RESEARCH ARTICLE

Dosing of Bedaquiline with Rifamycins reduces Rifabutin Plasma Concentrations

Authors

Amanda M. Healan,[a*] J. Mcleod Griffiss,[b#] Mary Ann O'Riordan,[c] Wesley A. Gray,[d] Robert A. Salata,[a] Jeffrey L. Blumer[d§]

Affiliations

Division of Infectious Diseases and HIV Medicine, Case Western Reserve University, Cleveland, OH, USA[a]

ClinicalRM, Hinckley, OH, and Dept. of Laboratory Medicine, University of California San Francisco, San Francisco, CA, USA[b]

Department of Pediatrics, Case Western Reserve University, Cleveland, OH, USA[c]

Department of Pediatrics, University of Toledo College of Medicine, Toledo, OH, USA[d]

Correspondence address:
J. McLeod Griffiss, MD,
Departtment of Laboratory Medicine
University of California San Francisco VA Medical Center (111W1)
4150 Clement St.
San Francisco, CA 94121
E-mail: crapaud@loursage.org

*Present address: Dr. Amanda M. Healan, 2816 Paden Dr., Nashville, TN 37206
§Deceased

Abstract

Bedaquiline (BDQ), a diarylquinoline mycobacterial ATP synthase inhibitor approved in the United States for drug-resistant tuberculosis, is metabolized by CYP3A4, an hepatic enzyme strongly induced by rifampin (RIF), an essential part of drug-sensitive tuberculosis treatment. BDQ is used more broadly in some other countries and has been evaluated for treatment of non-tuberculosis mycobacterial infections, often in combination with rifabutin (RBT). We examined the pharmacokinetic interactions of BDQ plus either RIF or RBT in 33 healthy volunteers. Subjects were randomly assigned to receive two single 400 mg doses of BDQ, given 29 days apart, and either RBT 300 mg or RIF 600 mg, given daily from day 20 to 41 after the first dose of BDQ. Blood samples were collected prior to dosing and at multiple subsequent time points to measure plasma drug concentrations, including those of the rifamycin primary metabolites. BDQ treatment had little effect on the disposition of RIF but resulted in a dramatic shortening of the half-life of RBT and decreased exposure to it. When the drugs were administered together (Day 29) the peak rifamycin concentrations and peak rifamycin metabolite concentrations were reduced significantly ($p < 0.001$). This appeared to result from reduced absorption and raises a concern that doses of BDQ and the rifamycins, particularly RBT should be staggered when the two drugs are given on the same day. The optimum time between dosing should be determined.

Introduction

Rifamycins, including rifabutin and rifampin, are cornerstones of first- line combination treatment regimens for patients with active *Mycobacterium tuberculosis* infection (TB). The pharmacokinetics and pharmacodynamics of rifampin and rifabutin have been well studied (1). These drugs are known inducers of drug transporters and cytochrome P450 (CYP) 3A4 (rifampin more so than rifabutin). Rifabutin is also a CYP3A4 substrate (2).

Given the importance of CYP3A4 mediated metabolism in the elimination of drugs in anti-TB regimens, drug-drug interaction studies between new drugs to treat TB and the rifamycins are common. However, these studies normally focus on the effects of rifamycins on the pharmacokinetics of the new drugs (3-5). The converse effects are seldom emphasized. Yet, as these remain important to the effectiveness and safety of the drug regimens, assessment of the impact of new drugs on rifamycin pharmacokinetics is important.

One study in immunodeficiency virus (HIV) andTB co-infected patients identified a 32% increase in rifabutin steady state plasma concentrations when 150 mg rifabutin was administered daily in conjunction with a lopinavir/ritonavir antiretroviral combination regimen, as compared to rifabutin monotherapy at twice the combination dose. Conversely, intermittent (thrice weekly) dosing of rifabutin at 150 mg daily with lopinavir/ritonavir reduced plasma concentrations of rifabutin by 44% (6). This study illustrates the need to consider rifamycin pharmacokinetics when developing combination TB treatment regimens.

Bedaquiline (BDQ), a novel diarylquinoline mycobacterial ATP synthase inhibitor, is approved in the United States as part of combination therapy to treat multi-drug resistant TB, but is used more broadly in other countries and has been evaluated for treatment of non-tuberculosis mycobacterial infections, often in combination with rifabutin (RBT) (7-9). BDQ is metabolized through CYP3A4 (10); thus, the potential for drug-drug interactions with the rifamycins exists. To date, there have been no studies published that reported on the effects of BDQ on rifamycin pharmacokinetics.

We recently reported results from a randomized, Phase 1 drug-drug interaction trial in healthy adult volunteers that evaluated the effects of steady-state dosing of rifampin (RIF) and RBT on the pharmacokinetics of BDQ (11, 12). Herein we report the effects of BDQ on the pharmacokinetics of RIF and RBT, and their primary desacetyl metabolites. The data were collected during the Phase 1 trial (11, 12); however, the sponsor stipulated that these observations were not to be included in the report of the trial, as drug-drug interactions between BDQ and the rifamycins were not specified in the original protocol but added after initiation of the trial. They are reported here because of their potential importance.

In the course of that study we noted that BDQ given at the same time as the rifamycins markedly reduced plasma concentrations of the latter. This effect appeared to result from decreased absorption and raised a concern that when BDQ and a rifamycin are part of combination therapy, dosing should be staggered. In addition, BDQ treatment resulted in a dramatic shortening of the half-life of RBT and decreased exposure to it.

Methods

Study. Data were collected during a randomized Phase 1 drug-drug interaction study that evaluated the safety, tolerability, and pharmacokinetics of BDQ when given in combination with RIF or RBT (11). The study was approved by the Institutional Review Board at University Hospitals Case Medical Center and all subjects provided written informed consent prior to participating in the study. Participating subjects received two single oral doses of 400 mg BDQ, first on Study Day 1 followed by a 28-day wash-out, the second on Study Day 29. RBT 300 mg (Group 1) or RIF 600 mg (Group 2) was administered once daily from Study Day 20 through Study Day 41. The study enrolled 33 subjects; 17 were randomly assigned to Group 1 and 16 to Group 2. One Group 1 subject discontinued and was replaced; pharmacokinetic

data from this subject are not included. There were no notable demographic differences between the two groups (11).

Blood samples for RBT, 25-*O*-desacetylrifabutin, RIF, and 25-*O*-desacetylrifampin pharmacokinetic analyses were obtained pre-rifamycin dose and two hours post-rifamycin dose on Study Days 27, 28, 29, 30, 35, and 41. This dosing regimen and sampling schedule were designed to ensure subjects were at steady state (1). Intensive sampling for rifamycins was performed on Day 29. Blood was drawn pre-dose and at 1, 2, 3, 4, 5, 6, 8, 12 and 24 hours after dosing. Subjects participated in a follow-up study visit on Day 57 (28 days after the last BDQ dose) for final safety assessments.

Materials. Water, methanol and acetonitrile were HPLC or LC/MS grade and purchased from Fisher Scientific (Pittsburgh, PA). Sigma-Aldrich (St. Louis, MO) was the supplier for RBT (CAS, 99% pure) and ascorbic acid (ACS grade). Other drug standards and internal standards including rifampicin, Lot # 1224-008a2, 98.4% pure, rifampicin-d4, Lot # 1182-093-A3, 25-desacetylrifampicin, Lot # 1071-017A4, 99.4% pure, 25-desacetylrifampicin-d4, Lot # 1233-015A2, 99.8% pure, 25-*O*-desacetylrifabutin, Lot # 1199-095A4, 99.8% pure, 25-*O*-desacetylrifabutin-d7, Lot # 1191-097A2, 99.7% pure, and rifabutin-d7, Lot # 1191-091A4, 98.5% pure were purchased from TLC PharmaChem (Ontario, Canada). Formic acid, 88%, was obtained from Mallinckrodt (St. Louis, MO) and human plasma, Na heparin, was purchased from Bioreclamation, LLC.

Sample Handling - Blood samples, 5 ml, were drawn into collection tubes containing sodium heparin at the times indicated and placed immediately on ice. The blood was centrifuged at 2500 x g within two hours of collection and the plasma fraction removed, divided into two aliquots and frozen at -70°C for shipment to the Analytical Pharmacology Laboratory at the University of Toledo. For analysis, plasma samples were allowed to thaw on ice in a covered ice bucket to protect from light exposure. Once thawed a 200 µl aliquot of study subject plasma was transferred to a labeled tube on ice. The remainder of the sample was refrozen immediately at -70°C.

Determination of rifamycins and their 25-*O*-desacetyl metabolites (13) - All bioanalytical standard and stock solutions were stored at -70°C and equilibrated to ambient temperature before use. To correct for purity, the weight of the compound obtained from the analytical balance was multiplied by the purity to yield the actual weight.

Detection and analysis were performed using a validated LC/MS/MS assay developed using a Varian 1200L Liquid Chromatograph Mass Spectrometer (Agilent Technologies, Inc., Santa Clara, CA) interfaced with a Shimadzu SIL-20AC HT autosampler (Shimadzu Scientific Instruments, Inc., Columbia, MD), a Varian ProStar HPLC system, Model 210 (Agilent Technologies, Inc. Santa Clara, CA) with a Brinkman CH-30 Column heater with a TC-50 Controller (Brinkmann Instruments, Inc., Westbury, NY) (7).

RIF and 25-*O*-desacetylrifampin. To the 1.5 ml microfuge tube containing the 200 µl of plasma, 20 µl of 0.1 M ammonium acetate was added with brief mixing. Then 100 µl of internal standard working solution containing 2000 ng/ml of each d4 compound was added. Following brief mixing, the sample was diluted with 500 µl of methanol and was thoroughly mixed and centrifuged at 16,400 x g for 15 minutes. The supernatant was transferred to a clean 16 x 100 mm borosilicate tube for evaporation to 200 µl under N_2. The supernatant was removed, transferred to an autosampler vial and centrifuged at 10,000 rpm; 75 µl was then transferred to an autosampler vial to which 25 µl of 1.0 mg/ml ascorbic acid (in 50/50 methanol/acetonitrile) was added. After mixing the sample was ready for injection.

The autosampler injection volume was 25 µl onto a Phenomenex Security Guard 4.0 x 3.0 mm C8 precolumn followed by a Supelco Discovery 50.0 x 2.1 mm 5 µ C18 column heated to 35°C. The gradient for elution was comprised of 75% 0.01 M ammonium acetate/25% acetonitrile and 0.5% formic acid in

LC/MS acetonitrile in the two reservoirs, respectively. The solvent flow rate began at 300 µl/minute and the ammonium acetate decreased from 75% to 5% over 0.57 minutes and at 4.57 minutes the flow rate increased to 400 µl/minute as the ammonium acetate reservoir increased to 75% over the next minute and then was reduced to 300 µl /minute over 2 minutes and the ammonium acetate declined to 0%. The total run time was 8.57 minutes.

Mass spectrometry was performed using selective ion monitoring by the transitions from the parent ion for rifampicin at 823.2 amu to its transition ion at 791.4 amu compared with the parent for the internal standard, rifampicin –d4 at 827.2 amu to its transition ion at 795.4 amu. For the primary 25-*O*-desacetylrifampicin the parent ion was 749.3 amu with a transition ion at 731.4 amu while the internal standard, 25-*O*-desacetylrifampicin-d4 yielded a parent ion at 753.3 amu with a transition ion at 734.4 amu. The intraday precision and accuracy for the determination of rifampin at its high quality control standard and at the lower limit of quantitation were 4.45 and 6.34% and 4.36 and 7.35% respectively. The between day values were 5.66 and 7.80% and 0.90 and 2.31% respectively. The comparable values for the 25-*O*-desacetyl metabolite were 1.94 and 14.02%, 11.48 and 2.16%, 2.60 and 12.20%, and 10.49 and 5.96%, respectively. The upper limits of quantitation for RIF and its desacetyl metabolite were 20,000 and 2000 ng/ml, respectively while the lower limits of quantitation were 50 and 20 ng/ml.

RBT and 25-*O*-desacetylrifabutin. Unknown plasma (150 µl) was transferred to a clean 12 x 75 mm borosilicate tube to which 300 µl of internal standard working solution was added. The latter contained 150 ng/ml rifabutin-d7 and 100 ng/ml 25-*O*-desacetylrifabutin-d7. The sample was then transferred to a Bond-Elut Plexa solid phase extraction column that had been preconditioned with 500 µl of LC/MS methanol followed by 500 µl HPLC water and drawn through using approximately 5 psi vacuum. The cartridge was rinsed with 500 µl of HPLC water and then the sample eluted into a clean 16 x 100 mm borosilicate tube using 1.50 ml LC/MS methanol. The sample was then evaporated to dryness at 30°C under a gentle N_2 stream.

The residue was redissolved in 75 µl of 0.1% citric acid in methanol, mixed and diluted further with 75 µl HPLC water and mixed for 30 seconds. Clear samples were transferred to the autosampler. Those that were cloudy were centrifuged for 5 minutes at 10,000 rpm and the supernatant was then transferred.

The autosampler injection volume was 35 µl onto a Phenomenex Security Guard 4.0 x 3.0 mm C8 precolumn followed by a Supelco Discovery 50.0 x 2.1 mm 5µ C18 column heated to 30°C. The gradient for elution was comprised of 0.5% formic acid in LC/MS methanol and 50% 0.01 M ammonium acetate/50% LC/MS methanol beginning at a flow rate of 210 µl/minute of the ammonium acetate and ramping to 55% and then 75% of the formic acid solution over approximately 1.6 and 7.6 minutes, respectively, before increasing the flow rate to 250 µl/minute and decreasing the formic acid to 0%. The entire run time per sample was 15.6 minutes.

Mass spectrometry was performed using selective ion monitoring following the transitions from 847.1 to 815.4 for rifabutin; 854.4 to 822.4 for rifabutin-d7; 805.4 to 773.4 for 25-*O*-deacetylrifabutin; 812.4 to 780.4 for 25-*O*-desacetylrifabutin-d7. The intraday precision and accuracy for the determination of rifabutin at its high quality control standard and at the lower limit of quantitation were 2.17 and 2.90% and -0.80 and 1.40% respectively. The between day values were 1.62 and 2.82% and -0.25 and -0.32%. Comparable values for the 25-*O*-desacetyl metabolite were 1.16 and 3.42%, -053 and 2.40%, 1.64 and 5.86% and 0.42 and -1.45%, respectively. The upper limits of quantitation for rifabutin and its desacetyl metabolite were 1000 ng/ml and 400 ng/ml, while the lower limits of quantitation were 10 and 5 ng/ml, respectively.

Pharmacokinetic Analysis - Concentration versus time profiles from blood draws were generated for RIF, 25-*O*-desacetylrifampin, RBT

and 25-*O*-desacetylrifabutin and plotted on the raw scale and semi-logarithmic scale for visual inspection. Concentrations were summarized over all subjects with the mean ± SD for each protocol time point and plots constructed for each compound. Pharmacokinetic parameters were estimated using each set of concentrations in separate analyses. Steady-state pharmacokinetic parameters were estimated at Day 29 with standard non-compartmental methods using Phoenix™ WinNonlin®, version 6.3 (Certara, St. Louis, MO). The apparent maximum concentration (C_{max}) and time to maximum concentration (T_{max}) were ascertained from visual inspection of the concentration versus time curves plotted for each subject. The area under the plasma drug concentration versus time curve was determined using the linear trapezoidal rule up to the final concentration point ($AUC_{0-\tau}$). The elimination half-life ($T_{1/2}$) was determined from the post-distributive terminal portion of the plasma concentration versus time curve (terminal slope). Clearance (Cl/F) was determined as Dose/$AUC_{0-\infty}$ and V_D/F calculated as Dose/$AUC_{0-\infty}$*$k_{el.}$ MRT_{last} was calculated as the ratio of the area under the moment curve, AUMC to the $AUC_{0-\tau}$.

Results.

Figure 1 shows the mean (± SD) of the peak (a) and trough (b) concentrations of RBT and RIF during daily dosing from Day 27 through Day 41 of the protocol. The peak and trough concentrations confirmed the attainment of steady-state concentrations and reflected large intra-and interindividual variability (Figure 1), consistent with previous reports (14-16). However, peak concentrations on the day when the rifamycins were co-administered with the second dose of BDQ were significantly lower than the steady state levels (Figure 1, Table 1). Table 1 presents the results of statistical analyses comparing mean (± SD) concentrations between different Days. The only values that differed significantly were those between Day 28, before co-administration of BDQ, and Day 29 when BDQ was given at the same time as the rifamycins (p = <0.001 for RBT and 0.001 for RIF). There were no differences between Day 28 and Day 30, when concentrations had returned to steady-state. Similar patterns were observed for the desacetylated metabolites of both rifamycins (data not shown). When sampled over the entire dosing interval the C_{max} and $AUC_{0-\tau}$ of RIF were much greater than those of RBT.

Table 1: Inferential Statistics of Rifamycin Peak Concentrations (*p* values for each comparison)

Comparison	Rifabutin	Rifampin	Comment
Day 28 and Day 27	0.65	0.27	Steady-state concentration achieved
Day 29 and Day 28	<0.001	0.001	Significantly different concentrations
Day 30 and Day 28	0.28	0.77	Return to steady-state concentration
Day 35 and Day 28	0.12	0.11	Maintain steady-state concentration
Day 41 and Day 28	0.20	0.46	Maintain steady-state concentration

Figure 1. Mean Rifamycin Peak and Trough Concentrations during Steady-state Dosing

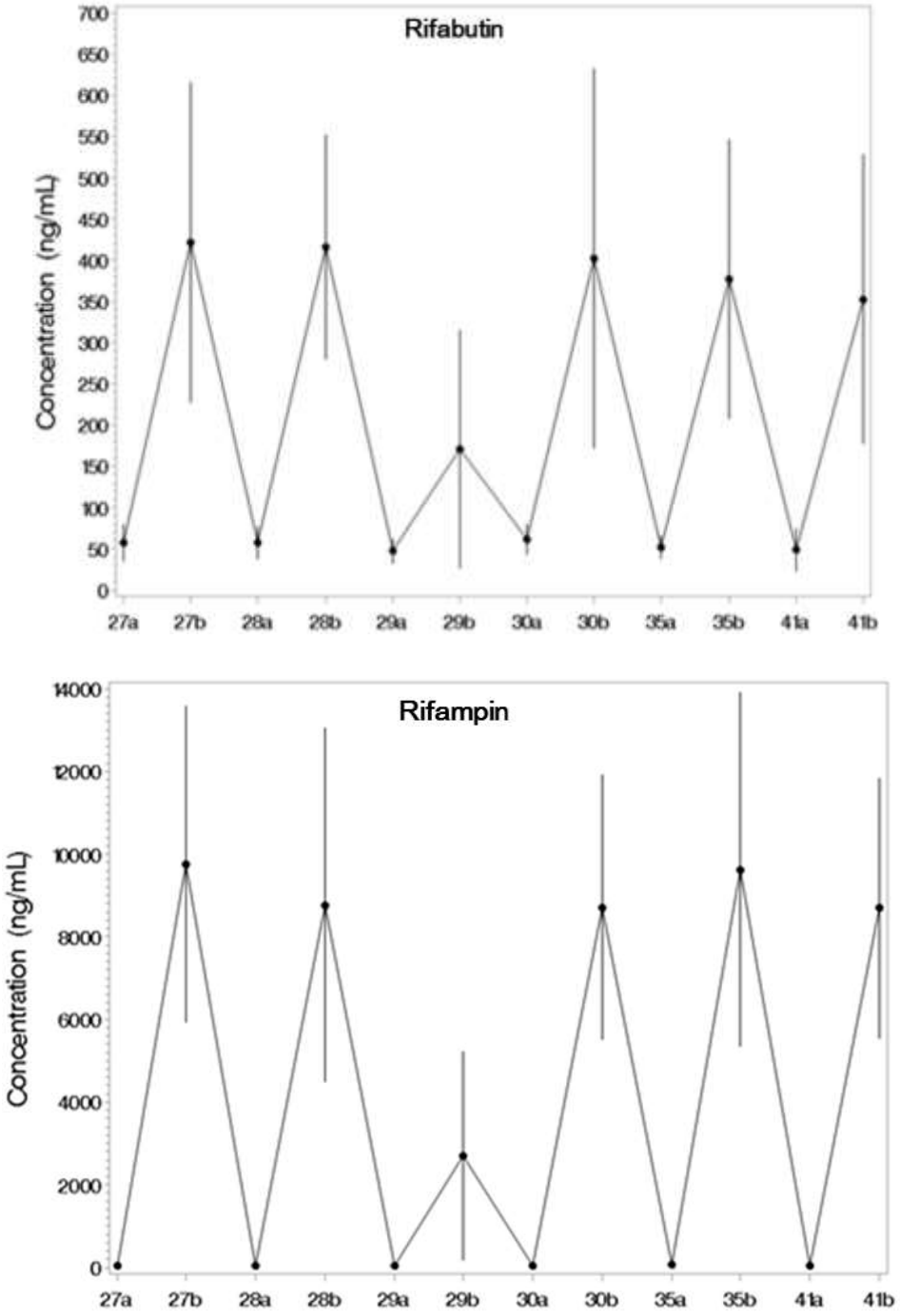

Figure 1: The figures depict the mean(± SD) of the peak (a) and trough (b) concentrations of RBT and RIF during daily dosing from Day 27 through Day 41 of the protocol.

Summary pharmacokinetic parameters for RBT and RIF and their respective 25-O-desacetyl primary metabolites are given in Table 2. Clearance of RIF and 25-*O*-desacetylrifampin was slower than that of RBT and 25-*O*-desacetylrifabutin. Time to maximal drug concentration (T_{max}) was not different between treatment groups.

Table 2. Summary pharmacokinetic parameters of rifamycins and their desacetyl metabolites*

	RBT *N* = 16	25-*O*-desacetylrifabutin *N* =16	RIF *N - 16*	25-*O*-desacetylrifampin *N* =16
C_{max} (ng/mL)	453.38 (144.852)	32.46 (14.779)	7503.76 (2861.806)	875.93 (565.758)
AUC_{0-t} (h*ng/mL)	3466.23 (708.481)	251.50 (74.138)	36058.37 (15680.192)	5147.29 (3768.828)
T_{max} (h)	4.47 (1.480)	4.97 (2.299)	3.58 (0.815)	4.52 (0.965)
$t_{1/2}$ (h)	10.86 (3.112)	9.51 (1.763)	2.93 (0.242)	4.21 (0.770)
Kel (10^{-4}/h)	680.27 (165.604)	754.54 (150.872)	2384.45 (194.118)	1701.38 (320.281)
V_D/F (L)	1057.6 (259.4)	NA**	86.0 (45.5)	NA
CL/F (L/h)	70.80 (17.794)	NA	19.85 (8.951)	NA
$MRT_{0-\square}$ (h)	8.93 (0.982)	8.95 (0.961)	5.54 (0.573)	6.86 (0.468)

*All values reported in mean (SD)
**NA = Not Applicable

Overall, 25-*O*-desacetylrifabutin represented 7% of the parent compound. RBT peak concentrations ranged from 36.36 ng/mL to 933.94 ng/mL (median: 324.54 ng/mL). 25-*O*-desacetylrifabutin peak concentrations ranged from 6.34 to 71.62 ng/mL (median: 23.25 ng/mL). Peak RIF and 25-*O*-desacetylrifampin concentrations ranged from below the lower limit of quantitation to 21,122.13 ng/mL and 3,109.07 ng/mL, respectively. 25-*O*-desacetylrifampin represented 11% of the parent compound.

Graphical representations of plasma concentrations over time for RBT and 25-*O*-desacetylrifabutin, and RIF and 25-*O*-desacetylrifampin, are shown in Figure 2.

Figure 2: Plasma rifamycin and desacetylrifamycin concentrations versus time

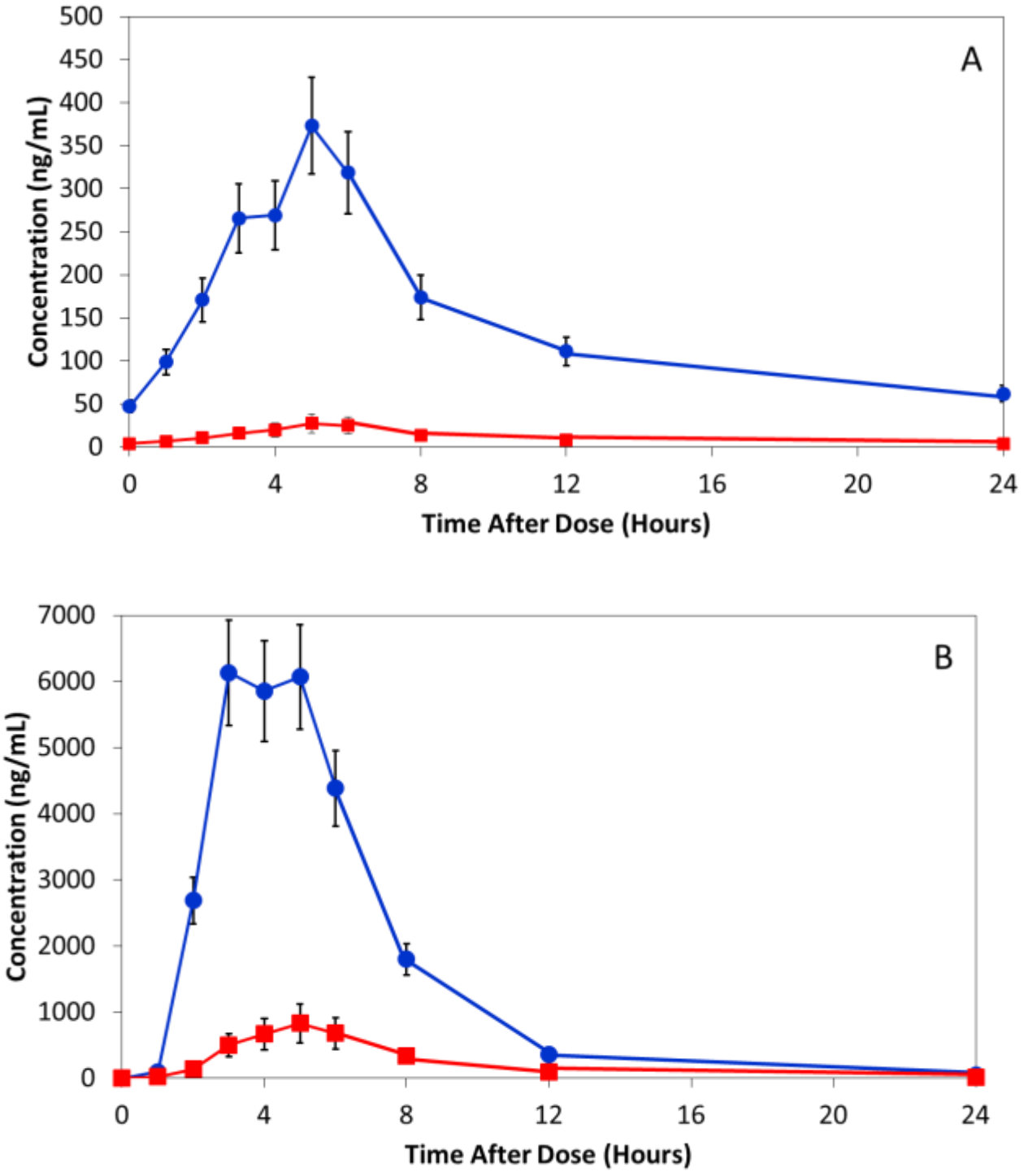

Figure 2: Plasma concentration versus time curves for the rifamycins and their primary desacetyl metabolites. RBT (Panel A) and RIF (Panel B). Parent drug --●-- and metabolite --▮--.

Discussion.

The rifamycins remain integral components of treatment regimens for patients with *M. tuberculosis* infections. They are known inducers of drug metabolism and their effects on the pharmacokinetics of other drugs often

are assessed to ensure that dosing adjustments are not required when used in combination (3-6,15,16). Less commonly are the effects of these other agents on exposure to the rifamycins measured as well. However, since the antimycobacterial effects of the rifamycins are important to patient outcomes, it is prudent to determine whether dosage adjustments of these agents might also be warranted when they are used in combinations.

In this study, BDQ pretreatment significantly affected RBT pharmacokinetics and resulted in a marked decrease in RBT exposure. Overall exposure to RBT was much lower than to RIF, due to enhanced clearance of RBT following BDQ pretreatment.

According to the manufacturer, single oral doses of 300 mg RBT administered to healthy adult volunteers reach a mean plasma C_{max} of 375 (±267) ng/mL (range: 141 to 1033 ng/mL) in 3.3 hours (±0.9 hours, T_{max} range: 2 to 4 hours) (17). These parameters are similar to those seen in the present study and consistent with other published reports (1, 14-16). *O-d*esacetylrifabutin C_{max} observed in the present study also is consistent with previously reported values (15, 16).

In healthy volunteers RBT is associated with a relatively long mean terminal half-life of 45 (± 17) hours following a single dose of 300 mg, which does not change following multiple doses (14,17). This reported half-life is more than four times that observed when BDQ was administered concomitantly in the present study. The substantially shorter half-life and lower $AUC_{0-\infty}$ of RBT observed in the present study is a direct result of BDQ treatment and likely due to enhanced clearance of RBT following BDQ pretreatment. This change in exposure does not appear to impact the ratio of the parent drug to its primary metabolite, 25-*O*-desacetylrifabutin suggesting the effects are presystemic or equally affect the metabolite elimination.

Pretreatment with BDQ did not have an effect on RIF disposition. The half-life, AUC and C_{max} observed in the present study are consistent with those outlined by the manufacturer and others following single 600 mg doses in healthy adult volunteers (average 2-3 h and 7,000 ng/mL, respectively) (18). At steady state in healthy males (14 daily doses of 600 mg), RIF C_{max} is slightly higher, 8,500 ng/mL, with ranges consistent with the present report (16). Data regarding desacetylrifampin pharmacokinetics in healthy volunteers are extremely llimited, making comparisons to parameters in the present study difficult, but there may be modest effects on half-life and AUC (19).

The most striking finding in the present study was the significant reduction in concentrations of the two rifamycins when administered together with BDQ. As this reduction was not accompanied by an increase in their 25-*O*-desacetyl metabolites, it likely resulted from interference by BDQ with absorption of the rifamycins from the gastrointestinal tract. Both rifamycins induce and interact with P-glycoprotein (20, 21) and to a lesser extent the organic acid transporters (22), but whether BDQ has significant functional interactions with these membrane proteins remains to be determined.

Importantly, RBT's primary metabolite, 25-*O*-desacetylrifabutin, has *in vitro* activity against mycobacterial species that is comparable to the parent compound (14). 25-*O*-desacetylrifampin also retains antibacterial activity (18).

BDQ-rifamycin interactions result in different outcomes when RIF and RBT are the subject rifamycins, as they were in this Phase 1 trial (11, 12). RIF, a strong inducer of CYP3A (1), accelerated clearance of BDQ, a CYP3A4 substrate (2), by doubling its desmethtylation to its M2 and M3 metabolites (12), but accelerated clearance resulted in decreased AUC and mean

residence time of BDQ and its metabolites (11, 12). Conversely, BDQ had little effect on the disposition of RIF.

In contrast, RBT, a weak inducer of CYP3A as well as a substrate of it (1), had little quantitative impact on exposure to BDQ (11). RBT did accelerate desmethylation, to a lesser degree than RIF, but unlike with RIF, clearance of the metabolites slowed, which resulted in sustained elevation of both M2 and M3 (12). Conversely BDQ dramatically shortened the half-life of RBT and decreased exposure to it.

The shortened RBT half-life when given with BDQ is especially important, as the combination of RBT plus BDQ produces sustained intracellular mycobactericidal activity that is greater than the sum of their individual effects (23). The same is not true for the combination of RIF and BDQ (23).

Increasing TB drug resistance necessitates exploring all possible treatment combinations to identify effective regimens. BDQ is a prime candidate for combination treatment regimens due to its proven tolerability and efficacy. It is critical that the effects of this new drug on the pharmacokinetics of the rifamycins are well understood, given that rifamycins are commonly used in combination regimens.

It is likely that RBT dosage will need to be increased in order to maintain effective exposure when the drug is employed in combination with BDQ. At a minimum the effect of BDQ dosing on rifamycin concentrations would seem to imply that dosing of BDQ with RBT or RIF should be staggered. The required interval between doses of BDQ and the rifamycins needs to be determined.

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