10, 27). The modest reductions in PA-824 with lopinavir/r were statistically significant but are likely not clinically relevant. In contrast, efavirenz substantially decreased PA-824 exposure, likely by upregulating CYP3A or other metabolizing enzymes. While CYP3A contributes only 20% to overall metabolism of PA-824, this percentage may increase when CYP3A is induced. Whether

interactions of efavirenz and rifampin with PA-824 will be clinically important can only be answered by PK/pharmacodynamics (PD) analysis of trial data in which different doses of PA-824 are tested for longer durations in patients with tuberculosis. PK and outcome data from an 8-week phase 2 clinical trial are expected soon, and with these data in hand, concentration-effect relationships can be explored more fully.

Rifampin has unique sterilizing activity against *M. tuberculosis*, making it a mainstay of first-line tuberculosis treatment. To date, there are no drugs clinically proven to have sterilizing activity equal to rifampin. Rifampin is, however, a potent inducer of metabolizing enzymes and drug transporters (28). Rifampin-induced drug interactions complicate drug development efforts for drug-sensitive tuberculosis in two ways. First, promising investigational drugs cannot be added to first-line antituberculosis regimens without evaluating PK effects of rifampin and other coadministered antituberculosis drugs. For example, rifampin reduces concentrations of the newest TB drug, bedaquiline, by 50% (29). Conversely, isoniazid can inhibit metabolizing enzymes, and unexpected effects may occur when rifampin and isoniazid are coadministered with a third drug (30, 31). Second, rifamycin antibiotics like rifampin and rifapentine have dose-dependent treatment-shortening potential, but evaluating high-dose rifamycins is challenging because the magnitude of drug interactions at increased rifamycin doses is unknown. That is, while it is generally believed that rifamycins' inductive capabilities are maximized at currently used doses, recent preliminary studies in human hepatocytes suggest that mRNA expression of CYP3A increases with higher rifamycin concentrations, within clinically relevant ranges (32). Whether or not higher mRNA expression will lead to greater enzyme activity or higher risk for clinically meaningful drug interactions is unknown.

Interpretation of drug interaction study results requires understanding of study drug pharmacodynamics (PD; i.e., correlations between PK parameters and efficacy). For tuberculosis, lack of a reliable biomarker of treatment response makes it difficult to define PK-PD relationships. PA-824 is being tested in a 2-month phase 2B treatment trial at doses of 100 mg and 200 mg daily because doses from 100 mg to 1,000 mg had similar activities in the 2-week dose-ranging phase 2A monotherapy studies; only at 50 mg daily was early bactericidal activity (EBA) decreased (33, 34). Preclinical studies suggest time-dependent activity of PA-824 against *M. tuberculosis* (35), suggesting that the AUC_{0-24} may be a key pharmacodynamic parameter; however, target AUC values have not been defined. The phase 2B study may help define dose-effect or concentration-effect relationships that will give our results context.

There are well-replicated associations between *CYP2B6* polymorphisms and efavirenz PK (11–15) and between an *SLCO1B1* polymorphism and lopinavir PK (16–18). An association has been reported between an *SLCO1B1* polymorphism and rifampin PK (20, 21). It is important to consider whether these polymorphisms affect drug interactions. In addition, because PA-824 is metabolized in part by CYP3A, we assessed a *CYP3A5* loss-of-function polymorphism. Our study did not show magnitudes of effects of efavirenz, lopinavir, and rifampin on PA-824 PK parameters to differ by the above genetic polymorphisms. In addition, we found no apparent associations between these polymorphisms and PA-824 C_{min} though the sample size was limited. We suspect that the apparent association between *CYP3A5*3* and lopinavir PK is spurious since this association has not been seen elsewhere (16–18).

There were limitations to the present study. Because study drugs were given for relatively brief intervals, the full safety profile could not be assessed. The effect of rifampin on PA-824 was not assessed in the context of full, multidrug first-line antituberculosis treatment. It is possible that effects of rifampin alone differ from effects when it is combined with other first-line antituberculosis drugs. None of the multiple metabolites of PA-824 were measured in this study, and the specific metabolizing enzyme(s) that mediates reductions in PA-824 seen in this study is not known.

In conclusion, PA-824 was well tolerated when given with efavirenz, lopinavir/r, or rifampin. Concomitant lopinavir/r only modestly reduced PA-824 plasma exposures, suggesting that the drugs can be coadministered without dose adjustment. Efavirenz reduced PA-824 exposures more substantially, and rifampin reduced PA-824 exposure even more. The clinical implications of these findings should be interpreted in light of results of ongoing phase 2 dose-ranging trials that will define dose-response relationships and identify target concentrations for maximal PA-824 effect so that use of PA-824 in first-line regimens and in patients requiring HIV-1 therapy can be optimized.

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