The Pharmacokinetics of Para-Aminosalicylic Acid in HIV-uninfected and HIV Co-Infected Tuberculosis Patients receiving antiretroviral therapy, managed for Multidrug-resistant and Extensively Drug-Resistant Tuberculosis

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Running Title: PAS population pharmacokinetics in TB-HIV co-infection
Abstract

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 co-infection 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 effect 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 co-infected. 41 pulmonary MDR or XDR tuberculosis patients received 4g PAS twice-daily or, in a second cross-over study, another 32 patients were randomized receiving 4g PAS twice-daily or 8g 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 minimal inhibitory concentration (MIC) during the dosing interval was estimated by simulation of once-, twice-, and thrice-daily dosing regimens not exceeding 12g daily. Efavirenz (EFV) concurrent medication or possibly HIV-seropositive status 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-TB co-infected patients. Current practice recommends maintenance of PAS concentrations \( \geq 1 \ \mu g/mL \) (the MIC of *M. tuberculosis*), but the model predicts that at only a minimum dose of 4g twice-daily
dosing can this PTA be achieved in at least 90% of the population whether or not EFV was concomitantly administered. Once-daily dosing of 12g PAS will not provide PAS concentrations exceeding MIC over the entire dosing interval if co-administered with EFV, while 4g twice-daily ensures concentrations exceeding MIC over the entire dosing interval, even in HIV-infected patients who received EFV.
**Introduction**

In sub-Saharan Africa, an estimated 79% of tuberculosis (TB) patients are also co-infected with human immunodeficiency virus (HIV) and approximately 50% of acquired immunodeficiency syndrome (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 TB/HIV co-infections, multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB are reaching epidemic proportions (3, 4). MDR-TB is resistant to at least the two first-line drugs, isoniazid and rifampin, while XDR-TB is additionally resistant to fluoroquinolone and at least one of three second-line injectable drugs (4). XDR-TB is associated with a high early mortality among HIV-infected individuals (5), and the scarcity of effective treatment options against MDR/XDR-TB 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 minimal inhibitory concentration (MIC) of approximately 1 µg/mL (9) throughout a dosing interval; current recommendations suggest a dosage of 8-12g PAS daily in two to three divided doses. However in early studies conducted by the British Medical Research Council, PAS was used at a dosage of 20g in four divided
dosages (10). When accompanied by streptomycin, PAS therapy was associated with a five 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 two decades a granular slow-release form of PAS (PASER® granules, para-aminosalicylic acid delayed release 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 the MIC of PAS when dosed at 4g twice daily. It was also shown that 4g once daily failed to maintain concentrations above MIC over a 24 hour 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 drug-drug interactions of PAS with other co-administered 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 uninfected, but this difference did not reach significance (14). Against the background of the very poor outcomes occurring amongst HIV-associated XDR-TB, 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 to evaluate whether once daily dosing can provide PAS concentrations above MIC for the greater part of a dosing interval.

We therefore explored PAS pharmacokinetics in adults with and without HIV infection in a dose of 4g twice daily, but also later in a second study wherein patients were randomized to receive either 4g PAS twice daily or 8g once daily. We aimed to evaluate whether once daily dosing with a slow-release preparation might provide satisfactory concentrations throughout a 24 hour 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 in nature 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.

Patients 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 PAS 4g twice-daily and thirty-two patients were enrolled in a randomized, cross-over, open-label study and received 4g twice-daily or an 8g once-daily regimen. Those with severe anemia, diarrhea or dehydration were excluded. Patients, between aged 18-64 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 (PASER® granules, para-aminosalicylic acid delayed release granules, Jacobus Pharmaceutical, Princeton, NJ, USA). In the first study, the subjects received 4g 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 pre-dose and 2, 3, 4, 5, 6, 8 and 12 hours post-dose.

The second study was a randomized cross-over study where patients received either 4g PAS twice daily or an 8g PAS once daily regimen on the first occasion followed by the alternative dosing scheme on the 2nd occasion. During both studies samples were taken only after patients had received PAS 4g twice daily for at least two weeks and patients who entered in the crossover study received the relevant regimen for another eight days before the pharmacokinetic study. Samples for pharmacokinetic analysis were collected at pre-dose and 1, 2, 3, 4, 5, 6, 8 and 12 hours after 4g twice daily; and at pre-dose and 1, 2, 3, 4, 6, 8, 12 and 24 hours after 8g once daily. To prevent early PAS release in the stomach, doses were routinely taken with acidic food or beverage. 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).
The analysis of quality control samples reported good reproducibility with coefficient of variation of ≤5% and accuracy with between-sample differences not exceeding 2.5%. The lower limit of quantification was 0.01 μg/mL. All of the observed drug concentrations were above this limit.

**Pharmacokinetic Analysis.** The nonlinear mixed-effects modeling of PAS disposition in TB-HIV co-infected 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) were explored for the steady-state concentration-time profiles of patients who were administered once-daily 8g or twice-daily 4g PAS. 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, $\eta_i$ and $\kappa_{ij}$ describe interindividual and interoccasion (within an individual) variability and were assumed to be independently and normally distributed, both with mean zero and variances $\omega_P^2$ and $\pi_P^2$, respectively. The subscript i represents the individual and j represents the occasion for the individual.
Dummy variables were introduced to distinguish separate occasions wherein 1 was designated for the first occasion wherein individuals received the first 4g twice-daily, 2 was indicated for the second 4g twice-daily and 3 was used for the 8g once-daily dosing 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 model.

Covariates that were evaluated for their potential effect on PAS disposition included the following: total body weight, age, HIV status, gender, race, and concomi-tant medications. The concomitant medications were evaluated individually for their effect on PAS disposition. Dummy variables consisting of 0 and 1 were introduced for absence or 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 pharmacokinetic, EFV effect was tested on the apparent oral clearance. The covariate model for the EFV effect on the apparent oral clearance was based on

$$TV_{CL/F} = \theta_1 \times \exp (\theta_2 \times I_{COV})$$ (2)

where $\theta_1$ is the typical value (TV) for the population CL/F in the absence of EFV concomitant medication, $\theta_2$ 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. Dose effect of EFV could not be evaluated as all patients who received EFV had 600 mg oral once-daily dose.
HIV status of the individual was also evaluated. The three additional HIV-seropositive individuals who were not on ART containing EFV were included in the category for HIV status. The evaluation process for the effect of HIV status on the pharmacokinetic 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 5000 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 dataset 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 level at p=0.05 and 0.01.

Monte Carlo Simulations of Dosage Regimens and Probability of Target Attainment. Since TB-HIV patients have compromised immunity, a conservative pharmacodynamic target of trough concentration of the free drug ($fC_{\text{min}} > 1 \mu g/mL$) 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 50-60% (19). In the simulation, the free PAS was assumed to be 50% of the observed total PAS concentration. PAS activity against Mycobacterium tuberculosis and Mycobacterium bovis is usually considered bacteriostatic (20); maintaining a PAS plasma concentration above the typical minimum inhibitory concentration (MIC) of MTB at 1 $\mu g/mL$ 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 PAS in
the slow-release formulation recommends an adult dosage of 12g/day in 3 divided
doses. World Health Organization in 2008 suggested a PAS dosage of 8 g daily for
patients weighing 33-70 kg, although at another point, a dosage of 10-12 g in two
divided doses is recommended (21).

Several dosing regimens were simulated with 5000 virtual subjects to determine
the probability of attaining the pharmacodynamic target: (1) 4g every 8 and 12 h; (2) 6g
every 12 and 24 h; (4) 8g once daily; and (5) 12g once daily. The target was attained
when 90% of the simulated $f_{C_{min}}$ was greater than the MIC. The probability of target
attainment (PTA) was computed over a range of 2-fold incrementing MICs (range: 0.128
to 4 μg/mL).

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
4g twice-daily study and 32 patients in the cross-over study; data from all 73 patients
were used in the analysis; 26% were HIV co-infected, 21% had XDR TB, and 79% 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 (Figure 1) (16). The model provided reasonable fits to the observed
concentration-time profiles (Figure 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 Figure 2. The conditional weighted residuals (CWRES) versus time and CWRES versus the population predicted PAS concentrations in Figure 2 show that most of the data lies within 2 units from 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 exception of the first-order absorption with lag-time model, the other absorption models were unstable and did not result in successful convergence, including those with boundary problems and undeterminable rounding of significant digits. The transit compartment model was selected as the objective function as markedly lower than that of the first-order absorption with lag time model. Allometric scaling by body weight did not improve interindividual variability, consequently 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=0.05 and 0.01, respectively to detect differences in PAS pharmacokinetics. Similarly, for an 80% power at significance level of p=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 \( p=0.01 \) level. 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 
estimated PAS AUCs over a day \( (\text{AUC}_{0-24h}) \) from the population PK analysis without and 
with EFV were 875 ± 424 and 621 ± 295 mg.h/L in the 4g twice-daily and 889 ± 493 and 
625 ± 338 mg.h/L in 8g 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 as 
not significant at \( p=0.05 \) level. 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 (CV\%) in 4g 
twice-daily and 8g 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 reduction in the PAS half-life in EFV co-medication 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 Figure 4. The top panels are 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 co-administered EFV was reflected in lower plasma PAS concentrations and areas under the concentration-time curve. In the computation of the free Cmin of PAS, the fraction unbound 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 Figure 5.

The PTA was based on the free trough PAS concentration (fCmin) above the MIC values during the entire dosing interval. When 4g PAS every 12 h was simulated, PTA >90% were obtained for MIC values of 0.5, 1 and 2 μg/mL in virtual patients with concomitant EFV. Those patients who are not taking EFV can achieve PTA >90% at the same dosage regimen even with hypothetical MIC values of 3 μg/mL. Higher PTAs were achieved when the dosing frequency was increased to thrice daily. With 6g twice daily, the PTA was achieved at MICs of 3 μg/mL and 5 μg/mL with and without EFV, respectively.
The once-daily dosage regimens of 6g, 8g and even 12g are at risk of not achieving PTA at MIC of 1 μg/mL. With the exception of 12g once-daily dosing without EFV, PTA for the other remaining dosage regimens were below 90% (Figure 5 middle and right panels). The 5th percentiles of free PAS trough concentrations in the once-daily dosing were below the 1 μg/mL for the three doses, regardless of whether EFV was coadministered or not.

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 TB-HIV co-infected 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 recently shown to be a prodrug that acts as a metabolic precursor of the folate biosynthetic pathway (23). More than 80% of absorbed PAS is excreted renally; the other 20% is cleared following metabolism (24). PAS is primarily metabolized by N-acetyltransferase (NAT) 1 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 of 3- to 4-fold increase in CYP3A4 activity was observed at 5 to 10 μM efavirenz concentration (35). Consequently, CYP3A4 substrates when administered with efavirenz resulted in a reduction in plasma levels of these drugs (36-38). EFV was thought to only affect the hepatic 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 TB-HIV patients (39). Even though their study was not designed to evaluate efavirenz effect 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 TB-HIV co-infection, 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 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 μg/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 μg/mL throughout the dosing interval is believed to at least maintain its bacteriostatic activity, as PAS lacks a post-antibiotic effect (12, 20, 42). Our results show that HIV-TB coinfected patients taking EFV are not at risk of PAS concentrations falling below 1 μg/mL at 4g PAS twice-daily dosing. Peloquin et al. evaluated 4g twice-daily and 4g once-daily PAS doses and determined that the 4g twice daily doses were adequate to maintain PAS concentrations >1 μg/mL throughout the dosing interval, but not 4 g once daily(7, 12). The Centers for Disease Control and Prevention recommendation for 8g – 12g daily in 2 – 3 equally divided doses (43) is consistent with our findings that equally divided doses of equal to or greater than a daily total dose of 8g would provide sufficient exposure above 1 μg/mL.

Several earlier studies compared once daily and divided dosing and found that adult patients tolerated gastro-intestinal side effects better with once daily dosage regimens (44-46). Single daily dosing 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 between maintenance of a plasma concentration above 1 μg/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 6g, 8g and 12g. The target attainment of at least 90% of the population maintaining greater
than 1 μg/mL free PAS concentration was unlikely for the once-daily dosage regimens, except for the 12g PAS without EFV concomitantly administered. Our study has shown that the 4g twice-daily PAS dosage achieves a 90% PTA for $f_{C_{\text{min}}}>1$ μg/mL even with concurrent EFV in HIV-positive patients with TB. However, the once-daily dosing even at 12g 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 (1980) studied the early bactericidal activity (EBA) of anti-TB agents by quantifying the serial reduction of colony forming units (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 only preceded 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 four 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 MIC (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 MIC 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 μg/mL (52). The pharmacokinetic-pharmacodynamic index of PAS is still debatable as to whether the improved action of PAS in preventing resistance in companion drugs was due to the maintenance of concentrations above MIC or to the higher peak concentrations. In the case of isoniazid when 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 TB-HIV patients who are on concurrent EFV. When ARV therapy contains EFV, once daily dosing up to 12g PAS in these patients is not recommended. If maintenance of PAS concentrations above MIC is considered advisable, a regimen of twice-daily 4 g provides sufficient pharmacodynamic coverage of PTA above 90% over the dosing interval and PTA of 100% is achieved with thrice-daily dosage regimens of 12g/day PAS. The possible advantages regarding increased efficacy and ease of programmatic administration of once daily higher doses of PAS and the role of PAS deserves further exploration particularly for the very vulnerable group of HIV-infected patients with MDR and XDR TB.
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Competing interests. The authors have no competing interests to declare.


Figure Legends:

Figure 1: Schematic representation of para-aminosalicylic acid pharmacokinetic model.

Figure 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 loess regression. The solid lines are the expected lines of unity (upper graphs) and zero lines (lower graphs).

Figure 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.

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

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

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

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

Table 3: Population pharmacokinetic model parameters of the final model evaluating pharmacokinetic interaction of para-aminosalicylic acid with efavirenz
Table 1. Characteristics of 73 patients enrolled in studies of the pharmacokinetics of para-aminosalicylic acid in tuberculosis patients with and without HIV infection

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<tr>
<th>Characteristics</th>
<th>Median/Count ( % of total or range)</th>
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<tr>
<td>Gender, n (% of total)</td>
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<td>Male</td>
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</tr>
<tr>
<td>HIV-infected</td>
<td>22 (30.1%)</td>
</tr>
<tr>
<td>HIV-infected and receiving efavirenz</td>
<td>19 (26.0%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Drug category</th>
<th>Drug names (number of individuals on the drug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-tuberculosis agents and antibiotics</td>
<td>Capreomycin (55), moxifloxacin (30), ethionamide (60), terizidone (69), pyrazinamide (64) ethambutol (64), isoniazid (35), clofazimine (7), dapsone (6) and kanamycin (4), clarithromycin (4)</td>
</tr>
<tr>
<td>Diabetic drugs</td>
<td>Metformin (3), glibenclamide (1), actraphane(1), humulin (3)</td>
</tr>
<tr>
<td>Antiretroviral therapy</td>
<td>Efavirenz (19), stavudine (18), zidovudine (2), lopinavir/ritonavir (3), lamivudine (21), lamivudine/zidovudine (1)</td>
</tr>
<tr>
<td>Antidepressants and psychotic drugs</td>
<td>Amitriptyline (15), fluoxetine (1), diazepam (1), haloperidol (1), chlorpromazine (7), lorazepam (1)</td>
</tr>
<tr>
<td>Supplements and other drugs</td>
<td>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), FeSO₄ (2), (sulfamethoxazole and trimethoprim (1)</td>
</tr>
</tbody>
</table>
Table 3. Population pharmacokinetic model parameters of the final model evaluating the pharmacokinetic interaction of para-aminosalicylic acid with efavirenz

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Final Model Mean (RSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural model parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Apparent Oral Clearance (CL/F, L/h)</td>
<td>9.42 (6.6%)</td>
</tr>
<tr>
<td>Apparent Volume of Distribution (V/F, L)</td>
<td>51.8 (6.7%)</td>
</tr>
<tr>
<td>Transit rate constant (Ktr, h⁻¹)</td>
<td>0.65 (6.8%)</td>
</tr>
<tr>
<td>Number of transit compartments</td>
<td>3</td>
</tr>
<tr>
<td><strong>Interindividual variability</strong></td>
<td></td>
</tr>
<tr>
<td>%CV of CL/F (ω_{CL})</td>
<td>47.5 (9%)</td>
</tr>
<tr>
<td>%CV of V/F (ω_{V})</td>
<td>74.8 (11.9%)</td>
</tr>
<tr>
<td>%CV of Ktr (ω_{Ka})</td>
<td>43.5 (13.2%)</td>
</tr>
<tr>
<td><strong>Interoccasion variability</strong></td>
<td></td>
</tr>
<tr>
<td>%CV of CL/F (κ_{CL})</td>
<td>49.8 (13.1%)</td>
</tr>
<tr>
<td>%CV of Ktr (κ_{Ktr})</td>
<td>47.7 (13.1%)</td>
</tr>
<tr>
<td><strong>Residual variability</strong></td>
<td></td>
</tr>
<tr>
<td>Proportional residual error</td>
<td>0.269 (12%)</td>
</tr>
<tr>
<td>Additive residual error (µg/mL)</td>
<td>7.90 (10.8%)</td>
</tr>
<tr>
<td><strong>Covariates</strong></td>
<td></td>
</tr>
<tr>
<td>Efavirenz on CL/F‡</td>
<td>0.419 (33%)</td>
</tr>
<tr>
<td>ΔOFV (from baseline model)</td>
<td>-8.94</td>
</tr>
</tbody>
</table>

Abbreviations: PI, prediction interval; OFV, minimum objective function value; RSE, relative standard error.

‡The covariate equation to describe the effect of efavirenz on CL/F is $\frac{CL/F_i}{CL/F_0} = \frac{CL/F_0 \exp(\theta_1 \cdot t_{ESV})}{CL/F_0}$. 
Oral Dose $\rightarrow$ PAS

3 transit compartments
Fig. 5