The effect of *SLCO1B1* polymorphisms on the pharmacokinetics of rifabutin in African HIV-infected patients with tuberculosis

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Abstract

Rifabutin used in HIV-infected tuberculosis shows highly variable drug exposure complicating dosing. Effects of *SLCO1B1* polymorphisms on rifabutin pharmacokinetic were investigated in 35 African HIV-infected tuberculosis patients after multiple dosing. Nonlinear mixed-effects modelling found influential covariates on the pharmacokinetics were weight, sex and a 30% increased bioavailability amongst heterozygous carriers of *SLCO1B1* rs1104581 (previously associated with low rifampicin concentrations). Larger studies are needed to understand the complex interactions of host genetics in HIV-infected tuberculosis patients.

Introduction

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 rifampicin, rifabutin does not reduce concentrations of concomitantly administered protease inhibitors (PI) significantly (1). The pharmacokinetics of rifabutin are highly variable. (2-4) As a CYP3A4 substrate rifabutin is subject to drug interaction with CYP3A4 inhibitors, such as PIs and increases the 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 is increasing in combination with PIs as 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 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 rifampicin 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*

63 rs2306283 variant is associated with increased OATP1B1 expression (15). The allele
64 frequencies of *SLCO1B1* vary markedly between different populations (16). We
65 recently showed that *SLCO1B1* rs4149032 is carried by 70% of South Africans in
66 whom it predicted reduced rifampicin concentrations (10). Since little is known about
67 pharmacogenomic determinants of rifabutin exposure, we investigated the frequencies
68 of *SLCO1B1* SNPs rs4149032, rs11045819, rs4149056 and rs2306283, and their
69 effects along with other covariate factors, on the pharmacokinetics of rifabutin in
70 HIV-infected patients with tuberculosis prior to initiation of ART.

71 ## *Methods*

72 The pharmacokinetics and safety of rifabutin was investigated in 44 patients with
73 HIV-associated tuberculosis as part of the ANRS 12150a trial (ClinicalTrials.gov
74 registration no. NCT00640887). After 6 weeks on standard antituberculosis treatment,
75 patients were switched from rifampicin to rifabutin 300 mg daily for the last 2 weeks
76 of the intensive phase (with standard isoniazid doses, pyrazinamide and ethambutol)
77 and for the first 2 weeks of the continuation phase (with standard isoniazid doses). All
78 study participants had microbiologically confirmed pulmonary tuberculosis, HIV
79 infection (CD4 lymphocyte count 50-200 cells/mm^3), weight ≥50kg or BMI > 18, a
80 Karnofsky score Q ≥80% and no grade 3 or 4 clinical or laboratory findings according
81 to DMID tables (17).
82 After 4 weeks on rifabutin 300 mg daily without ART, patients were admitted for
83 pharmacokinetic evaluation. Following an overnight fast, blood samples were drawn
84 immediately before dosing and at 2, 3, 4, 5, 6, 8, 12 and 24 h after dosing. A standard
85 hospital breakfast (oats with 2 slices of toast and tea) was served >2 hours after
86 dosing. Samples were placed on ice, until the plasma was separated and stored
87 at -80^0C, within 30 minutes of sampling. Forty-two patients provided 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 LC/MS/MS as described previously (18). For both analytes, inter-batch accuracy (%Nom) was 99.1-109.0% and precision (%CV) was <9.2% at low, medium and high QC levels. The calibration ranges were 3.91-1000 ng/ml and 0.780-200 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 (Supplementary material).

The pharmacokinetics of rifabutin and 25-desacetyl rifabutin were described using a population nonlinear mixed-effects model in NONMEM (19) (Supplementary material). Structural base model building was followed by covariate model development. Firstly, the influence of 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). Influence of age, sex and the SNPs (*SLCO1B1* rs414903, rs2306283 and rs11045819 respectively) 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 simulation of the original study design) to estimate area under the concentration–time curve (AUC) over the 24h dosing interval ($AUC_{0–24}$) for rifabutin, and 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 non-carriers were evaluated using the Mann-Whitney Wilcoxon test (Rstudio Version 0.98.501).

Results

Forty-four patients (61% males) with mean (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 Karnovsky score was 100 in all patients. All patients were of Black African ethnicity. Genetic samples were not available for 7 of these patients and in a further 2 patients analysis of rs4149032 was unsuccessful (Table 1).

A 2-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 2-compartments with linear elimination from the central compartment (Fig. S1 in the supplemental material). The final population parameter estimates are shown in Table 2. Body weight allometrically scaled (20) on rifabutin apparent clearances (from the central compartment; inter-compartmental clearance; clearance to the metabolite) and apparent central and peripheral volume of distribution improved the model (change in objective function value (ΔOFV)=-6.79). Males had a 1.84 times higher central volume of distribution for rifabutin than females (ΔOFV=-20.9), accounting for a 31.3% reduction in between subject variability (BSV) on V/F. After weight and gender were included in the model the effects of rs4149032, rs2306283 and rs11045819 were evaluated on bioavailability (F; with the nominal population value of 1), apparent oral metabolism clearance to des-rifabutin (CL_e/F), rifabutin

intercompartmental clearance (Q), apparent oral clearance of des-rifabutin (CL_m/F), and volumes of distribution of the parent and metabolite. The rs11045819 SNP was associated with a 30% increase in F (ΔOFV=-6.5) and reduced BSV on 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 (Fig. S2 in the supplemental material) displayed good model predictability and other goodness-of-fit plots (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 supplemental material Table S1.

Discussion

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 amongst heterozygous carriers of the *SLCO1B1* rs11045819 polymorphism compared to non-carriers. The effect on bioavailability was significant within the model, resulting in a significant difference between the estimated exposures for carriers and non-carriers (Supplemental material Table 1). Larger studies are needed to confirm the effect and characterize its effect on rifabutin exposure in patients. Interestingly, prior studies associated this polymorphism with reduced rifampicin 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, the low frequency of this SNP in our study (Table 1) is consistent with the reported population frequency (0.7-11.5%) 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, rifampicin inhibits OATP1B1 (13), while rifampicin but not rifabutin induced 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 rifampicin and rifabutin. As lopinavir is a substrate and inhibitor of OATP1B1 (22) and, like rifabutin and 25-desacetyl rifabutin, is a CYP3A4 substrate, further studies are needed to evaluate the impact of genetic variants on rifabutin pharmacokinetic, 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 could 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 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 could be used to optimize dosing.

Tables

Table 1. Allele Frequencies

Allele	***Numbers of patients with the polymorphism (Number 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)

188 **Table 2:** Population pharmacokinetic parameter estimates of the base model and the final model for rifabutin and des-rifabutin

	Final model		***Bootstrap results***		***Base model***	
			Median (RSE %)			
OFV	-1413.8		-1432.5 (7.8)		-1386.4	
Rifabutin parameters		***BSV (%)***		***BSV (%)***		***BSV (%)***
Clearance Cl/F (L/h/70 kg)	116.5	12.0	108.2 (18.2)	11.6 (126.8)	114.3	16.0
Central volume of distribution V/F (L/70kg)	117.8	49.0	121.7.6 (53.8)	52.3 (54.0)	148.8	58.0
Absorption rate constant 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
Bioavailability F (Fixed)	1	33.0	1	28.3 (35.2)	1	34.0
Q/F (L/h/70 kg)	123.8		121.9 (23.0)		111.9	
V_{per}/F (L/70kg)	4897.8		4904.8 (116.1)		4663.8	
Cl_e/F (metabolism of RBN to des-RBN)	21.2		21.2 (52.5)		18.8	
des-Rifabutin parameters						
Cl_m/F (L/h)	196.7	30.0	200.4 (53.8)	27.5 (20.5)	174.1	30.0

V_m/F (L)	3.9	3.8 (77.8)	3.5
Q_m/F (L/h) (Fixed)	0.15		0.15
$V_{m\text{-}per}$/F (L) (Fixed)	536.8		536.8
Residual error			
Proportional error rifabutin (%)	34.6	33.8 (18.3)	34.6
Proportional error des-rifabutin (%)	34.6	34.2 (28.6)	33.2
Additive error rifabutin (ng/ml)	14.0	12.8 (13.9)	14.4
Additive error des-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 bioavailability F (%) for rs11045819 genotype	30.4	39.7 (71.6)	

Cl-clearance, V= volume of distribution for rifabutin in the central compartment, V_{per}- volume of distribution for rifabutin in the peripheral compartment, ka- first order absorption rate constant, F- bioavailability, Q- intercompartmental clearance for rifabutin, Cl_e- clearance of rifabutin to des-rifabutin, BSV –

between subject variability, Cl_m- clearance of des-rifabutin, V_m- volume of distribution for des-rifabutin in the central compartment, $V_{m\text{-}per}$- volume of distribution for des-rifabutin in the peripheral compartment, the base model included weight allometricaly scaled on CL/F, V/F, Q/F and V_{per}/F

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Transparency declarations

None of the authors have any conflict of interest to declare.

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Supplemental Material

Detailed methods used to detect single nucleotide polymorphisms (SNPs):

Total Genomic DNA was isolated using the QIAamp DNA mini kit according to manufacturer's instructions. Purity was assessed following extraction by comparing the A260 and A280 ratio. DNA was normalised to 20ng/µl. Genotyping for *SLCO1B1* rs4149032, rs2306283, rs4149056 and rs11045819 was performed by real-time PCR allelic discrimination by standard methodology (95˚C for 15 min, then 40 cycles of 95˚C for 15 sec and 60˚C for 1 min). The Applied Biosystems assay IDs for the first three SNPs were C_1901709_10, C_1901697_20 and C_30633906_10, respectively. For rs11045819, the forward primer, reverse primer, VIC probe and FAM probe were 5′CAGTGATGTTCTTACAGTTACAGGTATTCTAA3′, 5′GAAGACTTTTTACTGTCAATATTAATTCTTACCTTTTCC3′, 5′-ACTATCTCAGGTGATGCT-VIC, and 5′-CACTATCTCAGTTGATGCT-FAM, respectively.

Detailed population modeling methods

Data was analysed using NONMEM (version 7.1.2)(19) and PSN v.3.4.2.(25). Population pharmacokinetic parameter estimates, between-subject variability modelled exponentially, and residual variability were obtained with the first-order estimation method with interaction (FOCE+I). The objective function value (OFV), 'goodness-of-fit' plots and visual predictive checks were used to evaluate and guide model building, a bootstrap (n=200) was performed for model validation. Proportional, additive and combined residual error models were tested separately for rifabutin and 25-desacetyl rifabutin. Nested models were hypothesis-tested using the

likelihood ratio test in which the change in OFV approximates the X^2 distribution ($X^2_{1,0.05} > 3.84$). Non-nested models were compared using the Akaike information criterion (AIC).

Table S1: Expected mean±SD area under the concentration–time curve over 24 h ($AUC_{0–24}$) for rifabutin and des-rifabutin ($AUCM_{0–24}$) for specific subpopulations based on 500 simulations from the final model.

	Female	*Male*	*Carrier*	*Non-Carrier*	*Female Carrier*	*Male Carrier*	*Female non-Carrier*	*Male non-Carrier*
$AUC_{0–24}$ (ng.h/L)	2830.4±160.2	2607.2±145.2	3050.4±187.3	2646.9±147.2	3142.9±176.8	2989.3±152.4	2788.1±153.6	2559.3±141.3
p-value	< 0.001		< 0.001		0.015		< 0.001	
					< 0.001[#]		< 0.001†	
$AUCM_{0–24}$ (ng.h/L)	277.1±18.9	272.3±19.3	327.8±26.6	267.1±19.2	328.5±25.4	327.2±29.2	270.3±17.5	265.3±19.2
p-value	0.032		< 0.001		0.70		0.035	
					< 0.001[#]		< 0.001†	

[#] comparing female heterozygous carriers of *SLCO1B1* rs1104581 versus female non-carriers, † comparing male heterozygous carriers of *SLCO1B1* rs1104581 versus male non-carriers

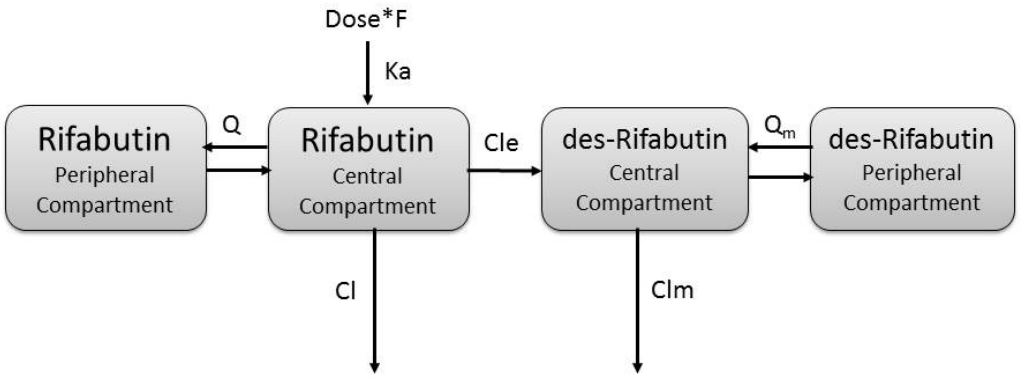

FIG S1. Structural model for rifabutin and 25-desacetyl rifabutin (des-Rifabutin).

F- bioavailability, ka- first order absorption rate constant, Q- intercompartmental clearance for rifabutin, Q_m- intercompartmental clearance for des-rifabutin, Cl-clearance of rifabutin, Cl_e- clearance of rifabutin to des-rifabutin, Cl_m- clearance of des-rifabutin

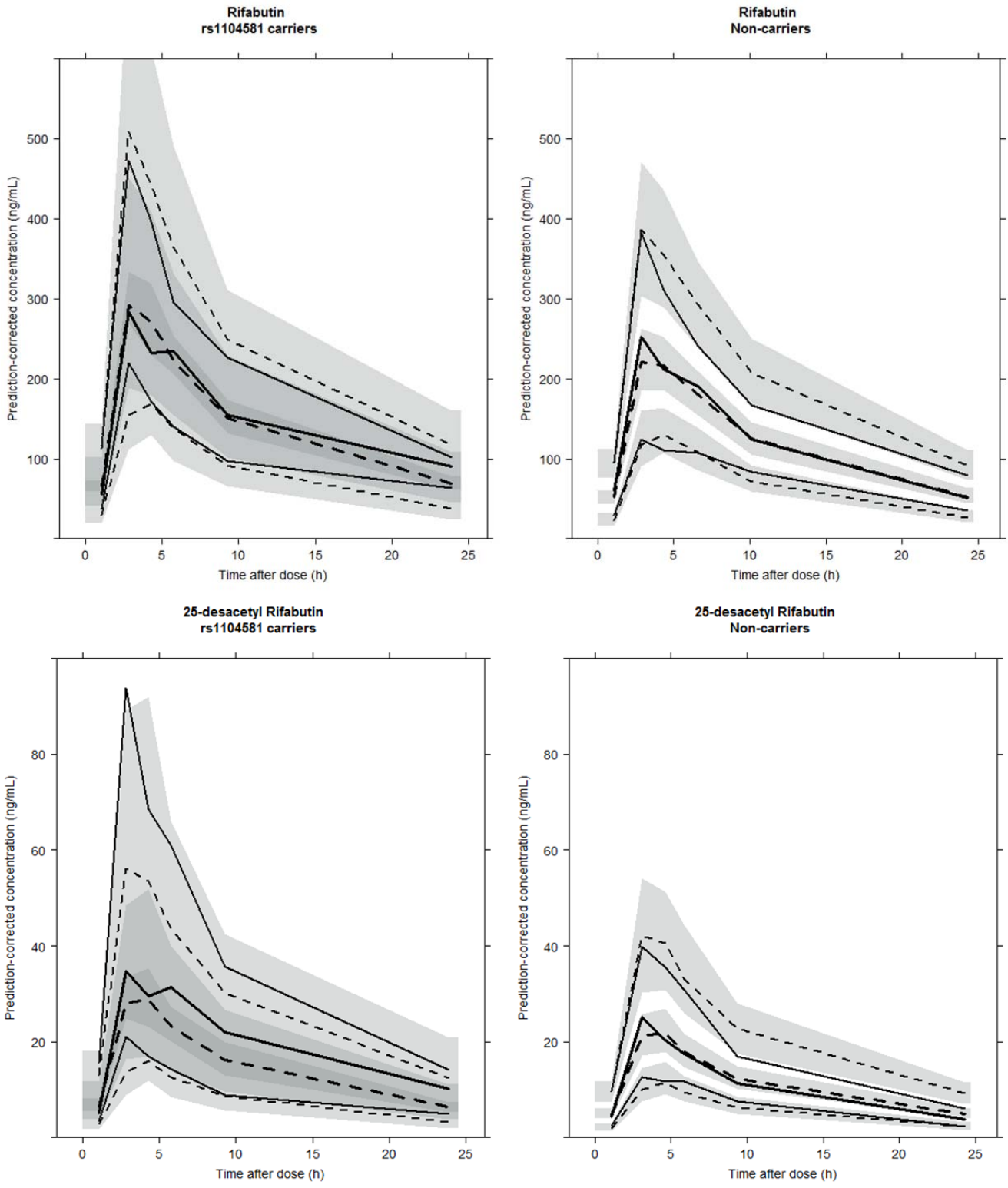

FIG S2. Prediction-corrected visual predictive check of the final model for rifabutin (top) and des-rifabutin (bottom) separated for rs1104581 carriers (left) and non-carriers (right). The solid upper, middle and lower lines represent the 90th, 50th and 10th percentile of the patients' observations. The dashed upper, middle and lower lines represents 90th , 50th and 10th percentile of simulated data. The grey shaded areas are the simulated confidence intervals for the corresponding percentiles.

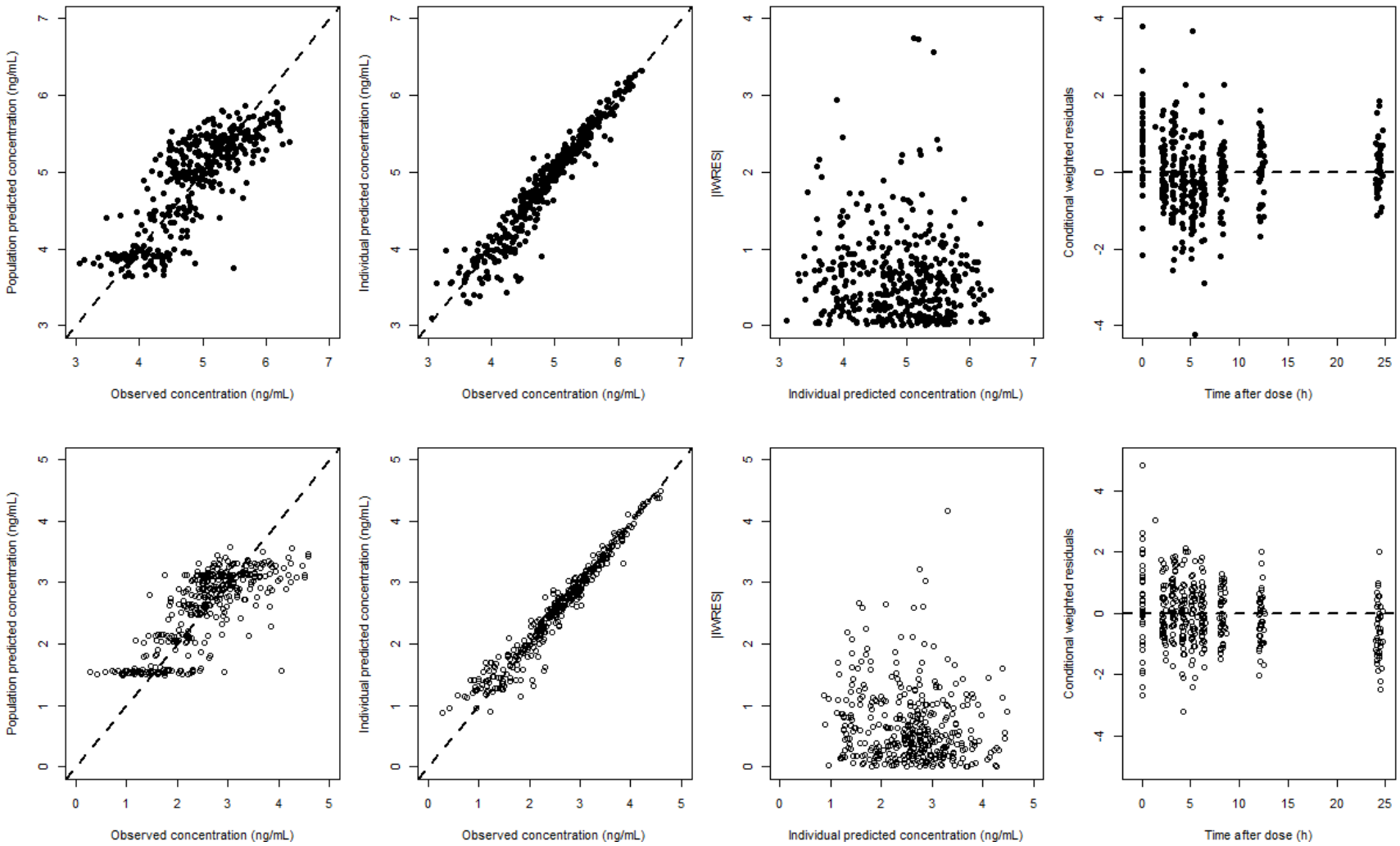

FIG S3. Goodness-of-fit plots for rifabutin (closed circles, top row) and des-rifabutin (open circles, bottom row) for the final model.