Pharmacokinetics of *para*-Aminosalicylic Acid in HIV-Uninfected and HIV-Coinfected Tuberculosis Patients Receiving Antiretroviral Therapy, Managed for Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis

Lizanne de Kock,^a Sherwin K. B. Sy,^b Bernd Rosenkranz,^a Andreas H. Diacon,^c Kim Prescott,^a Kenneth R. Hernandez,^b Mingming Yu,^b Hartmut Derendorf,^b Peter R. Donald^d

Division of Clinical Pharmacology, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa^a; Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, Florida, USA^b; Department of Biomedical Science, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa^c; Pediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa^d

The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* prompted the reintroduction of *para*-aminosalicylic acid (PAS) to protect companion anti-tuberculosis drugs from additional acquired resistance. In sub-Saharan Africa, MDR/XDR tuberculosis with HIV coinfection is common, and concurrent treatment of HIV infection and MDR/XDR tuberculosis is required. Out of necessity, patients receive multiple drugs, and PAS therapy is frequent; however, neither potential drug interactions nor the effects of HIV infection are known. Potential drug-drug interaction with PAS and the effect of HIV infection was examined in 73 pulmonary tuberculosis patients; 22 (30.1%) were HIV coinfecting. Forty-one pulmonary MDR or XDR tuberculosis patients received 4 g PAS twice daily, and in a second crossover study, another 32 patients were randomized, receiving 4 g PAS twice daily or 8 g PAS once daily. A PAS population pharmacokinetic model in two dosing regimens was developed; potential covariates affecting its pharmacokinetics were examined, and Monte Carlo simulations were conducted evaluating the pharmacokinetic-pharmacodynamic index. The probability of target attainment (PTA) to maintain PAS levels above MIC during the dosing interval was estimated by simulation of once-, twice-, and thrice-daily dosing regimens not exceeding 12 g daily. Concurrent efavirenz (EFV) medication resulted in a 52% increase in PAS clearance and a corresponding >30% reduction in mean PAS area under the concentration curve in 19 of 22 HIV-*M. tuberculosis*-coinfecting patients. Current practice recommends maintenance of PAS concentrations at $\geq 1 \mu\text{g/ml}$ (the MIC of *M. tuberculosis*), but the model predicts that at only a minimum dose of 4 g twice daily can this PTA be achieved in at least 90% of the population, whether or not EFV is concomitantly administered. Once-daily dosing of 12 g PAS will not provide PAS concentrations exceeding the MIC over the entire dosing interval if coadministered with EFV, while 4 g twice daily ensures concentrations exceeding MIC over the entire dosing interval, even in HIV-infected patients who received EFV.

In sub-Saharan Africa, an estimated 79% of tuberculosis (TB) patients are also coinfecting with human immunodeficiency virus (HIV), and approximately 50% of AIDS-associated death is TB related (1); concurrent treatment with combination chemotherapy against both infections is the standard of care (2). Complicating this high prevalence of *M. tuberculosis*-HIV coinfections, multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Mycobacterium tuberculosis* strains are reaching epidemic proportions (3, 4). MDR *M. tuberculosis* is resistant to at least the two first-line drugs, isoniazid and rifampin, while XDR *M. tuberculosis* is additionally resistant to fluoroquinolone and at least one of three second-line injectable drugs (4). XDR *M. tuberculosis* is associated with a high early mortality among HIV-infected individuals (5), and the scarcity of effective treatment options against MDR and XDR *M. tuberculosis* caused the reintroduction of second-line TB drugs, including *para*-aminosalicylic acid (PAS).

PAS is a second-line antimycobacterial agent generally reserved for treatment of XDR TB (6) and for management of complicated forms of MDR TB but is notorious for gastrointestinal intolerance, including nausea, vomiting, abdominal cramping, and diarrhea (7, 8). Current recommendations for dosing of PAS are based on the supposition that, as PAS is bacteriostatic, it is thus necessary to maintain PAS concentrations above the MIC of ap-

proximately $1 \mu\text{g/ml}$ (9) throughout a dosing interval; current recommendations suggest a dosage of 8 to 12 g PAS daily in two or three divided doses. However, in early studies conducted by the British Medical Research Council, PAS was used at a dosage of 20 g in four divided dosages (10). When accompanied by streptomycin, PAS therapy was associated with a 5-year mortality of 19% (10). This rate can be compared with a mortality of 80% reported for some series of XDR HIV-associated TB (11).

For nearly 2 decades, a granular slow-release form of PAS (*para*-aminosalicylic acid delayed-release granules [PASER granules]) has been available and has been found to cause less intolerance in several studies in TB patients and healthy volunteers (12, 13). This formulation still provides PAS concentrations exceeding

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Address correspondence to Bernd Rosenkranz, rosenkranz@sun.ac.za.

L.D.K. and S.K.B.S. contributed equally to this work and are joint first authors.

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the MIC of PAS when given at 4 g twice daily. It was also shown that 4 g once daily failed to maintain concentrations above the MIC over a 24-h dosing interval (12, 13). Although PAS has been in use for more than 60 years and was extensively studied, little is known about the pharmacokinetics of PAS in HIV-infected patients and potential interactions of PAS with other coadministered second-line TB drugs and antiretroviral (ARV) medications, and the currently available PAS formulation (PASER) has been the subject of relatively few pharmacokinetic studies (12–14).

In a previous study of the pharmacokinetics of PAS in a small number of children and adults, we noted that HIV-infected adults and children had lower PAS concentrations than those who were uninfected, but this difference did not reach significance (14). Against the background of the very poor outcomes occurring among HIV-associated XDR TB infections, we have reviewed some of the early PAS literature and studied the pharmacokinetics of PAS in larger numbers of TB patients, both HIV infected and HIV uninfected, and also assessed the possible role of concomitant medication in reducing exposure to PAS, particularly in those with HIV infection. We were also interested in the possible role of once-daily PAS dosing, as this would greatly ease the role of health services in managing therapy in patients with MDR TB and XDR TB, as well as in evaluating whether once-daily dosing can provide PAS concentrations above the MIC for the greater part of a dosing interval.

We therefore explored PAS pharmacokinetics in adults with and without HIV infection at a dosage of 4 g twice daily and also later in a second study, wherein patients were randomized to receive either 4 g PAS twice daily or 8 g PAS once daily. We aimed to evaluate whether once-daily dosing with a slow-release preparation might provide satisfactory concentrations throughout a 24-h dosing interval and whether there are factors that may affect the pharmacokinetics of PAS. Given the large variety of possible drug combinations to study in TB patients and those with HIV infection, this study was exploratory, and no *a priori* evaluation of specific drug combinations for drug-drug interaction was planned. In this paper, we summarize our findings regarding the population pharmacokinetics (PK) of PAS and screening of concomitant medications for possible effects on PAS disposition in our patients.

MATERIALS AND METHODS

Study population and design. Seventy-three patients with MDR or XDR TB from the Brooklyn Hospital for Chest Disease in Cape Town, South Africa, were prospectively enrolled in two PAS therapeutic monitoring studies. Forty-one patients were studied twice after receiving 4 g PAS twice daily, and 32 patients were enrolled in a randomized, crossover, open-label study and received 4 g twice daily or 8 g once daily. Those with severe anemia, diarrhea, or dehydration were excluded. Patients, ages 18 to 64 years, provided written informed consent, and their age, sex, weight, height, HIV antibody test results, serum creatinine, concomitant medications, and concurrent disease diagnosis were documented.

Patients were prescribed oral PAS (*para*-aminosalicylic acid delayed-release granules [PASER granules]; Jacobus Pharmaceutical, Princeton, NJ, USA). In the first study, the subjects received 4 g PAS twice daily on two occasions to evaluate interoccasion variability, with at least 1 week elapsing between the two occasions. Their blood samples were collected at predose and 2, 3, 4, 5, 6, 8, and 12 h postdose. The second study was a randomized crossover study where patients received either 4 g PAS twice daily or 8 g PAS once daily on the first occasion followed by the alternative dosing scheme on the second occasion. During both studies, samples were taken only after patients had received PAS 4 g twice daily for at least 2 weeks, and patients who entered the crossover study received the relevant regimen for another 8 days before the pharmacokinetic study. Samples for

pharmacokinetic analysis were collected at predose and 1, 2, 3, 4, 5, 6, 8, and 12 h after 4 g twice daily and at predose and 1, 2, 3, 4, 6, 8, 12, and 24 h after 8 g once daily. To prevent early PAS release in the stomach, doses were routinely taken with acidic food or beverages. Patients received breakfast an hour after dosing. These studies were conducted according to the principles of the South African Guidelines for Good Clinical Practice and were approved by the Ethics Committee for Human Research of Stellenbosch University (N09/08/212; M12/01/006). The protocols conformed to the Declaration of Helsinki and subsequent amendments.

Bioanalytical method. The concentrations of PAS were quantified using a validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay as previously described (14). The analysis of quality control samples reported good reproducibility with coefficient of variation of $\leq 5\%$ and accuracy, with between-sample differences not exceeding 2.5%. The lower limit of quantification was 0.01 $\mu\text{g/ml}$. All of the observed drug concentrations were above this limit.

Pharmacokinetic analysis. The nonlinear mixed-effects modeling of PAS disposition in *M. tuberculosis*-HIV-coinfected patients was performed using NONMEM (version 7.2) with first-order conditional estimation and with η interaction. The one-compartment model with first-order absorption with lag time or transit compartment followed by first-order absorption (15, 16) or mixed Michaelis-Menten and first-order absorption (17) was explored for the steady-state concentration-time profiles of patients who were administered 8 g PAS once daily or 4 g PAS twice daily. The final structural model was parameterized on the PK parameters: transit rate constant (K_{tr}) with 3 transit compartments, apparent oral clearance (CL/F), and the apparent volume of distribution (V/F). The relative bioavailability parameter (F) assumed the value of 1. Exponential interindividual variability terms were included in the pharmacokinetic parameters. Interoccasion variability was introduced to both the apparent oral clearance and transit rate constant: $P_{ij} = \bar{P} \times \exp(\eta_i + \kappa_{ij})$, where $\bar{P}$ represents the mean parameter value and η_i and κ_{ij} describe interindividual and interoccasion (within an individual) variability and were assumed to be independently and normally distributed, both with mean zero and variances of ω_p^2 and π_p^2 , respectively. The subscript i represents the individual, and j represents the occasion for the individual. Dummy variables were introduced to distinguish separate occasions, where 1 designated the first occasion wherein individuals received the first 4-g twice-daily regimen, 2 indicated the second 4-g twice-daily regimen, and 3 was used for the 8-g once-daily regimen. All three occasions were consolidated to determine the interoccasion variability. The residual variability, which is a composite of the other unexplained errors, was estimated by a mixture of additive and proportional error models.

Covariates that were evaluated for their potential effect on PAS disposition included the following: total body weight, age, HIV status, gender, race, and comedications. The concomitant medications were evaluated individually for their effects on PAS disposition. Dummy variables consisting of 0 and 1 were introduced for absence and presence of drug, respectively. The hypothesis testing to discriminate among alternative hierarchical structural models was based on the P values for the forward inclusion and backward elimination at 0.05 and 0.01, respectively. After the covariate screening determined that EFV has a significant effect on PAS pharmacokinetics, EFV effect was tested on the apparent oral clearance. The covariate model for the EFV effect on the apparent oral clearance was based on the following: $TV_{CL/F} = \theta_1 \times \exp(\theta_2 \times I_{COV})$, where θ_1 is the typical value (TV) for the population CL/F in the absence of EFV concomitant medication, θ_2 is the exponential factor to adjust for the population CL/F in concomitant EFV medication, and I_{COV} represents the indicator function for the presence or absence of concomitant EFV. The dose effect of EFV could not be evaluated, as all patients who received EFV had a 600-mg oral once-daily dose.

The HIV status of the individual was also evaluated. The three additional HIV-seropositive individuals who were not on antiretroviral therapy containing EFV were included in the category for HIV status. The

TABLE 1 Characteristics of 73 patients enrolled in studies of the pharmacokinetics of para-aminosalicylic acid in tuberculosis patients with and without HIV infection^a

Characteristic	Value (% of total or range)
Gender	
No. of males	39 (53.4)
No. of females	34 (46.6)
Race/ethnicity	
Black	17 (23.3)
Mixed	56 (76.7)
Median wt, kg	56 (33–86)
Median ht, m	1.7 (1.5–1.8)
Median BMI, kg m ⁻²	20 (14–35)
Median age, yr	33 (18–64)
Median creatinine clearance, ml/min	87 (35 to >150)
Median serum creatinine, μmol/liter	72.5 (44–152)
No. with concurrent disease	
MDR TB	58 (79)
XDR TB	15 (21)
Total HIV infected	22 (30.1)
HIV infected and receiving efavirenz	19 (26.0)

^a Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; TB, tuberculosis; MDR, multidrug resistant; XDR, extensively drug resistant; CV, coefficient of variation.

evaluation process for the effect of HIV status on the pharmacokinetics of PAS was similar to that which was carried out for EFV.

Given the exploratory nature of the study, model selection was based on maximum-likelihood statistics (defined as negative twice the log likelihood), goodness of fit, and visual predictive checks (VPC; with 5,000 simulated profiles).

A Monte Carlo mapped power (MCMP) analysis (18) was conducted to determine the power of the study. The full model incorporating the EFV effect, the base model, and the current data set were used to determine whether the number of subjects in the study was sufficient to identify the covariate relationship with a power of at least 80% and significance at *P* values of 0.05 and 0.01.

Monte Carlo simulations of dosage regimens and probability of target attainment. Since *M. tuberculosis*-HIV-coinfected patients have compromised immunity, a conservative pharmacodynamic target of trough concentration of the free drug (*fC*_{min}) of >1 μg/ml was considered. Free drug refers to the portion of the drug concentration that is not bound to plasma proteins. PAS has significant plasma protein binding between 50 and 60% (19). In the simulation, the free PAS was assumed to be 50% of the observed total PAS concentration. PAS activity against *M. tuberculosis* and *M. bovis* is usually considered bacteriostatic (20); maintaining a PAS plasma concentration above the typical MIC of *M. tuberculosis* at 1 μg/ml is believed to be important. Thus, the current practice is to target a sustained PAS concentration above 1 μg/ml throughout the dosing interval (12), and the package insert of the slow-release PAS formulation recommends an adult dosage of 12 g/day in 3 divided doses. In 2008, the World Health Organization suggested a PAS dosage of 8 g daily for patients weighing 33 to 70 kg, although at another point, a dosage of 10 to 12 g in two divided doses was recommended (21).

Several dosing regimens were simulated with 5,000 virtual subjects to determine the probability of attaining the pharmacodynamic target: (i) 4 g every 8 and 12 h; (ii) 6 g every 12 and 24 h; (iii) 8 g once daily; and (iv) 12 g once daily. The target was attained when 90% of the simulated *fC*_{min} was greater than the MIC. The probability of target attainment (PTA) was computed over a range of incremental MICs (range, 0.5 to 5 μg/ml).

TABLE 2 Concomitant drugs administered to 73 patients enrolled in studies of the pharmacokinetics of para-aminosalicylic acid in tuberculosis patients with and without HIV infection

Drug category	Drug(s) (no. of individuals)
Anti-tuberculosis agents and antibiotics	Capreomycin (55), moxifloxacin (30), ethionamide (60), terizidone (69), pyrazinamide (64) ethambutol (64), isoniazid (35), clofazimine (7), dapsone (6) and kanamycin (4), clarithromycin (4)
Diabetic drugs	Metformin (3), glibenclamide (1), actraphane (1), humulin (3)
Antiretroviral therapy	Efavirenz (19), stavudine (18), zidovudine (2), lopinavir/ritonavir (3), lamivudine (21), lamivudine/zidovudine (1)
Antidepressants and psychotic drugs	Amitriptyline (15), fluoxetine (1), diazepam (1), haloperidol (1), chlorpromazine (7), lorazepam (1)
Supplements and other drugs	Pyridoxine (43), vitamin B complex (13), vitamin C (2), folic acid (2), eltroxin (2), maxolon (5), ranitidine (6), orphenadrine (2), codeine phosphate (2), aspirin (1), amlodipine (2), FeSO ₄ (2), (sulfamethoxazole and trimethoprim (1)

RESULTS

Patient characteristics. The patients’ characteristics and administration of concomitant drugs are listed in Tables 1 and 2. Forty-one patients were recruited in the 4-g twice-daily study and 32 patients in the crossover study; data from all 73 patients were used in the analysis; 26% were HIV coinfectd, 21% had XDR TB, and 79% had MDR TB.

The time course of PAS concentrations was well described by a one-compartment disposition model with its absorption described by a 3-transit-compartment in series (Fig. 1) (16). The model provided reasonable fits to the observed concentration-time profiles (Fig. 2). The large interindividual and interoccasion variability in PAS disposition resulted in a large difference between the population fit and the observed data, as shown in the upper right graph of Fig. 2. The conditional weighted residuals (CWRES) versus time and CWRES versus the population predicted PAS concentrations in Fig. 2 show that most of the data lie within 2 units of the zero ordinate. Figure 3 shows a visual predictive check of the final model stratified on concomitant efavirenz administration. The concentration profiles for both dosing regimens were simultaneously described with a single set of population PK parameter estimates (Table 3). With the excep-

FIG 1 Schematic representation of the para-aminosalicylic acid pharmacokinetic model.

FIG 2 Model diagnostics showing individual (upper left) and population (upper right) fitted versus observed PAS concentrations and time (lower left) and population fitted values (lower right) versus conditional weighted residuals. The dashed lines indicate smoothing using LOWESS (locally weighted scatter plot smoothing) regression. The solid lines are the expected lines of unity (top) and zero lines (bottom).

tion of the first-order absorption with the lag time model, the mixed Michaelis-Menten and first-order absorption model was unstable and did not result in successful convergence, including boundary problems and undeterminable rounding of significant digits. The transit compartment model selected as the objective function was markedly lower than that of the first-order absorption with lag time model. Allometric scaling by body weight did not improve interindividual variability, resulting in no significant reduction in the minimum objective function value.

The MCMP analysis indicated that the sample size of 73, with 19 and 54 patients receiving and not receiving EFV, respectively, for the covariate analysis had 95% and 88% power at P values of 0.05 and 0.01, respectively, to detect differences in PAS pharmacokinetics. Similarly, for an 80% power at a significance level corresponding to a P value of 0.05, 10 and 30 individuals on PAS with or without EFV, respectively, would be sufficient to detect the EFV effect. Because of the exploratory and uncontrolled nature of the study, there was an unbalanced allocation of subjects in each treatment group. For this reason, a formal bioequivalence-type analysis was not performed. Given the sample size, however, the study was deemed sufficiently powered to detect pharmacokinetic interactions, and MCMP analysis indicated that the sample size of the present study was sufficient to detect an EFV effect with a statistical power of 95%. The other demographic variables were not influential factors of PAS pharmacokinetics.

Efavirenz interaction with PAS. During the screening for drug-drug interaction, the EFV effect in reducing PAS exposure was significant. The difference in objective function value was 8.94 from the base model, which is significant at a P value of 0.01. EFV was associated with an approximately 52% increased PAS clearance in patients who took this concurrent medication. The exponential coefficient for the effect of EFV on PAS CL/F was 0.419, which translated to 1.52 in the linear scale (Table 3). The areas under the concentration-time curve (AUCs) over a day (AUC_{0-24} ; mean $\pm$ standard deviation) for PAS without and with EFV were 875 ± 424 and 621 ± 295 mg $\cdot$ h/liter in the group receiving 4 g twice daily and 889 ± 493 and 625 ± 338 mg $\cdot$ h/liter in the group receiving 8 g once daily, respectively. The HIV-infected patients on EFV had an overall >30% reduction in PAS mean exposure.

When HIV status was evaluated as a covariate of apparent oral clearance and bioavailability, the resulting change in objective function values was -3.151 , which is not significant at a P value of 0.05. Adding HIV status on top of EFV effect did not result in additional reduction in objective function values. Thus, the model incorporating the EFV effect on PAS pharmacokinetic was selected.

The half-lives of PAS in the individual observed concentration-time profiles were determined using log-linear regression. Without EFV, the mean half-lives (coefficient of variation [CV]) in the 4-g twice-daily and 8-g once-daily dosing regimens were 4.09 h (50.8%) and 3.25 h (54.5%), respectively. In EFV comedication group, PAS half-lives were 2.85 h (36.2%) and 2.56 h (37.3%), respectively. The

FIG 3 Visual predictive check showing the 5th, 50th, and 95th percentiles (lines) of the observed PAS concentration data (dots) and the 95% confidence intervals (shaded areas) of the percentiles based on the pharmacokinetic model stratified by concomitant efavirenz administration.

TABLE 3 Population pharmacokinetic model parameters of the final model evaluating the pharmacokinetic interaction of *para*-aminosalicylic acid with efavirenz^a

Parameter	Mean value (% RSE) in final model
Structural model parameters	
Apparent oral clearance (CL/F, liters/h)	9.42 (6.6)
Apparent volume of distribution (V/F, liters)	51.8 (6.7)
Transit rate constant (K_{tr} , h ⁻¹)	0.65 (6.8)
Number of transit compartments	3
Interindividual variability	
% CV of CL/F (ω_{CL})	47.5 (9)
% CV of V/F (ω_V)	74.8 (11.9)
% CV of K_{tr} ($\omega_{K_{tr}}$)	43.5 (13.2)
Interoccasion variability	
% CV of CL/F (π_{CL})	49.8 (13.1)
% CV of K_{tr} ($\pi_{K_{tr}}$)	47.7 (13.1)
Residual variability	
Proportional residual error	0.269 (12)
Additive residual error ($\mu\text{g/ml}$)	7.90 (10.8)
Covariates	
Efavirenz on CL/F ^b	0.419 (33)
ΔOFV (from baseline model)	-8.94

^a Abbreviations: OFV, minimum objective function value; RSE, relative standard error.
^b The covariate equation to describe the effect of efavirenz on CL/F is $\text{CL}/F_i = \overline{\text{CL}/F} [\exp(\theta_i \times I_{\text{EFV}})]$.

reduction in the PAS half-life in EFV comedication group indicated that EFV effect on PAS CL/F is more likely than its effect on PAS relative bioavailability. There are some indications that EFV induction is primarily hepatic and not intestinal (22).

Monte Carlo simulations and probability of target attainment. The plasma PAS concentrations were simulated for several dosing regimens, and the resulting exposure parameters are summarized in Fig. 4. The top panels show the pharmacokinetic parameters in virtual patients who had concomitant EFV, whereas the lower panels are for those without EFV. The increase in PAS clearance in patients who were coadministered EFV was reflected in lower plasma PAS concentrations and areas under the concentration-time curve. In the computation of the free $C_{\min}$ of PAS, the unbound fraction is assumed to be 50% of the total drug concentration (19). There is an overall >30% reduction in PAS exposure with EFV. These simulated profiles were used to determine the probability of achieving free PAS levels greater than the MIC against *M. tuberculosis*, as shown in Fig. 5.

The PTA was based on the free trough PAS concentration ($fC_{\min}$) above the MICs during the entire dosing interval. When 4 g PAS every 12 h was simulated, PTA values of >90% were obtained for hypothetical MICs of 0.5 up to 5 $\mu\text{g/ml}$ in virtual patients with concomitant EFV. Patients who are not taking EFV can achieve PTA values of >90% at the same dosage even with hypothetical MICs of 3 $\mu\text{g/ml}$. Higher PTAs were achieved when the dosing frequency was increased to thrice daily. With 6 g twice daily, the PTA was achieved at MICs of 3 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$ with and without EFV, respectively.

The once-daily dosage regimens of 6 g, 8 g, and even 12 g are at risk of not achieving PTA at a MIC of 1 $\mu\text{g/ml}$. With the exception

FIG 4 Pharmacokinetic exposure parameters (AUC_{0-24} , C_{max} , C_{min} , and $free C_{min}$) from simulated PAS profiles for 4 g every 8 and 12 h, 6 g every 12 and 24 h, and 8 g and 12 g every 24 h. (Top) PAS profiles with concomitant efavirenz administration; (bottom) profiles without efavirenz comedication.

of the 12-g once-daily dosing without EFV, PTA for the dosage regimens were below 90% (Fig. 5, middle and right). The 5th percentiles of free PAS trough concentrations in the once-daily dosing were below 1 $\mu\text{g/ml}$ for the three doses, regardless of whether EFV was coadministered.

DISCUSSION

The most important findings of this study are that PAS concentrations are reduced in HIV-infected TB patients and that concomitant administration of EFV likely plays a role in this deficit.

Our population pharmacokinetic model predicts a 52% increase in PAS clearance. A corresponding reduction of 30% in mean PAS exposure was observed in *M. tuberculosis*-HIV-coinfected patients on EFV. To our knowledge, there has been no *in vitro* or animal experiment to evaluate drug interactions between second-line anti-tuberculosis and anti-retroviral therapies.

The mechanism of the drug-drug interaction between efavirenz and PAS remains to be elucidated. PAS was previously shown to be a prodrug that acts as a metabolic precursor of the folate biosynthetic pathway (23). More than 80% of absorbed PAS

FIG 5 Probability of target attainment of an fC_{min} greater than the MIC for PAS dosage regimens of 4 g every 8 and 12 h (left), 6 g every 12 and 24 h (center) and 8 g and 12 g every 24 h (right), with (solid lines) and without (dashed lines) efavirenz. The gray dotted line corresponds to a PTA value of 90%, which corresponds to the success probabilities.

is excreted renally; the other 20% is cleared following metabolism (24). PAS is primarily metabolized by *N*-acetyltransferase 1 (NAT1) and to a lesser extent by NAT2 (25, 26) to form *N*-acetyl-*p*-aminosalicylates; conjugation with glycine to form *p*-aminosalicyluric acid accounts for the remaining 25% of PAS metabolism (27). It is not clear whether efavirenz affects the NAT metabolic pathway of PAS. Efavirenz is known to affect both phase I and phase II metabolic enzymes, including induction of CYP3A4, 2B6, 2C19, UGT1A1 2B7, and bile efflux transporters and inhibition of CYP3A5, 2C9, 2C19 and UDP 1A4 and 1A9 (28–34). The effect of efavirenz on CYP3A4 was previously shown to be due to the activation of the human pregnane X receptor, which regulates the CYP3A4 transcription (35). An average 3- to 4-fold increase in CYP3A4 activity was observed at 5 to 10 μ M efavirenz (35). Consequently, CYP3A4 substrates when administered with efavirenz resulted in a reduction in plasma levels of these drugs (36–38). EFV was thought to affect only the hepatic CYP3A4 enzymes and not intestinal CYP3A4 (22). One study indicated that there may be some association between efavirenz systemic exposure and NAT2 genotypes as risk factors of liver injury in *M. tuberculosis*-HIV patients (39). Even though that study was not designed to evaluate the effect of efavirenz on NAT2 enzymes, we hypothesize that modulation of NAT2 activities by efavirenz may contribute to toxicity of drugs and agents that are metabolized by NAT2. Given the wide variety of drug-metabolic enzymes that were shown to be affected by efavirenz, it is likely that the increase in PAS clearance could be due to NAT1 or NAT2 induction by efavirenz. This hypothesis needs to be validated clinically by evaluating the extent of increase in *N,N*-acetyl-PAS formation in a controlled trial, as the current study was not designed to evaluate the effect of efavirenz on the metabolism of PAS. Nonetheless, this limitation does not affect the results of the present study.

With the high rate of *M. tuberculosis*-HIV coinfection, drug interactions between ARV and TB drugs are increasingly documented, primarily for the first-line anti-TB drugs (40, 41). Given that EFV appears to increase PAS clearance, it is likely that PAS efficacy could be compromised when PAS is administered with EFV. In this study, Monte Carlo simulations were conducted to evaluate several dosage regimens that could achieve drug concentrations of at least 1 μ g/ml throughout the dosing interval. With its primary purpose, the prevention of further resistance to companion anti-tuberculosis drugs in MDR-TB and XDR-TB patients, maintaining PAS plasma concentration above the typical MIC against *M. tuberculosis* at 1 μ g/ml throughout the dosing interval is believed to at least maintain its bacteriostatic activity, as PAS lacks a postantibiotic effect (12, 20, 42). Our results show that HIV-*M. tuberculosis*-coinfecting patients taking EFV are not at risk of PAS concentrations falling below 1 μ g/ml in a 4-g twice-daily PAS regimen. Peloquin et al. evaluated PAS dosages of 4 g twice daily and 4 g once daily and determined that the 4-g twice-daily dosage, but not the 4-g once-daily dosage, was adequate to maintain PAS concentrations at >1 μ g/ml throughout the dosing interval (7, 12). The Centers for Disease Control and Prevention recommendation for 8 g to 12 g daily in 2 or 3 equal doses (43) is consistent with our findings that equal doses equal to or greater than a daily total dose of 8 g would provide sufficient exposure above 1 μ g/ml.

Several earlier studies compared once-daily and divided dosing and found that adult patients tolerated gastrointestinal side effects better with once-daily dosage regimens (44–46). Single daily dos-

ing was also shown to be as effective as divided daily doses in both TB patients (44) and guinea pigs (47). In deciding the most appropriate PAS dose and frequency of administration, one needs to balance maintenance of a plasma concentration above 1 μ g/ml and the possible risk of increased intolerance due to more frequent dosing or using higher PAS doses.

For this reason, we chose to evaluate once-daily dosing regimens, including 6 g, 8 g, and 12 g. The target attainment of at least 90% of the population maintaining greater than 1 μ g/ml free PAS concentration was unlikely for the once-daily dosage regimens, except for the 12 g PAS without EFV concomitantly administered. Our study has shown that the 4-g twice-daily PAS dosage achieves a 90% PTA for fC_{min} of >1 μ g/ml even with concurrent EFV in HIV-positive patients with TB. However, the once-daily dosing even at 12 g is not compatible with EFV concurrent medication. We did not simulate higher doses, so as not to go above the total daily dose recommended in the product's package insert.

Despite the current conviction that PAS is bacteriostatic, there is evidence that PAS has bactericidal effects at higher doses probably associated with peak concentrations. Thus, Jindani et al. (48) studied the early bactericidal activity (EBA) of anti-*M. tuberculosis* agents by quantifying the serial reduction of CFU of *M. tuberculosis* in sputum of pulmonary TB patients and reported that at a single dose of 15 g in the first two treatment days, PAS caused a fall in CFU per ml sputum per day of 0.25 $\log_{10}$ /ml, a rank that is preceded only by isoniazid (48). In a study of streptomycin resistance prevention, the evaluation of PAS daily dosages of 5, 10 or 20 g divided in four doses showed that following 4 months of treatment with the highest dosage of 20 g PAS in divided doses only 4% of patients developed streptomycin resistance but the proportion increased to 21% and 47% following lower PAS dosages of 10 g or 5 g daily (49). In a subsequent study following the introduction of the more powerful drug isoniazid, it was found that a daily PAS dosage of 10 g was adequate to prevent the development of resistance to isoniazid (50). An early *in vitro* study also reported that low PAS concentrations only delayed the emergence of PAS resistance, but that higher concentrations suppressed the growth of resistant mutants (51). There is also recent evidence that PAS resistance occurs over a wide range of MICs (52), some of which are well within the range of the peak concentration achieved by many of our patients receiving 8 g once daily. The MICs of PAS-resistant *M. tuberculosis* complex strains containing mutations in the *thyA* gene, which encodes enzymes of the folate pathway (53), ranged from 8 to over 128 μ g/ml (52). With regard to the pharmacokinetic-pharmacodynamic index of PAS, it is still debatable whether the improved action of PAS in preventing resistance in companion drugs was due to the maintenance of concentrations above the MIC or to the higher peak concentrations. In the case of isoniazid administered with the purpose of preventing resistance development of other companion TB drugs, its pharmacokinetic-pharmacodynamic index is related to the peak concentrations (54).

In conclusion, this study demonstrates a possible effect of EFV on the pharmacokinetics of PAS in HIV-infected patients with TB. An overall reduction of approximately 30% PAS exposure should be expected in HIV-infected TB patients who are on concurrent EFV. When ARV therapy contains EFV, once-daily dosing up to 12 g PAS in these patients is not recommended. If maintenance of PAS concentrations above the MIC is considered advisable, a regimen of 4 g twice daily provides sufficient pharmacodynamic coverage of a PTA above 90% over the dosing interval, and a PTA of

100% is achieved with thrice-daily dosage regimens of 12 g/day. The possible advantages regarding increased efficacy and ease of programmatic administration of once-daily higher doses of PAS and the role of PAS deserve further exploration, particularly for the very vulnerable group of HIV-infected patients with MDR and XDR TB.

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We have no competing interests to declare.

REFERENCES

- Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Ravigione MC, Dye C. 2003. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch. Intern. Med.* 163:1009–1021. <http://dx.doi.org/10.1001/archinte.163.9.1009>.
- Havir DV, Kendall MA, Ive P, Kumwenda J, Swindells S, Qasba SS, Luetkemeyer AF, Hogg E, Rooney JF, Wu X, Hosseinipour MC, Lalloo U, Veloso VG, Some FF, Kumarasamy N, Padayatchi N, Santos BR, Reid S, Hakim J, Mohapi L, Mugenyi P, Sanchez J, Lama JR, Pape JW, Sanchez A, Asmelash A, Moko E, Sawe F, Andersen J, Sanne I. 2011. Timing of antiretroviral therapy for HIV-1 infection and tuberculosis. *N. Engl. J. Med.* 365:1482–1491. <http://dx.doi.org/10.1056/NEJMoa1013607>.
- Cox H, Ford N, McDermid C, van Cutsem G, Goemaere E. 2010. Extensively drug-resistant tuberculosis in South Africa. *Lancet* 376:681. [http://dx.doi.org/10.1016/S0140-6736\(10\)61327-X](http://dx.doi.org/10.1016/S0140-6736(10)61327-X).
- Cox HS, McDermid C, Azevedo V, Muller O, Coetzee D, Simpson J, Barnard M, Coetzee G, van Cutsem G, Goemaere E. 2010. Epidemic levels of drug resistant tuberculosis (MDR and XDR-TB) in a high HIV prevalence setting in Khayelitsha, South Africa. *PLoS One* 5:e13901. <http://dx.doi.org/10.1371/journal.pone.0013901>.
- Gandhi NR, Shah NS, Andrews JR, Vella V, Moll AP, Scott M, Weissman D, Marra C, Lalloo UG, Friedland GH. 2010. HIV coinfection in multidrug- and extensively drug-resistant tuberculosis results in high early mortality. *Am. J. Respir. Crit. Care Med.* 181:80–86. <http://dx.doi.org/10.1164/rccm.200907-0989OC>.
- Raviglione MC, Smith IM. 2007. XDR tuberculosis—implications for global public health. *N. Engl. J. Med.* 356:656–659. <http://dx.doi.org/10.1056/NEJMp068273>.
- Peloquin CA, Zhu M, Adam RD, Singleton MD, Nix DE. 2001. Pharmacokinetics of para-aminosalicylic acid granules under four dosing conditions. *Ann. Pharmacother.* 35:1332–1338. <http://dx.doi.org/10.1345/aph.1A088>.
- Berte SJ, Dewlett HJ. 1959. Isoniazid and para-aminosalicylic acid toxicity in 513 cases: a study including high doses of INH and gastrointestinal intolerance to PAS. *Dis. Chest* 36:146–151. <http://dx.doi.org/10.1378/chest.36.2.146>.
- Heifets LB. 1991. Antituberculosis drugs: antimicrobial activity in vitro, p 13–58. In Heifets LB (ed), *Drug susceptibility in the chemotherapy of mycobacterial infections*. CRC Press, Boca Raton, FL.
- Fox W, Sutherland I. 1956. A five-year assessment of patients in a controlled trial of streptomycin, para-aminosalicylic acid, and streptomycin plus para-aminosalicylic acid, in pulmonary tuberculosis. *Q. J. Med.* 25:221–243.
- Gandhi NR, Andrews JR, Brust JC, Montreuil R, Weissman D, Heo M, Moll AP, Friedland GH, Shah NS. 2012. Risk factors for mortality among MDR- and XDR-TB patients in a high HIV prevalence setting. *Int. J. Tuberc. Lung Dis.* 16:90–97. <http://dx.doi.org/10.5588/ijtld.11.0153>.
- Peloquin CA, Berning SE, Huitt GA, Childs JM, Singleton MD, James GT. 1999. Once-daily and twice-daily dosing of p-aminosalicylic acid granules. *Am. J. Respir. Crit. Care Med.* 159:932–934.
- Peloquin CA, Henshaw TL, Huitt GA, Berning SE, Nitta AT, James GT. 1994. Pharmacokinetic evaluation of para-aminosalicylic acid granules. *Pharmacotherapy* 14:40–46.
- Liwa AC, Schaaf HS, Rosenkranz B, Seifart HI, Diacon AH, Donald PR. 2013. Para-aminosalicylic acid plasma concentrations in children in comparison with adults after receiving a granular slow-release preparation. *J. Trop. Pediatr.* 59:90–94. <http://dx.doi.org/10.1093/tropej/fms053>.
- Savic RM, Jonker DM, Kerbusch T, Karlsson MO. 2007. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. *J. Pharmacokinet. Pharmacodyn.* 34:711–726. <http://dx.doi.org/10.1007/s10928-007-9066-0>.
- Upton RN, Mould DR. 2014. Basic concepts in population modeling, simulation, and model-based drug development: part 3—introduction to pharmacodynamic modeling methods. *CPT Pharmacometrics Syst. Pharmacol.* 3:e88. <http://dx.doi.org/10.1038/psp.2013.71>.
- Bulitta JB, Landersdorfer CB, Kinzig M, Holzgrabe U, Sorgel F. 2009. New semiphysiological absorption model to assess the pharmacodynamic profile of cefuroxime axetil using nonparametric and parametric population pharmacokinetics. *Antimicrob. Agents Chemother.* 53:3462–3471. <http://dx.doi.org/10.1128/AAC.00054-09>.
- Vong C, Bergstrand M, Nyberg J, Karlsson MO. 2012. Rapid sample size calculations for a defined likelihood ratio test-based power in mixed-effects models. *AAPS J.* 14:176–186. <http://dx.doi.org/10.1208/s12248-012-9327-8>.
- Way EL, Smith PK, Howie DL, Weiss R, Swanson R. 1948. The absorption, distribution, excretion and fate of para-aminosalicylic acid. *J. Pharmacol. Exp. Ther.* 93:368–382.
- Bastian I, Portaels F. 2000. Multidrug-resistant tuberculosis. Kluwer, Dordrecht, The Netherlands.
- World Health Organization. Guidelines for programmatic management of drug-resistant tuberculosis. WHP/HTM/TB/2008.402. World Health Organization, Geneva, Switzerland.
- Mouly S, Lown KS, Kornhauser D, Joseph JL, Fiske WD, Benedek IH, Watkins PB. 2002. Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans. *Clin. Pharmacol. Ther.* 72:1–9. <http://dx.doi.org/10.1067/mcp.2002.124519>.
- Zheng J, Rubin EJ, Bifani P, Mathys V, Lim V, Au M, Jang J, Nam J, Dick T, Walker JR, Pethe K, Camacho LR. 2013. para-Aminosalicylic acid is a prodrug targeting dihydrofolate reductase in *Mycobacterium tuberculosis*. *J. Biol. Chem.* 288:23447–23456. <http://dx.doi.org/10.1074/jbc.M113.475798>.
- Peloquin CA. 2002. Therapeutic drug monitoring in the treatment of tuberculosis. *Drugs* 62:2169–2183. <http://dx.doi.org/10.2165/00003495-200262150-00001>.
- Hong L, Jiang W, Zheng W, Zeng S. 2011. HPLC analysis of para-aminosalicylic acid and its metabolite in plasma, cerebrospinal fluid and brain tissues. *J. Pharm. Biomed. Anal.* 54:1101–1109. <http://dx.doi.org/10.1016/j.jpba.2010.11.031>.
- Cummins CL, O'Neil WM, Soo EC, Lloyd DK, Wainer IW. 1997. Determination of p-aminosalicylic acid and its N-acetylated metabolite in human urine by capillary zone electrophoresis as a measure of in vivo N-acetyltransferase 1 activity. *J. Chromatogr. B Biomed. Sci. Appl.* 697:283–288. [http://dx.doi.org/10.1016/S0378-4347\(97\)00084-4](http://dx.doi.org/10.1016/S0378-4347(97)00084-4).
- Wan SH, Pentikainen PJ, Azarnoff DL. 1974. Bioavailability of aminosalicylic acid and its various salts in humans. 3. Absorption from tablets. *J. Pharm. Sci.* 63:708–711.
- Michaud V, Kreutz Y, Skaar T, Ogburn E, Thong N, Flockhart DA, Desta Z. 2014. Efavirenz-mediated induction of omeprazole metabolism is CYP2C19 genotype dependent. *Pharmacogenomics J.* 14:151–159. <http://dx.doi.org/10.1038/tpj.2013.17>.
- Svensson EM, Aweeka F, Park JG, Marzan F, Dooley KE, Karlsson MO. 2013. Model-based estimates of the effects of efavirenz on bedaquiline pharmacokinetics and suggested dose adjustments for patients coinfecting with HIV and tuberculosis. *Antimicrob. Agents Chemother.* 57:2780–2787. <http://dx.doi.org/10.1128/AAC.00191-13>.
- Lee LS, Pham P, Flexner C. 2012. Unexpected drug-drug interactions in human immunodeficiency virus (HIV) therapy: induction of UGT1A1 and bile efflux transporters by efavirenz. *Ann. Acad. Med. Singapore* 41:559–562.
- Turpeinen M, Zanger UM. 2012. Cytochrome P450 2B6: function, genetics, and clinical relevance. *Drug Metab. Drug Interact.* 27:185–197.
- Habtewold A, Amogne W, Makonnen E, Yimer G, Nylen H, Riedel KD, Aderaye G, Bertilsson L, Burhenne J, Diczfalussy U, Aklilu E. 2013. Pharmacogenetic and pharmacokinetic aspects of CYP3A induction by efavirenz in HIV patients. *Pharmacogenomics J.* 13:484–489. <http://dx.doi.org/10.1038/tpj.2012.46>.

33. Ji HY, Lee H, Lim SR, Kim JH, Lee HS. 2012. Effect of efavirenz on UDP-glucuronosyltransferase 1A1, 1A4, 1A6, and 1A9 activities in human liver microsomes. *Molecules* 17:851–860. <http://dx.doi.org/10.3390/molecules17010851>.
34. Ngaimisi E, Mugusi F, Haefeli WE, Bertilsson L, Burhenne J, Aklillu E. 2011. Effect of rifampicin and CYP2B6 genotype on long-term efavirenz autoinduction and plasma exposure in HIV patients with or without tuberculosis. *Clin. Pharmacol. Ther.* 90:406–413. <http://dx.doi.org/10.1038/clpt.2011.129>.
35. Hariparsad N, Nallani SC, Sane RS, Buckley DJ, Buckley AR, Desai PB. 2004. Induction of CYP3A4 by efavirenz in primary human hepatocytes: comparison with rifampin and phenobarbital. *J. Clin. Pharmacol.* 44:1273–1281. <http://dx.doi.org/10.1177/0091270004269142>.
36. Falloon J, Piscitelli S, Vogel S, Sadler B, Mitsuya H, Kavlick MF, Yoshimura K, Rogers M, LaFon S, Manion DJ, Lane HC, Masur H. 2000. Combination therapy with amprenavir, abacavir, and efavirenz in human immunodeficiency virus (HIV)-infected patients failing a protease-inhibitor regimen: pharmacokinetic drug interactions and antiviral activity. *Clin. Infect. Dis.* 30:313–318. <http://dx.doi.org/10.1086/313667>.
37. Aarnoutse RE, Grintjes KJ, Telgt DS, Stek M, Jr, Hugen PW, Reiss P, Koopmans PP, Hekster YA, Burger DM. 2002. The influence of efavirenz on the pharmacokinetics of a twice-daily combination of indinavir and low-dose zidovudine in healthy volunteers. *Clin. Pharmacol. Ther.* 71:57–67. <http://dx.doi.org/10.1067/mcp.2002.121424>.
38. Clarke SM, Mulcahy FM, Tjia J, Reynolds HE, Gibbons SE, Barry MG, Back DJ. 2001. The pharmacokinetics of methadone in HIV-positive patients receiving the non-nucleoside reverse transcriptase inhibitor efavirenz. *Br. J. Clin. Pharmacol.* 51:213–217. <http://dx.doi.org/10.1046/j.1365-2125.2001.00342.x>.
39. Yimer G, Ueda N, Habtewold A, Amogne W, Suda A, Riedel KD, Burhenne J, Aderaye G, Lindquist L, Makonnen E, Aklillu E. 2011. Pharmacogenetic & pharmacokinetic biomarker for efavirenz based ARV and rifampicin based anti-TB drug induced liver injury in TB-HIV infected patients. *PLoS One* 6:e27810. <http://dx.doi.org/10.1371/journal.pone.0027810>.
40. McIlleron H, Meintjes G, Burman WJ, Maartens G. 2007. Complications of antiretroviral therapy in patients with tuberculosis: drug interactions, toxicity, and immune reconstitution inflammatory syndrome. *J. Infect. Dis.* 196(Suppl 1):S63–S75. <http://dx.doi.org/10.1086/518655>.
41. Dybul M, Fauci AS, Bartlett JG, Kaplan JE, Pau AK. 2002. Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. *Ann. Intern. Med.* 137:381–433. http://dx.doi.org/10.7326/0003-4819-137-5_Part_2-200209031-00001.
42. Berning SE, Peloquin CA. 1998. Antimycobacterial agents: para-aminosalicylic acid, p 663–668. In Yu VL, Merigan TC, Barriere S, White NJ (ed), *Antimicrobial chemotherapy*. Williams and Wilkins, Baltimore, MD.
43. Blumberg HM, Burman WJ, Chaisson RE, Daley CL, Etkind SC, Friedman LN, Fujiwara P, Grzemska M, Hopewell PC, Iseman MD, Jasmer RM, Koppaka V, Menzies RI, O'Brien RJ, Reves RR, Reichman LB, Simone PM, Starke JR, Vernon AA. 2003. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am. J. Respir. Crit. Care Med.* 167:603–662. <http://dx.doi.org/10.1164/rccm.167.4.603>.
44. Bridge EV, Carr DT. 1958. Clinical use of a single daily dose of para-aminosalicylic acid in association with isoniazid. *Am. Rev. Tuberc.* 78:749–752.
45. Riska N, Tennberg C. 1962. Optimal PAS dosage. *Am. Rev. Respir. Dis.* 86:430–433.
46. Lehmann J. 1969. The role of the metabolism of p-aminosalicylic acid (PAS) in the treatment of tuberculosis. Interaction with the metabolism of isonicotinic acid hydrazide (INH) and the synthesis of cholesterol. *Scand. J. Respir. Dis.* 50:169–185.
47. Karlsn AG, Carr DT. 1958. Effect of single and of double daily doses of para-aminosalicylic acid in tuberculosis of guinea pigs. *Am. Rev. Tuberc.* 78:753–759.
48. Jindani A, Aber VR, Edwards EA, Mitchison DA. 1980. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. *Am. Rev. Respir. Dis.* 121:939–949.
49. *British Medical Journal*. 1952. Prevention of streptomycin resistance by combined chemotherapy. *Br Med. J.* 1:1157–1162. <http://dx.doi.org/10.1136/bmj.1.4769.1157>.
50. *British Medical Journal*. 1953. Isoniazid in combination with streptomycin or with P.A.S. in the treatment of pulmonary tuberculosis. *Br Med. J.* 2:1005–1014. <http://dx.doi.org/10.1136/bmj.2.4844.1005>.
51. Singh B, Mitchison DA. 1955. Bactericidal activity of streptomycin and isoniazid in combination with p-aminosalicylic acid against *Mycobacterium tuberculosis*. *J. Gen. Microbiol.* 12:76–84. <http://dx.doi.org/10.1099/00221287-12-1-76>.
52. Mathys V, Wintjens R, Lefevre P, Bertout J, Singhal A, Kiass M, Kurepina N, Wang XM, Mathema B, Baulard A, Kreiswirth BN, Bifani P. 2009. Molecular genetics of para-aminosalicylic acid resistance in clinical isolates and spontaneous mutants of *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 53:2100–2109. <http://dx.doi.org/10.1128/AAC.01197-08>.
53. Rengarajan J, Sasseti CM, Naroditskaya V, Sloutsky A, Bloom BR, Rubin EJ. 2004. The folate pathway is a target for resistance to the drug para-aminosalicylic acid (PAS) in mycobacteria. *Mol. Microbiol.* 53:275–282. <http://dx.doi.org/10.1111/j.1365-2958.2004.04120.x>.
54. Mitchison D. 1973. Plasma concentrations of isoniazid in the treatment of tuberculosis, p 171–182. In Davies D, Pritchard B (ed), *Biological effects of drugs in relation to their plasma concentrations*. Macmillan, London, United Kingdom.