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Effect of *SLCO1B1* Polymorphisms on Rifabutin Pharmacokinetics in African HIV-Infected Patients with Tuberculosis

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Rifabutin, used to treat HIV-infected tuberculosis, shows highly variable drug exposure, complicating dosing. Effects of *SLCO1B1* polymorphisms on rifabutin pharmacokinetics were investigated in 35 African HIV-infected tuberculosis patients after multiple doses. Nonlinear mixed-effects modeling found that influential covariates for the pharmacokinetics were weight, sex, and a 30% increased bioavailability among heterozygous carriers of *SLCO1B1* rs1104581 (previously associated with low rifampin concentrations). Larger studies are needed to understand the complex interactions of host genetics in HIV-infected tuberculosis patients. (This study has been registered at ClinicalTrials.gov under registration no. NCT00640887.)

Rifabutin is an alternative rifamycin for tuberculosis treatment. It is also used to treat other mycobacterial infections and to prevent *Mycobacterium avium* complex in patients with AIDS. Unlike rifampin, rifabutin does not reduce concentrations of concomitantly administered protease inhibitors (PIs) significantly (1). The pharmacokinetics of rifabutin are highly varied (2–4). As a CYP3A4 substrate, rifabutin is subject to drug interaction with CYP3A4 inhibitors, such as PIs, and increases in exposure can result in an increased risk for adverse effects, particularly uveitis. Toxicity, including uveitis, neutropenia, and hepatotoxicity, are a concern with high exposures (5). Conversely, low rifabutin exposures are associated with relapse and acquired rifamycin resistance (6). While therapeutic drug monitoring is advocated, it is seldom available (5). Although a lack of suitable formulations and cost limit the widespread use of the drug in resource-constrained settings, its use in combination with PIs is increasing as antiretroviral therapy (ART) programs mature and more patients are started on 2nd-line PI-based regimens.

In a process subject to autoinduction, arylacetamide deacetylase converts rifabutin to the active primary metabolite 25-desacetyl rifabutin, which is in turn metabolized by CYP3A4 (7, 8). Organic anion-transporting polypeptide 1B1 (OATP1B1) mediates hepatocellular influx of diverse xenobiotics prior to their excretion in bile (9). Functional single nucleotide polymorphisms (SNPs) in *SLCO1B1*, the gene encoding OATP1B1, have been associated with significant alterations in drug pharmacokinetics. *SLCO1B1* rs4149032 and rs11045819 have been associated with lower rifampin and lopinavir concentrations (10–12), while the rs4149056 SNP is associated with higher concentrations of lopinavir and other drugs, including statins (13, 14). The *SLCO1B1* rs2306283 variant is associated with increased OATP1B1 expression (15). The allele frequencies of *SLCO1B1* vary markedly between different populations (16). We recently showed that *SLCO1B1* rs4149032 is carried by 70% of South Africans, in whom its presence predicts reduced rifampin concentrations (10). Since little is known about pharmacogenomic determinants of rifabutin exposure, we investigated the frequencies of *SLCO1B1* SNPs rs4149032, rs11045819, rs4149056, and rs2306283 and their effects, along with those of other covariate factors, on the pharmacokinetics of rifabutin in HIV-infected patients with tuberculosis prior to initiation of ART.

The pharmacokinetics and safety of rifabutin were investigated in 44 patients with HIV-associated tuberculosis as part of the ANRS 12150a trial (ClinicalTrials.gov registration no. NCT00640887). After 6 weeks on standard antituberculosis treatment, patients were switched from rifampin to rifabutin (300 mg daily) for the last 2 weeks of the intensive phase (with standard isoniazid, pyrazinamide, and ethambutol doses) and for the first 2 weeks of the continuation phase (with standard isoniazid doses). All study participants had microbiologically confirmed pulmonary tuberculosis, HIV infection (CD4 lymphocyte count, 50 to 200 cells/mm^3), a weight of $\geq$50 kg or a body mass index of $>$18, a Karnofsky score of $\geq$80%, and no grade 3 or 4 clinical or laboratory findings according to DMID tables (17).

After 4 weeks on rifabutin at 300 mg daily without ART, patients were admitted for pharmacokinetic evaluation. Following an overnight fast, blood samples were drawn immediately before dosing and at 2, 3, 4, 5, 6, 8, 12, and 24 h after dosing. A standard hospital breakfast (oats with 2 slices of toast and tea) was served $>$2 h after dosing. Samples were placed on ice, until the plasma was separated and stored at −80°C, within 30 min of sampling. Forty-two patients provided

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TABLE 1 Allele frequencies

Allele	No. of patients with the indicated polymorphisms (no. of patients with missing data regarding the polymorphism)
SLCO1B1 rs4149032	CC = 5, CT = 11, TT = 17 (9)
SLCO1B1 rs2306283	AG = 8, GG = 27 (7)
SLCO1B1 rs4149056	CC = 34, CT = 1 (7)
SLCO1B1 rs11045819	AC = 5, CC = 30 (7)

additional written informed consent for the pharmacogenetic testing. A whole-blood sample was collected and stored for genetic analysis.

Rifabutin and 25-desacetyl rifabutin were assayed by liquid chromatography-tandem mass spectrometry (LC/MS/MS) as described previously (18). For both analytes, interbatch accuracy (percent of the nominal concentration [%Nom]) was 99.1 to 109.0%, and the precision (percent coefficient of variation [%CV]) was <9.2% at low, medium, and high quality control (QC) levels. The calibration ranges were 3.91 to 1,000 ng/ml and 0.780 to 200 ng/ml for rifabutin and 25-desacetyl rifabutin, respectively.

Genotyping for *SLCO1B1* rs4149032, rs2306283, rs4149056, and rs11045819 was performed using real-time PCR allelic discrimination by standard methodology (see the supplemental material).

The pharmacokinetics of rifabutin and 25-desacetyl rifabutin were described using a population nonlinear mixed-effects model in NONMEM (19; see also the supplemental material). Structural base model building was followed by covariate model development. First, the influence of a patient's weight and lean body weight, respectively, were investigated on all apparent clearance and apparent volume parameters of rifabutin using allometric scaling *a priori* (20). The influences of age, sex, and the SNPs (*SLCO1B1* rs414903, rs2306283, and rs11045819) on model parameters were then each investigated in a stepwise fashion. As only 1 subject carried rs4149056, this SNP was not included in the covariate analysis.

The final model was used in Monte Carlo simulations (500 simulations of the original study design) to estimate the areas under the concentration-time curve over the 24-h dosing interval (AUC_{0-24}) for rifabutin and the metabolite ($AUCM_{0-24}$) and investigate the relevance of dose adjustment based on significant covariate factors. Differences in AUC measures between males and females and rs11045819 carriers and noncarriers were evaluated using the Mann-Whitney-Wilcoxon test (RStudio version 0.98.501).

Forty-four patients (61% males) with a mean (standard deviation [SD]) weight, height, body mass index, age, and CD4 lymphocyte count of 60.7 (8.7) kg, 159.6 (7.7) cm, 22.8 (3.3) kg/m^2, 32.7 (5.9) years, and 126.1 (44.0) cells/mm^3, respectively, contributed 780 pharmacokinetic observations. The Karnofsky score was 100 for all patients. All patients were of black African ethnicity. Genetic samples were not available for 7 of these patients, and for a further 2 patients, analysis of rs4149032 was unsuccessful (Table 1).

A two-compartment model with first-order absorption after a lag time and first-order elimination from the central compartment best described rifabutin pharmacokinetics. Simultaneously, metabolism to the 25-desacetyl rifabutin metabolite was modeled via a first-order process. The metabolite model was also best described by two compartments, with linear elimination from the central compartment (see Fig. S1 in the supplemental material). The final population parameter estimates are shown in Table 2. Body weight allometrically scaled (20) on the basis of rifabutin apparent clearances (from the central compartment, intercompartmental clearance, clearance to the metabolite), and apparent central and peripheral volume of distribution (V and V_{per}, respectively) improved the model (change in objective function value [ΔOFV] = −6.79). Males had a 1.84-times-higher V for rifabutin than females (ΔOFV = −20.9), accounting for a 31.3% reduction in between-subject variability (BSV) on the central volume of distribution (V over bioavailability [F]). After weight and gender were included in the model, we evaluated the effects of rs4149032, rs2306283, and rs11045819 on F (with the nominal population value of 1), the apparent oral clearance of rifabutin via metabolism to 25-desacetyl rifabutin (CL_e/F), the rifabutin intercompartmental clearance (Q), the apparent oral clearance of 25-desacetyl rifabutin (CL_m/F), and the volumes of distribution of the parent and the metabolite. The rs11045819 SNP was associated with a 30% increase in F (ΔOFV = −6.5) and a reduced BSV for F by 8.9%.

Although the drop in OFV was significant, the changes visible in the visual predictive check (VPC) were minor. Inclusion of other SNP effects on the pharmacokinetic parameters did not statistically improve the model fit. The VPC (see Fig. S2 in the supplemental material) displayed good model predictability, and other goodness-of-fit plots (see Fig. S3 in the supplemental material) further validated the final model. The influence of all covariate effects on overall exposures (AUC_{0-24}, $AUCM_{0-24}$) of rifabutin and the metabolite for subpopulations can be found in Table S1 in the supplemental material.

Conclusions. We investigated the effect of *SLCO1B1* polymorphisms, weight, and sex on rifabutin concentrations in an African population with HIV-associated tuberculosis. We found that rifabutin bioavailability was 30% higher among heterozygous carriers of the *SLCO1B1* rs11045819 polymorphism than among noncarriers. The effect on bioavailability was significant within the model, resulting in a significant difference between the estimated exposures for carriers and noncarriers (see Table S1 in the supplemental material). Larger studies are needed to confirm the effect and to characterize its effect on rifabutin exposure in patients. Interestingly, prior studies associated this polymorphism with reduced rifampin and lopinavir concentrations (11, 12). *SLCO1B1* rs4149032 was also associated with reduced rifampin exposure in South African tuberculosis patients (10). We did not find this polymorphism to affect rifabutin exposure; however, our study included insufficient carriers of this polymorphism to exclude an association. While rs4149056 is more frequent in Asian and Caucasian populations than in other populations, the low frequency of this SNP in our study (Table 1) is consistent with the reported population frequency (0.7 to 11.5%) in sub-Saharan Africa (21).

Relationships between drug concentrations and OATP1B1 variants are complex. SNPs may be associated with altered OATP1B1 expression or loss of function (12–14). Moreover, rifampin inhibits OATP1B1 (13), while rifampin but not rifabutin induces OATP1B1 mRNA expression in hepatocytes incubated with 0.5, 5, or 10 μM concentrations of the drugs (9). A better understanding of these complex factors is necessary to explain the disparate effects of *SLCO1B1* rs1104581 on rifampin and rifabutin. As lopinavir is a substrate and inhibitor of OATP1B1 (22) and,

TABLE 2 Population pharmacokinetic parameter estimates of the base model and the final model for rifabutin and 25-desacetyl rifabutin[a]

Parameter	Final-model result with an OFV of −1,413.8		Median bootstrap result with an OFV of −1,432.5 and 7.8% RSE		Base-model result with an OFV of −1,386.4	
	Value	% BSV	Value (% RSE)	% BSV (% RSE)	Value	% BSV
Rifabutin parameters						
CL/*F* (liters/h/70 kg)	116.5	12.0	108.2 (18.2)	11.6 (126.8)	114.3	16.0
V/F (liters/70 kg)	117.8	49.0	121.6 (53.8)	52.3 (54.0)	148.8	58.0
k_a (1/h)	0.24	23.9	0.22 (47.7)	24.9 (81.5)	0.21	26.0
Lag time (h)	1.6	24.7	1.7 (8.6)	20.2 (69.2)	1.5	25.0
F (fixed)	1	33.0	1	28.3 (35.2)	1	34.0
Q/F (liters/h/70 kg)	123.8		121.9 (23.0)		111.9	
V_{per}/F (liters/70 kg)	4,897.8		4,904.8 (116.1)		4,663.8	
CL_e/F (metabolism of RBN to des-RBN)	21.2		21.2 (52.5)		18.8	
25-Desacetyl rifabutin parameters						
CL_m/F (liters/h)	196.7	30.0	200.4 (53.8)	27.5 (20.5)	174.1	30.0
V_m/F (liters)	3.9		3.8 (77.8)		3.5	
Q_m/F (liters/h) (fixed)	0.15				0.15	
$V_{m\text{-per}}/F$ (liters) (fixed)	536.8				536.8	
Residual errors						
Proportional error for rifabutin (%)	34.6		33.8 (18.3)		34.6	
Proportional error for 25-desacetyl rifabutin (%)	34.6		34.2 (28.6)		33.2	
Additive error for rifabutin (ng/ml)	14.0		12.8 (13.9)		14.4	
Additive error for 25-desacetyl rifabutin (ng/ml)	1.2		1.3 (24.7)		1.17	
Covariate effects						
Increase of *V/F* for males (factor)	1.8		1.3 (33.3)			
Increase in *F* (%) for rs11045819 genotype	30.4		39.7 (71.6)			

[a] CL, clearance; *V*, volume of distribution for rifabutin in the central compartment; V_{per}, volume of distribution for rifabutin in the peripheral compartment; k_a, first-order absorption rate constant; *F*, bioavailability; *Q*, intercompartmental clearance for rifabutin; CL_e, clearance of rifabutin to 25-desacetyl rifabutin; RBN, rifabutin; des-RBN, 25-desacetyl rifabutin; CL_m, clearance of 25-desacetyl rifabutin; V_m, volume of distribution for 25-desacetyl rifabutin in the central compartment; $V_{m\text{-per}}$, volume of distribution for 25-desacetyl rifabutin in the peripheral compartment; Q_m, intercompartmental clearance for des-rifabutin; BSV, between-subject variability; RSE, relative standard error; OFV, objective function value. The base model included weight allometrically scaled on the basis of CL/*F*, *V/F*, *Q/F*, and V_{per}/F.

like rifabutin and 25-desacetyl rifabutin, is a CYP3A4 substrate, further studies are needed to evaluate the impact of genetic variants on rifabutin pharmacokinetics, safety, and efficacy with concomitant lopinavir-ritonavir.

A further finding in this study is the effect of gender on the distribution of rifabutin after adjusting for weight. A possible explanation for the importance of both weight and gender effects may be differences in body composition between men and women (23, 24). However, allometric scaling using lean body weight and total body weight, respectively, were tested in the model, and total body weight was superior.

In conclusion, we explored factors contributing to a wide variability in rifabutin exposures in HIV-infected patients with tuberculosis. The *SLCO1B1* rs1104581 polymorphism, weight, and gender appear to play important roles; however, larger studies are needed to confirm these effects before they can be used to optimize dosing.

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