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CLINICAL THERAPEUTICS

Impact of Rifabutin or Rifampin on Bedaquiline Safety, Tolerability, and Pharmacokinetics Assessed in a Randomized Clinical Trial with Healthy Adult Volunteers

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ABSTRACT Bedaquiline is a diarylquinoline that specifically inhibits mycobacterial ATP synthase. Bedaquiline has been used to effectively treat tuberculosis (TB) caused by drug-susceptible and drug-resistant *Mycobacterium tuberculosis*. Rifamycins are a cornerstone of combination drug regimens for the treatment of TB. This phase 1, open-label, randomized, controlled trial evaluated the effect of steady-state dosing of rifabutin or rifampin on the safety, tolerability, and pharmacokinetics of bedaquiline given as a single dose. Thirty-three healthy subjects were enrolled to receive a 400-mg single oral dose of bedaquiline at two time points, on study days 1 and 29. Subjects were randomly assigned to once daily oral doses of rifabutin (300 mg/day, $n = 17$) or rifampin (600 mg/day, $n = 16$) during period 2 from days 20 to 41. Serial blood sampling for bedaquiline measurement occurred on days 1 and 29 through 336 h after bedaquiline administration. The day 29 bedaquiline pharmacokinetic parameter estimates were compared to the corresponding day 1 estimates for each rifamycin group. Steady-state rifampin reduced bedaquiline AUC_{0-336} approximately 45%, from 47.69 h·μg/ml in period 1 to 26.33 h·μg/ml in period 2. Bedaquiline apparent clearance accelerated 24% in rifampin-treated subjects from 6.59 liters/h in period 1 to 8.19 liters/h in period 2. Steady-state rifabutin resulted in little quantitative impact on bedaquiline exposure but was associated with grade 3 and 4 adverse events before and after the day 29 bedaquiline dose. Dosage adjustments may therefore be necessary to ensure that bedaquiline plasma concentrations reach therapeutic levels safely when combining bedaquiline and rifamycins in TB treatment regimens. (This single-site, randomized, open-label, prospective study in healthy adult volunteers was registered at Clinicaltrials.gov under registration no. NCT01341184.)

KEYWORDS *Mycobacterium tuberculosis*, bedaquiline, clinical trials, pharmacokinetics, rifabutin, rifampin

Initially known as TMC207, bedaquiline was approved in 2012 by the U.S. Federal Food and Drug Administration for the treatment of pulmonary multidrug-resistant tuberculosis (MDR-TB) infections. Bedaquiline is the first drug to be approved in a new class, diarylquinolines, and ended a 40-year drought in the development of new anti-TB drugs. Bedaquiline's unique antimycobacterial activity derives from specific inhibition of the proton pump of mycobacterial ATP synthase. Binding of bedaquiline to the oligomeric and proteolipic subunit-c of mycobacterial ATP synthase leads to the inhibition of ATP synthesis, which subsequently results in bacterial death (1, 2). The

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drug is structurally and mechanistically different from fluoroquinolone antibiotics and other related quinoline classes of drugs. For this reason, resistance to fluoroquinolones, which are a part of standard treatment of MDR-TB, does not confer resistance to bedaquiline (2). Bedaquiline has demonstrated *in vitro* and *in vivo* antituberculosis properties. It is bactericidal against drug-susceptible and drug-resistant *M. tuberculosis*, with a reported MIC of 0.06 μg/ml (2).

While recent progress has been made in controlling the incidence of TB globally, the World Health Organization still reported 10.4 million new TB cases in 2015 and 1.4 million associated deaths (3). An estimated 20% of individuals worldwide treated previously for TB are harboring MDR-TB, and this number rises to more than 50% in resource-limited settings (3). Growing resistance necessitates using multiple antimycobacterial drugs in combination. When incorporated into combination treatment regimens, bedaquiline has the potential to reduce the duration of treatment for MDR-TB (4, 5), a particular benefit in resource-limited settings where the disease is endemic.

Treatment regimens for TB have historically included rifamycins, specifically rifampin or, less frequently, rifabutin. Current treatment guidelines for HIV-TB coinfection recommend rifabutin as the rifamycin of choice in cases where HIV protease inhibitors must be used (6). The pharmacokinetics and pharmacodynamics of rifampin and rifabutin have been established. Rifampin and rifabutin are known inducers of drug transporters and cytochrome P450 (CYP3A4), with rifampin displaying these properties more strongly. Rifabutin is also a CYP3A4 substrate (7).

Bedaquiline is metabolized through CYP3A4 (8); thus, the potential for drug-drug interactions with rifamycins exists. In a two-period, sequential-design phase 1 pharmacokinetic study, combining bedaquiline with a different CYP3A4 inducer, efavirenz, an increased clearance of bedaquiline in healthy adult volunteers was noted. There was a single grade 3 adverse event (AE) in this study, when one subject developed asymptomatic grade 3 serum transaminase elevations. This occurred 2 weeks after a single 400-mg dose of bedaquiline and following a single 600-mg dose of efavirenz (9).

We hypothesized that combining steady-state rifabutin or rifampin with bedaquiline may similarly affect bedaquiline plasma concentrations due to the effects on CYP3A4. The present study was designed to evaluate the safety, tolerability, and pharmacokinetics of bedaquiline when administered in such a combination regimen to healthy adult volunteers. Information gained from this study will inform appropriate dose adjustments to ensure the safe and efficacious dosing of rifamycins and bedaquiline in combination.

RESULTS

Subject demographics. Thirty-three healthy adults aged 19 to 38 years participated in the study from November 2011 to April 2012. Of 49 adults screened, 33 were enrolled and randomized into one of two rifamycin treatment groups. Reasons for exclusion are shown in Fig. 1. Twenty-one of 33 subjects were male, and 28 of 33 subjects self-identified as Caucasian. The mean age of all participating subjects at screening was 24.9 years. Demographics and screening laboratory characteristics did not differ significantly between rifamycin treatment groups (Table 1). Additional screening analyses, including total bilirubin, uric acid, pancreatic lipase, alkaline phosphatase, creatine phosphokinase, fasting glucose, and troponin, also did not differ significantly between treatment groups, and the findings were within the acceptable range of normal. Hematology and coagulation parameters, which included complete blood counts (Table 1), as well as urinalysis analyzed on all subjects at screening, were also within the normal ranges and not statistically different between treatment groups.

Subject safety and tolerability. The overall proportion of subjects experiencing any AE during any period was 26/33 (78.8%). This includes 14 subjects in the rifabutin group and 12 subjects in the rifampin group. A total of 144 AEs were reported over the course of the study: 69 in the rifabutin group and 75 in the rifampin group. The vast majority of AEs (121/144) occurred during period 2 (days 20 to 41). A list of AEs reported by two or more subjects is displayed by period and treatment group in Table 2.

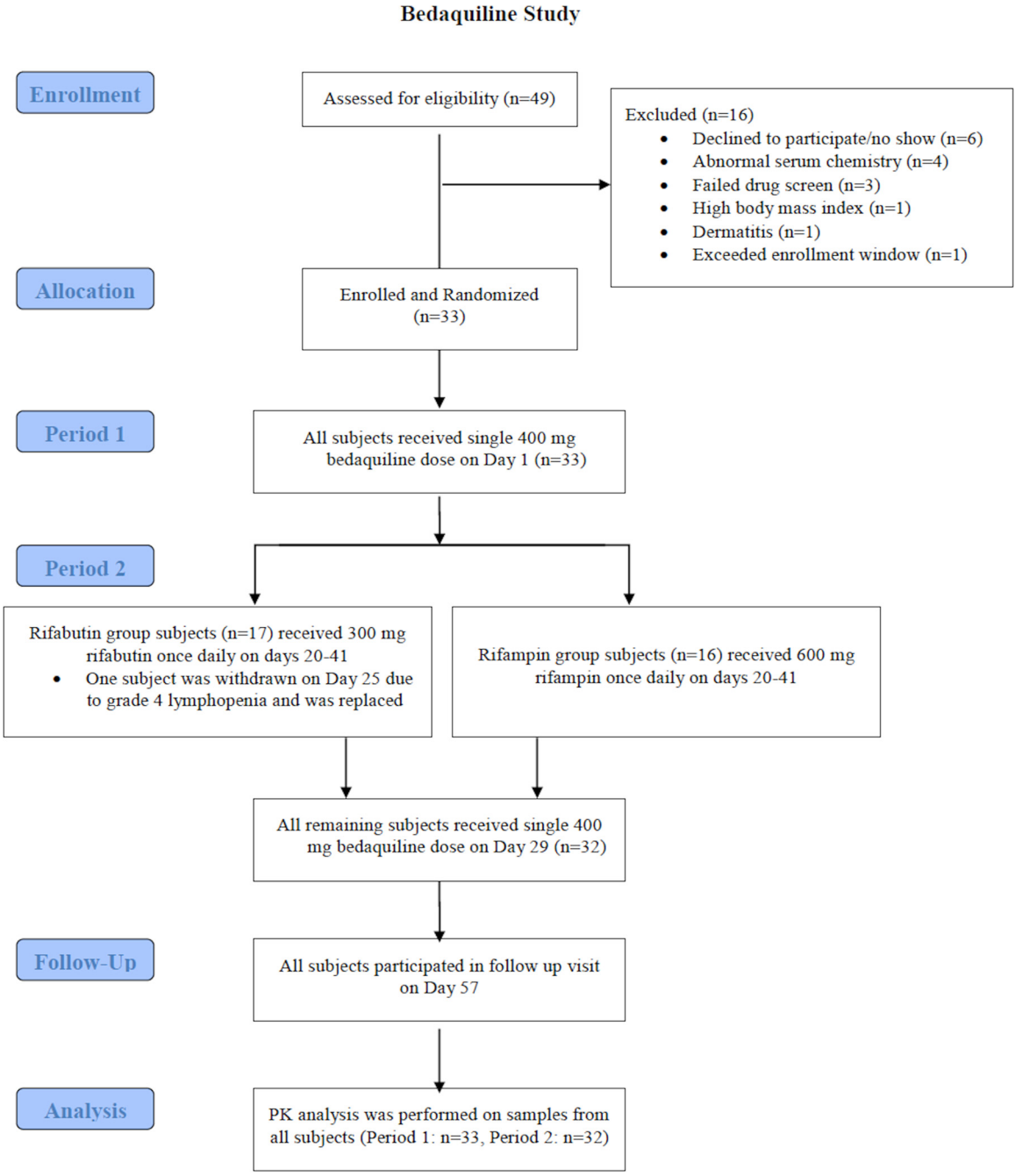

FIG 1 Study flow diagram.

In the rifabutin treatment group, the number of subjects experiencing any AE during period 1 (bedaquiline only, 3/17) was significantly less than the number of subjects experiencing any AE during period 2 (rifabutin plus bedaquiline, 13/17 [$P = 0.0063$ by McNemar's test]). Adverse events reported in the rifabutin treatment group during period 1 included pyrexia, headache, and oropharyngeal pain. Headache was the most common AE reported by rifabutin-treated subjects during period 2 (9/17 subjects, 52.9%), followed by lymphopenia (8/17 subjects, 47.1%), arthralgia (3/17 subjects, 17.6%), and neutropenia (3/17 subjects, 17.6%). Fatigue, nausea, pyrexia, chills, increased gamma-glutamyl transpeptidase, myalgia, dizziness, leukopenia, and pain were reported by two rifabutin subjects each during period 2 (Table 2). Lymphocyte counts of $<$500 cells/mm^3 (grade 3 or higher lymphopenia) were observed in eight subjects in

TABLE 1 Demographics and mean laboratory values at screening for the study population

Characteristic	Treatment group		*P*
	Rifabutin	Rifampin	
Patient demographics			
No. of subjects	17	16	
Age (yr)			
Mean (SD)	25.2 (5.7)	24.6 (4.8)	0.7655
Median	23.0	23.5	
Minimum, maximum	19, 38	20, 38	
Gender, no. (%)			
Male	10 (58.8)	11 (68.8)	0.8178
Female	7 (41.2)	5 (31.3)	
Race, no. (%)			
White	13 (76.5)	15 (93.8)	
Black or African-American	1 (5.9)	0	
Asian	2 (11.8)	0	
Other	1 (5.9)	1 (6.3)	
Ethnicity, no. (%)			
Hispanic or Latino	0	1 (6.3)	
Non-Hispanic or Latino	17 (100.0)	15 (93.8)	
Laboratory values (SD)[a]			
Body mass index (kg/m^2)	25.3 (3.9)	25.7 (3.7)	0.7640
Hemoglobin (g/dl)	14.37 (1.43)	14.92 (1.32)	0.2631
White blood cell count (cells/liter)			
ALT (IU/liter)	30.9 (11.9)	34.9 (19.8)	0.4848
AST (IU/liter)	15.5 (7.7)	20.5 (15.0)	0.2303
BUN (mg/dl)	13.0 (3.4)	12.8 (3.7)	0.8416
Creatinine (mg/dl)	0.858 (0.152)	0.887 (0.148)	0.5884

[a]AST, aspartate aminotransferase; ALT, alanine transaminase; BUN, blood urea nitrogen.

the rifabutin group as early as day 20 and as late as day 57 (before and after single-dose bedaquiline administration on day 29).

Four rifabutin subjects experienced AEs that led to study treatment modification or discontinuation during period 2. One subject in the rifabutin group received 6 of 21 doses of rifabutin and was withdrawn from the study on day 25 due to grade 4 lymphopenia (<250 cells/mm^3). The subject did not receive a second dose of bedaquiline on day 29 and was replaced. Three additional subjects in the rifabutin group discontinued rifabutin dosing after receiving the second dose of bedaquiline. The reasons for rifabutin discontinuation during period 2 for these subjects included acute mononucleosis, fatigue, and grade 4 lymphocytopenia; the latter was documented as related to the study drug.

In the rifampin treatment group, 5/16 (31.3%) of subjects experienced at least one AE during period 1 (bedaquiline only). More than twice as many subjects in the rifampin group experienced at least one AE during period 2 (rifampin plus bedaquiline) (11/16, 68.8%); however, this increase was not statistically significant as assessed by McNemar's test ($P = 0.0703$). The most commonly reported AEs in the rifampin group during period 1 were headache, fatigue, and pulmonary congestion. During period 2, the most commonly reported AE in the rifampin group was headache (7/16 subjects, 43.8% of the treatment group), followed by fatigue, nausea, infection, increased AST, and increased bilirubin (reported by 2/17 subjects each, 12.5% of the treatment group). Cytopenias, including lymphopenia, neutropenia, and leukopenia, were not observed in any subjects in the rifampin group during either period. At the follow-up visit, one subject in the rifampin group was diagnosed with an asymptomatic right eye retinal cotton-wool spot. No treatment was provided. The AE was considered nonserious, moderate in severity, and related to study treatment; this finding resolved with further follow-up. One subject in the rifampin group elected to discontinue rifampin dosing in period 2 due to nausea.

Effect of rifamycins on bedaquiline pharmacokinetics. The only statistically significant difference in the day 1 pharmacokinetic parameter estimates for bedaquiline

TABLE 2 Adverse events reported by at least two subjects in order of decreasing overall frequency[a]

Adverse event[b]	No. of patients (%)			
	Period 1 (days 1–14), bedaquline alone		Period 2 (days 20–41), bedaquline + rifamycin	
	Rifabutin group	Rifampin group	Rifabutin group	Rifampin group
Headache	2 (11.8)	3 (18.8)	9 (52.9)	7 (43.8)
Lymphopenia	0	0	8 (47.1)	0
Fatigue	0	3 (18.8)	2 (11.8)	2 (12.5)
Arthralgia	0	2 (12.5)	3 (17.6)	1 (6.3)
Pulmonary congestion	0	3 (18.8)	1 (5.9)	1 (6.3)
AST increased	0	1 (6.3)	1 (5.9)	2 (12.5)
Nausea	0	0	2 (11.8)	2 (12.5)
Oropharyngeal pain	2 (11.8)	0	1 (5.9)	1 (6.3)
Pyrexia	1 (5.9)	0	2 (11.8)	1 (6.3)
Chills	0	0	2 (11.8)	1 (6.3)
Infection	0	0	1 (5.9)	2 (12.5)
GGT increased	0	0	2 (11.8)	1 (6.3)
Myalgia	0	0	2 (11.8)	1 (6.3)
Neutropenia	0	0	3 (17.6)	0
ALT increased	0	0	1 (5.9)	1 (6.3)
Blood ALP increased	0	0	1 (5.9)	1 (6.3)
Blood bilirubin increased	0	0	0	2 (12.5)
Blood LDH increased	0	0	1 (5.9)	1 (6.3)
Dizziness	0	0	2 (11.8)	0
Eye disorders	0	0	0	2 (12.5)
Leukopenia	0	0	2 (11.8)	0
Pain	0	0	2 (11.8)	0

[a]All data represent the numbers of subjects (per treatment group). A subject was counted once if one or more events were reported for each adverse event category.
[b]ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase.

between the groups randomized to ultimately receive either rifabutin or rifampin was in C_{max} ($P = 0.04$). Subjects randomized to receive rifabutin during period 2 had a higher period 1 bedaquiline C_{max} (4.10 μg/ml) than subjects randomized to receive rifampin in period 2 (3.57 μg/ml). The observed difference was small and likely an imbalance of randomization. Statistical comparisons of all other parameters were not significant. Summary bedaquiline pharmacokinetic parameter estimates for subjects in the rifabutin and rifampin treatment groups, as well as within-group statistical comparisons, are provided in Tables 3 and 4.

Effects of steady-state rifabutin on bedaquiline pharmacokinetic parameter estimates are shown in Table 3. Parameter estimates significantly affected by steady-state rifabutin treatment at day 29 compared to day 1 included $t_{1/2}$ ($P = 0.004$), k_{el} ($P = 0.004$), CL/F ($P = 0.01$), MRT_{336} ($P < 0.001$), and MRT_{inf} ($P < 0.001$). Mean residence times (MRT) for bedaquiline increased modestly in this treatment group between days

TABLE 3 Effect of rifabutin administration on the pharmacokinetics of bedaquiline

Parameter	Mean (SD)		*P*
	Day 1	Day 29	
C_{max} (μg/ml)	4.10 (1.21)	3.66 (1.05)	0.23
AUC_{0-336} (h·μg/ml)	57.34 (14.91)	50.33 (12.93)	0.11
$AUC_{0-\infty}$ (h·μg/ml)	59.54 (15.79)	53.28 (13.98)	0.18
T_{max} (h)	4.78 (1.30)	4.99 (1.26)	0.73
$t_{1/2}$ (h)	56.11 (4.78)	61.98 (6.66)	0.004
k_{el} (10^{-4}/h)	124 (8)	113 (12)	0.004
CL/F (liters/h)	7.23 (2.12)	8.08 (2.39)	0.01
MRT_{336} (h)	56.84 (7.22)	62.73 (6.94)	<0.001
MRT_{inf} (h)	69.75 (10.18)	82.23 (14.01)	<0.001

TABLE 4 Effect of rifampin administration on the pharmacokinetics of bedaquiline

Parameter	Mean (SD)		*P*
	Day 1	Day 29	
C_{max} (μg/ml)	3.57 (13.93)	2.54 (0.78)	0.003
AUC_{0-336} (h·μg/ml)	47.69 (14.85)	26.33 (7.84)	<0.001
$AUC_{0-\infty}$ (h·μg/ml)	49.52 (15.33)	27.81 (7.92)	<0.001
T_{max} (h)	4.26 (1.35)	4.75 (1.01)	0.17
$t_{1/2}$ (h)	56.15 (3.56)	59.37 (5.06)	0.04
k_{el} (10^{-4}/h)	124 (8)	118 (11)	0.04
CL/F (liters/h)	8.81 (2.60)	15.54 (4.51	<0.001
MRT_{336} (h)	57.62 (7.08)	54.31 (8.12)	0.21
MRT_{inf} (h)	71.26 (9.28)	75.24 (14.11)	0.38

1 and 29, and apparent clearance was slightly enhanced (7.23 liters/h during period 1 versus 8.08 liters/h during period 2). Plots of bedaquiline plasma concentration versus time for periods 1 (Fig. 2A) and 2 (Fig. 3A) bedaquiline concentrations over time for rifabutin-treated subjects are also provided. These plots demonstrate an exponential decline in bedaquiline concentration for approximately 72 h. Beyond 72 h, drug elimination appears complex in rifabutin-treated subjects, showing a more gradual decline punctuated by an apparent oscillatory behavior with continued measurable plasma concentrations up to 14 days (336 h) after a single bedaquiline dose in both periods (insets). In the rifabutin treatment group, the disposition kinetics of bedaquiline appear qualitatively similar during period 1 (Fig. 2A) and period 2 (Fig. 3A) and Fig. 4A.

Steady-state rifampin had more pronounced effects on bedaquiline disposition (Table 4) than did steady-state rifabutin. The mean AUC_{0-336} in the rifampin group dropped nearly half from 47.69 h·ng/ml at day 1 after single-dose bedaquiline treatment to 26.33 h·ng/ml at day 29 ($P < 0.001$). The mean C_{max} was also reduced significantly by day 29 in the rifampin group to 2.54 μg/ml (compared to 3.57 μg/ml at day 1, $P = 0.003$). Bedaquiline apparent clearance (CL/F) increased 76% in the rifampin group between day 1 and day 29 from 8.81 to 15.54 liters/h ($P < 0.001$). Other bedaquiline pharmacokinetic parameter estimates significantly affected between periods 1 and 2 in rifampin-treated subjects included $AUC_{0-\infty}$ ($P < 0.001$), $t_{1/2}$ ($P = 0.04$), and k_{el} ($P = 0.04$). Period 1 bedaquiline disposition in rifampin-treated subjects is displayed in Fig. 2B, demonstrating a bedaquiline disposition similar to that observed in the group randomized to receive rifabutin during period 2 (Fig. 2A). Figure 3B and 4B illustrate the reduction in bedaquiline C_{max} and the overall bedaquiline exposure in the rifampin treatment group compared to period 1 disposition for either treatment group (Fig. 2A). Steady-state rifampin also decreased the oscillatory behavior of bedaquiline concentrations beyond 72 h postdose (Fig. 3B, inset).

Enhancement or suppression of key bedaquiline summary pharmacokinetic parameter estimates in both the rifabutin- and rifampin-treated groups is demonstrated in Fig. 5. Among this rather small cohort, interindividual variability is apparent; however, the modest effects of rifabutin are easily contrasted with the relatively profound effects of rifampin (Table 5 and Fig. 4).

DISCUSSION

This randomized, controlled trial successfully evaluated the safety, tolerability, and pharmacokinetics of bedaquiline when combined with steady-state rifabutin or rifampin in healthy adult volunteers. The effect of steady-state rifabutin or rifampin on bedaquiline pharmacokinetic parameter estimates was also evaluated. The most notable finding is that steady-state rifampin during period 2 reduced overall bedaquiline exposure compared to bedaquiline given alone as a single dose during period 1. This may be due in part to increased clearance of bedaquiline in the face of steady-state rifampin. Steady-state rifabutin resulted in little quantitative impact on the pharmacokinetics of bedaquiline comparatively. It is important to note that in the present study, 15/16 subjects in the rifampin group self-identified as white. Bedaquiline exposure is

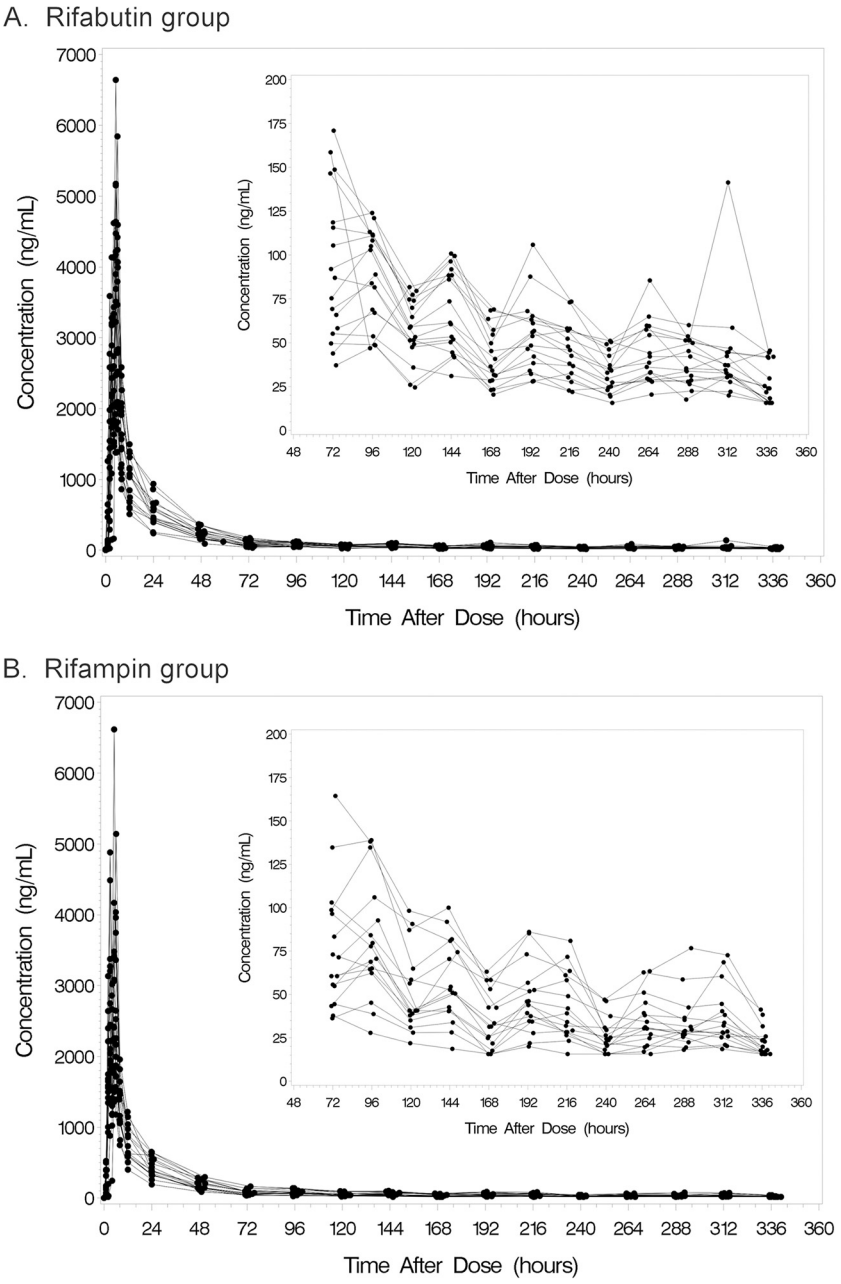

FIG 2 Period 1 bedaquiline plasma concentrations over time.

affected by race, with blacks experiencing increased clearance of the drug and a corresponding 34% reduction in exposure compared to nonblacks (10). Therefore, the increase in bedaquiline clearance and decreased overall exposure to bedaquiline observed in the rifampin treatment group may actually under represent effects which would be seen in black subjects. This is an important point to consider when developing dosing strategies for African populations.

Bedaquiline was well tolerated by study subjects, in accordance with previous studies (11–13). Nausea, arthralgias, and headache were the most common adverse events observed in patients who received bedaquiline in combination with other drugs used to treat MDR-TB in clinical trials (11). Headache was also the most common AE reported across treatment groups in the present study. During preclinical testing, the liver, skeletal muscle, heart, pancreas, stomach, and eyes were all identified by the

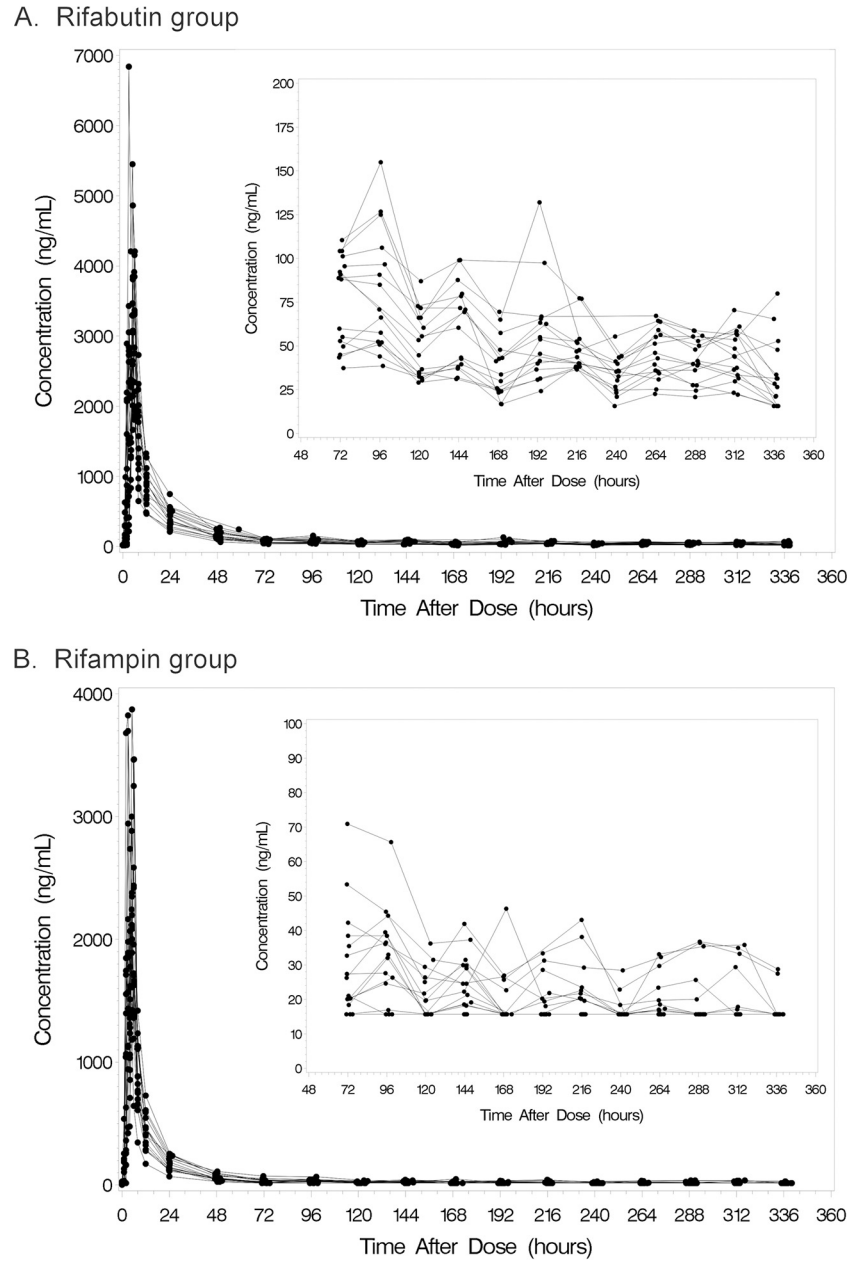

FIG 3 Period 2 bedaquiline plasma concentrations over time.

manufacturer as target organs for bedaquiline toxicity. In dogs, corneal eye lesions and intolerance to bright light were observed after bedaquiline treatment at various doses (11). Extensive ocular assessments were incorporated into the present study. A single asymptomatic right-eye retinal cotton-wool spot was observed in one subject in the rifampin treatment group at follow-up which was believed to be related to the study drug, but no other effects on the eye were observed in either treatment group.

In a phase 2, randomized, controlled trial, 47 patients newly diagnosed with MDR-TB received either bedaquiline (400 mg daily for 2 weeks, followed by 200 mg three times a week for 6 weeks) or placebo in combination with a standard five-drug, second-line antituberculosis regimen (kanamycin, ofloxacin, ethionamide, pyrazinamide, and cycloserine or terizidone). Adverse events were similar between the two groups, although 26% ($n = 6$) of subjects receiving bedaquiline reported nausea compared to just 4% ($n = 1$) of subjects receiving the five drugs without bedaquiline. The racial distribution of subjects in this study was 26 black, 1 white, and 20 other (47 total subjects) (12).

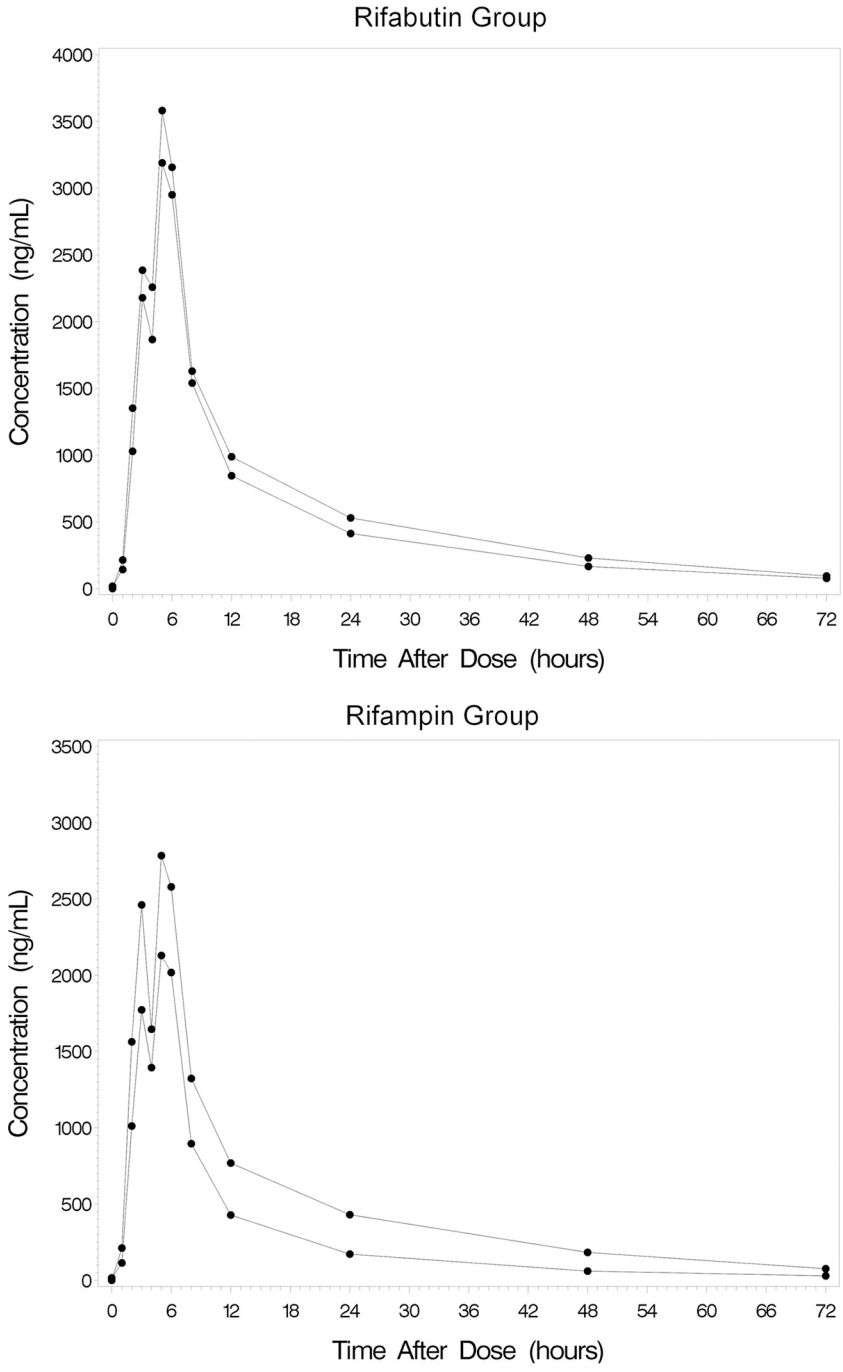

FIG 4 Effect of steady-state rifamycin on mean bedaquiline exposure during the 72 h after dosing. The upper line in each panel is for period 1; the lower line is for period 2.

In the present study, combining steady-state rifampin, and even more so rifabutin, with a single oral dose of bedaquiline in period 2 was associated with an increased frequency and severity of adverse events compared to bedaquiline treatment alone. The majority of grade 3 or higher adverse events observed in this study occurred in the rifabutin group. This includes lymphopenia, which was observed in 8 subjects in the rifabutin group. Lymphopenia onset in rifabutin-treated subjects was after the single oral dose of bedaquiline on day 29 in 6/8 affected subjects, suggesting the observation may be related to the combination regimen. Rifabutin has been historically associated with blood and lymphatic disorders which include cytopenias (leukopenia, lymphope-

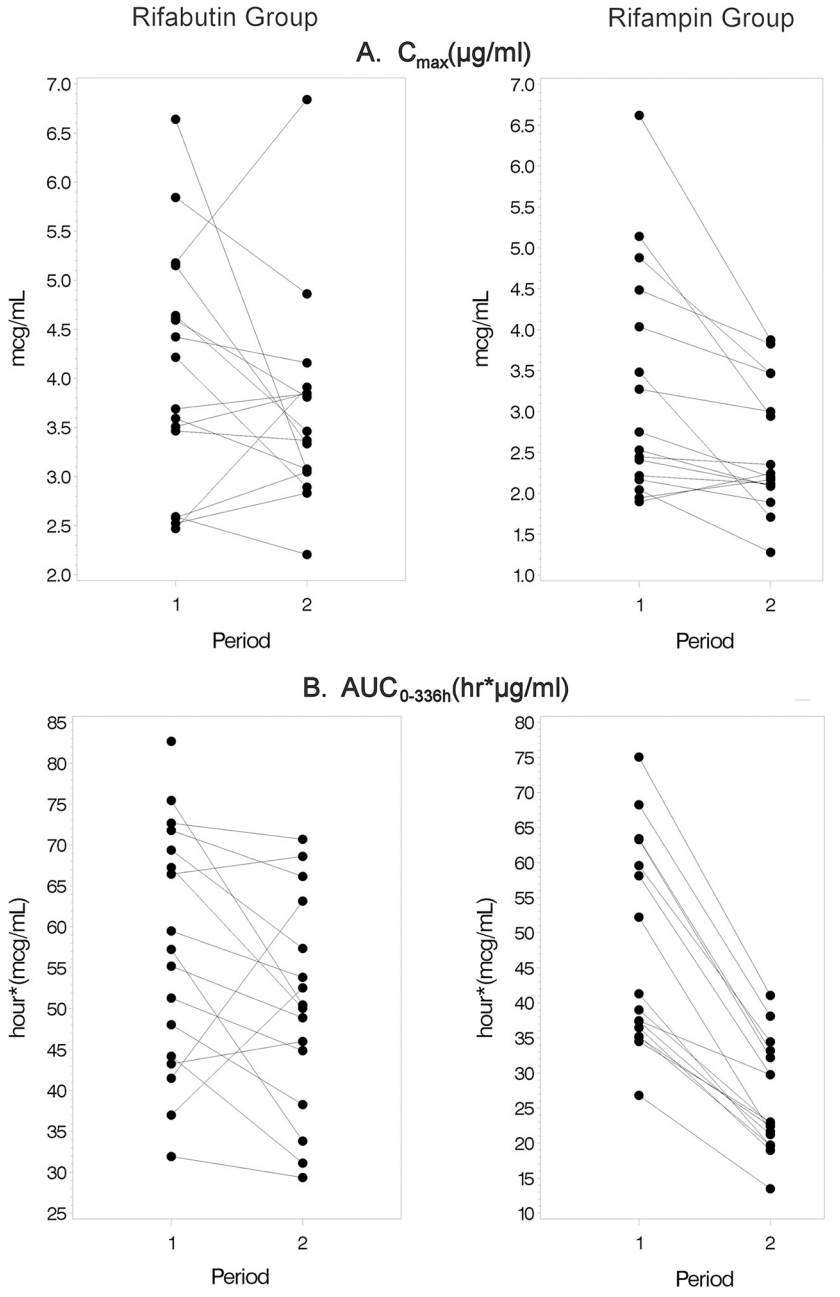

FIG 5 Effect of steady-state rifamycin treatment on key bedaquiline pharmacokinetic parameter estimates.

nia, and neutropenia) (14, 15). Combining rifabutin with other anti-infectives has been shown to enhance these effects by further reducing neutrophil (15, 16) and lymphocyte (17, 18) counts in healthy adults. However, the frequency of lymphopenia observed in the present study (47% of the rifabutin group affected) has not been generally observed with bedaquiline (11). It is not clear whether bedaquiline enhanced known toxicities associated with rifabutin or whether the observed toxicities are attributable to rifabutin only.

In a study assessing bedaquiline disposition following a single 400-mg dose given to 26 white, 8 black, 2 Hispanic, and 1 Asian healthy adult volunteers, median bedaquiline C_{max} was 3.4 μg/ml (interquartile range, 2.4 to 4.3 μg/ml) (9). Peak plasma bedaquiline concentrations observed here following a single 400-mg oral dose of bedaquiline during period 1 are compatible with this range (rifabutin group, C_{max} = 4.10 μg/ml; rifampin

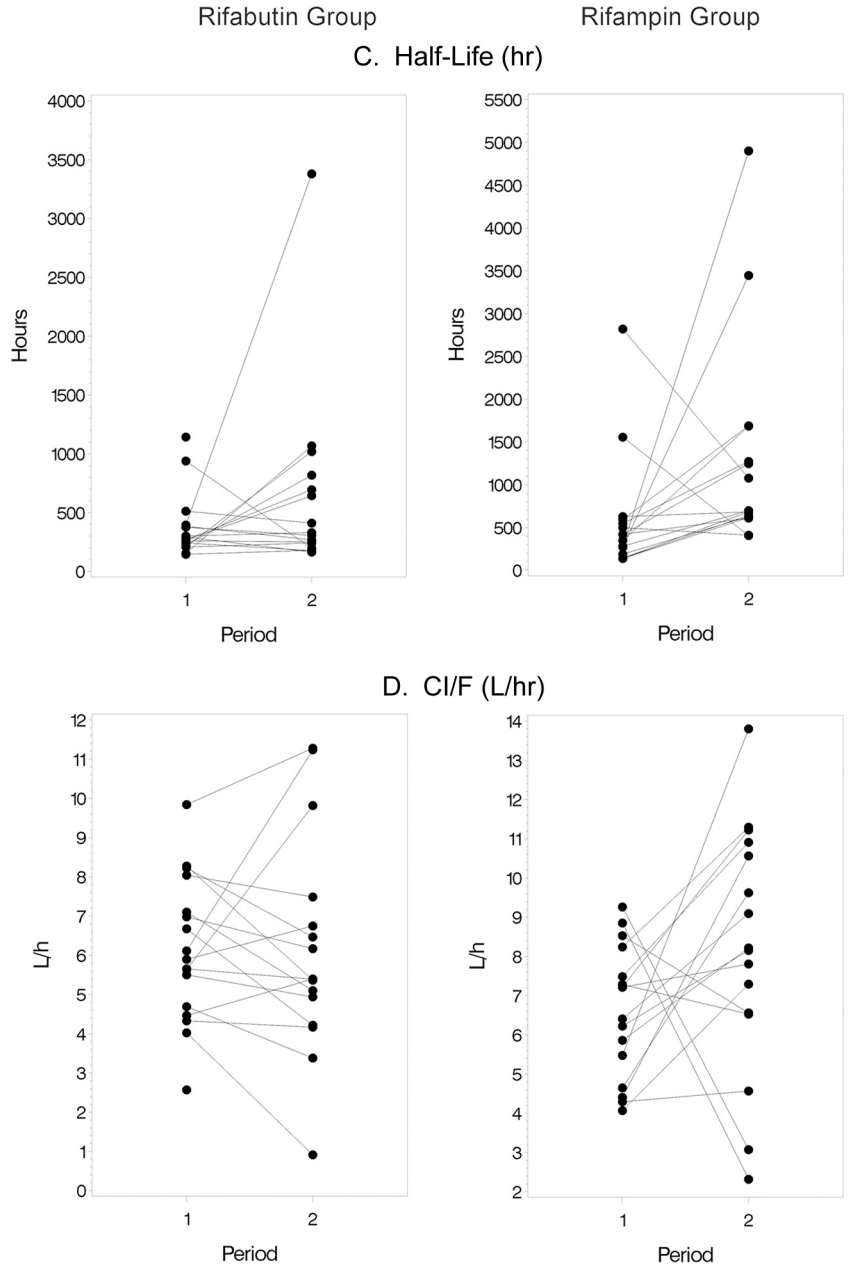

FIG 5 (Continued)

group, C_{max} = 3.57 μg/ml). Bedaquiline is associated with a time to maximum concentration of approximately 5 h, regardless of the dose (9, 11). The calculated T_{max} values during period 1 in the present study were 4.78 and 4.26 h for the rifabutin and rifampin treatment groups, respectively. Steady-state rifamycin treatment during period 2 did not have a significant effect on T_{max} in either treatment group (P = 0.17 and 0.73, respectively). Bedaquiline has a reported elimination half life of approximately 5 months (2, 9). Measureable bedaquiline concentrations were still present at 336 h postdose in our study. The saw tooth pattern of bedaquiline clearance observed in this study (Fig. 2 and 3) suggests that pharmacokinetic parameter estimates of elimination (e.g., $t_{1/2}$ and k_{el}) will have limited utility when describing bedaquiline disposition. Although a precise mechanism for this unique saw tooth clearance pattern remains unknown, the oscillations suggest sequential redistribution of bedaquiline from and to the intravascular compartment.

TABLE 5 Effect of steady-state rifamycin on bedaquiline pharmacokinetic parameter estimates

Parameter	Treatment group[a]			
	Rifabutin		Rifampin	
	GMR	CI	GMR	CI
C_{max}	0.910	0.776–1.068	0.803	0.705–0.915
$t_{1/2}$	1.012	1.037–1.172	1.056	1.002–1.112
AUC_{0-336}	0.901	0.789–1.028	0.554	0.519–0.599
$AUC_{0-\infty}$	0.918	0.808–1.044	0.565	0.523–0.610
CL/F	1.089	0.958–1.238	1.771	1.640–1.912
V/F	1.200	1.067–1.350	1.869	1.689–2.068

[a]GMR, geometric mean ratio; CI, confidence interval.

Steady-state rifampin during period 2 reduced overall exposure (AUC_{0-336}) of bedaquiline by nearly half and increased apparent clearance 76%, compared to period 1 values within the same treatment group (Table 4). This is similar to reduced antiviral exposure seen when rifampin is coadministered with other CYP3A4 substrates, including amprenavir (19), efavirenz (9), and lersivirine (20). Winter et al. (21), reported that the bedaquiline C_{max} and AUC were reduced by approximately 58% in healthy subjects following a single 400-mg dose of bedaquiline combined with steady-state rifapentine or rifampin (22 days of 600-mg daily dosing) compared to a single 400-mg dose of bedaquiline alone. Together, these data indicate that bedaquiline metabolism is induced by steady-state rifampin. It remains unclear whether bedaquiline exposure will be further affected after longer-term coadministration with rifabutin or rifampin.

A number of limitations should be noted when reviewing the present study results. Most importantly, the study population consisted predominantly of Caucasian subjects in contrast to the racial and ethnic backgrounds of patients likely to receive bedaquiline as part of an antimycobacterial regimen. Pharmacokinetics and drug-drug interactions in the target population may differ somewhat from the results reported here. The conclusions drawn also reflect single-dose administrations of bedaquiline rather than the daily or every other day dosing anticipated during drug treatment. Because the unique pharmacokinetic characteristics of bedaquiline preclude rigorous project of the single-dose data to the multidose regimen, the significance of the data in the context of a therapeutic regimen will require further study. The study is also impacted by the absence of some key information, such as the minimal bedaquiline exposure required to achieve antimycobacterial effectiveness and baseline levels of cytopenias related to the rifamycins in the target population that are essential to explaining the clinical importance of some of the results reported.

Rifabutin and rifampin are central to anti-tuberculosis regimens, and known inducers of CYP3A4 (7). Coadministration of inducers of CYP isoenzymes with bedaquiline is expected to reduce overall exposure of bedaquiline (10). By day 29, rifabutin- and rifampin-treated subjects differed quantitatively with respect to the majority of calculated pharmacokinetic parameters (Tables 3 and 4). Steady-state rifabutin did not affect bedaquiline exposure as appreciably as steady-state rifampin in period 2. This may be due to the fact that rifabutin is a weaker inducer of CYP3A4 compared to rifampin (7, 19). The minimal effect of rifabutin on bedaquiline exposure is similar to that observed when rifabutin is administered daily with amprenavir, another weak CYP3A4 substrate (19). Conversely, rifabutin significantly reduces lersivirine half-life and steady-state plasma concentrations when administered in combination. These effects are enhanced following steady-state rifampin coadministration (20), similar to effects on bedaquiline pharmacokinetics seen here in the rifampin treatment group.

The effect of the rifamycins on the pharmacokinetics of other anti-TB agents, as well as various anti-infectives utilized in the treatment of coinfections such as HIV, continues to be important. It is critical that the effects of rifamycin on the pharmacokinetics of bedaquiline are thoroughly understood if bedaquiline is to be applied for first line anti-TB therapy. At present, the use of bedaquiline is indicated primarily in combination

drug regimens for MDR-TB. The reduction in exposure to bedaquiline observed following steady-state rifampin administration, and observed toxicities following steady-state rifabutin administration, suggests dosage adjustments may be necessary to ensure bedaquiline plasma concentrations reach therapeutic levels safely when these drugs are coadministered. Such adjustments will ensure effective use of this novel diarylquinolone in the treatment of *M. tuberculosis* infection.

MATERIALS AND METHODS

Materials. Bedaquiline (100-mg oral tablets) was provided by Tibotec, now Janssen Therapeutics. Rifabutin and rifampin (150-mg oral capsules) were obtained from Fisher BioServices. Water and elution solvents for drug analysis, including methanol and acetonitrile, were high-pressure liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS) grade and purchased from Fisher Scientific (Pittsburgh, PA). Sigma-Aldrich (St. Louis, MO) was the supplier for ascorbic acid (ACS grade). Other drug standards and internal standards 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 IVT, LLC.

Trial design and study population. This was a single-site, randomized, open-label, prospective study in healthy adult volunteers (Clinicaltrials.gov registration number NCT01341184). All study procedures were conducted at the Case Western Reserve University and University Hospitals Case Medical Center (UHCMC) in Cleveland, OH. The study was approved by the Institutional Review Board at the UHCMC, and all subjects provided written informed consent prior to participating in the study. The primary objectives of the study were to (i) evaluate the safety and tolerability of bedaquiline when given in combination with rifabutin or rifampin and (ii) assess the effect of steady-state rifamycin dosing on the pharmacokinetics of bedaquiline given as just two single 400-mg doses separated in time by 28 days.

The study design included two study periods and two rifamycin treatment groups. All subjects received a single 400-mg dose of bedaquiline on study days 1 (period 1) and 29 (period 2). After enrollment, subjects were randomized in simple randomization blocks of four to one of two treatment groups to receive either 300 mg of rifabutin or 600 mg of rifampin once daily from days 20 to 41 (period 2). Subjects from both rifamycin treatment groups were enrolled and treated in parallel. A study flow diagram is included in Fig. 1.

Study participants were eligible for inclusion if they met the following criteria: aged 18 to 45 years (inclusive); nonsmoker; no illicit drug use; body mass index of 18 to 35 kg/m^2; negative screens for hepatitis B and C, and human immunodeficiency virus; and healthy on the basis of clinical assessment (including physical exam, medical history, electrocardiogram [ECG], vital signs, ophthalmologic exam, blood biochemistry and hematology, and urinalysis). Subjects with clinical evidence of acute illness, diarrhea, history of skin or ophthalmologic disease, current use of azoles or concomitant medications affecting CYP3A4 pathways, or blood donation or similar loss of blood within 56 days of enrollment or who had taken another investigational drug within 60 days prior to enrollment were excluded. Participating female subjects were required to use two forms of acceptable birth control throughout the study and pregnant women were not enrolled.

Clinical safety assessments. Subjects remained inpatient in the Dahm's Clinical Research Unit at the UHCMC for at least 24 h prior to and after each bedaquiline dose (days −1 to 2 and days 28 to 30). In period 2, subjects in both rifamycin treatment groups returned to the ambulatory unit at the UHCMC for rifamycin dosing, outpatient blood draws, and safety assessments. Safety assessments, including physical examinations, vital signs, ECGs, serum chemistry, hematology, coagulation, and urinalysis (pH, specific gravity, protein, glucose, microalbumin, ketones, bilirubin, nitrite, urobilinogen, and leukocyte esterase) were completed at screening, at enrollment, at 24 and 336 h after each bedaquiline dose, and six additional times throughout the rifamycin dosing period. Eye exams with fundoscopy, slit lamp, and retinal photos were completed at screening and on days 2, 19, and 29 at the Retinal Diseases Image Analysis Reading Center at UHCMC. A follow-up study visit occurred on day 57 (28 days after the last bedaquiline dose) for final safety assessments listed above, including eye examination.

Measurement of bedaquiline in plasma. Serial blood samples for bedaquiline measurement were collected before bedaquiline dosing on days 1 and 29, as well as 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 h after each bedaquiline dose. Three-milliliter samples were drawn into collection tubes containing sodium heparin and placed immediately on ice. The blood was centrifuged at 2,500 × *g* within 2 h of collection, and the plasma fraction was 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 175-μl aliquot of unknown plasma was transferred to a labeled tube on ice. The remainder of the sample was refrozen immediately at −70°C.

Determination of bedaquiline. 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.

To the 1.5-ml microfuge tube containing the 175 μl of plasma, 300 μl of internal standard working solution containing 500 ng/ml TMC207-d6 in methanol containing 1% formic acid was added. After brief mixing, the sample was applied to a preconditioned (500 μl of methanol, followed by 500 μl of water) Bond-Elut Plexa solid-phase extraction cartridge (1-ml volume with 30 mg of absorbent) on an SPE vacuum manifold (at −5 psi). The cartridge was rinsed with 500 μl of water. The eluate was transferred to a clean 16-by-100-mm borosilicate tube with 1.50 ml of LC-MS methanol for evaporation to 200 μl

under N_2 at 30°C. After the samples were dry, they were reconstituted in 75 μl of 0.1% citric acid methanol. The reconstituted sample was diluted with 75 μl of HPLC water, transferred to a "nonfooted" HPLC insert vial, and centrifuged for 5 min at 10,000 rpm. The supernatant was used for injection onto the HPLC apparatus.

Detection and analysis were performed using a validated LC-MS/MS assay developed using a Varian 1200L liquid chromatograph mass spectrometer (Palo Alto, CA) and a SIL-A20 AC HT Autosampler (Shimadzu Instruments, Inc., Columbia, MD) interfaced with a Pro Star HPLC system model 210 (Varian, Inc., Palo Alto, CA). The autosampler injection volume was 25 μl placed on a Phenomenex Security Guard C_8 precolumn (4.0 by 3.0 mm) and then a Supelco Discovery C_{18} column (50.0 by 2.1 mm; 5 μm) heated to 30°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 0.21 ml/min and was stepped up to 0.25 ml/min at 9.57 min into the run as the percentage of ammonium acetate declined from 100 to 25%. This flow rate was maintained from 9.57 min until 11.57 min and then was ramped to 0.21 ml/min as the gradient recovered to its initial solvent composition. The total run time for each sample was 15.57 min.

Multiple reaction monitoring for bedaquiline followed transitions from the two precursor ions at 555.2 and 557.2 atomic mass units (amu) to the product ions at 523.1 and 525.1 amu, respectively. The upper and lower limits of quantitation for bedaquiline were 8,000 and 20 ng/ml, respectively. The intraday precision and accuracy of the high-, mid-, and low-quality control standards were 7.24, 5.62, and 6.79% and −3.68, 7.06, and 12.14%, respectively. The corresponding interday values were 6.45, 6.58, and 10.90% and −3.52, 2.47, and 4.85%, respectively.

Pharmacokinetic analysis. Plasma concentration versus time profiles from blood draws were generated for bedaquiline and plotted on the raw scale and semilogarithmic scale for visual inspection. Concentrations were summarized over all subjects with the means ± the standard deviations for each protocol time point and plots constructed. There were minimal protocol deviations as a result of samples not being drawn at protocol times. Two subjects in period 1 were missing concentration data at one time point due to an insufficient quantity to assay. An additional five missing concentrations across four subjects were also omitted from period 2 analyses due to an insufficient quantity to assay.

Pharmacokinetic parameters were estimated using each subject's time-concentration data in separate analyses. First-dose pharmacokinetic parameters were estimated at both day 1 and day 29. Standard noncompartmental methods using Phoenix WinNonlin (version 6.3) were used, with observed values for the apparent maximum concentration (C_{max}) and time to maximum concentration (T_{max}). The area under the plasma drug concentration versus time curve was determined using the linear trapezoidal rule up to the final measurable concentration point (AUC_{0-336}) and then extrapolated to infinity ($AUC_{0-\infty}$). The apparent clearance (CL/F) was determined according to the following formula: dose/$AUC_{0-\infty}$. The MRT_{336} (the MRT at 336 h) and MRT_{inf} are both reported. MRT is defined as the average total time molecules of a given dose spend in the body. MRT is calculated as the AUMC (not reported here)/AUC using the respective measures of AUMC and AUC. The ratios for C_{max}, both measures of AUC, the terminal elimination half-life, and the CL/F were calculated as the value from period 2/the value from period 1.

On observation, time-concentration profiles appear to exhibit multiple compartments. This observation has also been made by others (9). In addition, the terminal portion of the curve does not exhibit a monotonic decrease in concentration as one would expect. Instead, the drug elimination beyond 72 h after dosing appears complex showing a gradual decline punctuated by an apparent oscillatory behavior with continued measurable plasma concentrations up to 14 days after a single dose. These characteristics suggest that single pharmacokinetic parameter estimates of elimination, e.g., $t_{1/2}$ and k_{el}, will have limited utility when describing TMC207 disposition. For this report, time points were selected to estimate the terminal slope and corresponding elimination half-life, overriding the algorithm in the software for choosing points. As a result, the pharmacokinetic parameters provided here were calculated using a second method, which estimated an average elimination slope and half-life over the entire profile, treating the data as one compartment. The last point used in calculation of the slope was the last observed concentration and the first was the maximum concentration (C_{max}). These measures allow for comparison of our data with other published data (9); however, it represents an estimate of a general elimination averaged over all phases.

Statistical analysis. Between-treatment group comparisons were performed using *t* tests for demographic and laboratory parameters, chi-squared tests (for gender), and *t* tests based on the logarithmically transformed values for plasma concentrations and pharmacokinetic summary parameters. Geometric mean ratios were calculated as the sample mean of the log-transformed data exponentiated to bring it back to the original scale. The confidence limits for the geometric mean ratio were calculated in a similar fashion from the 95% confidence interval of the log-transformed data. The calculations were carried out in PROC TTEST, SAS v 9.4 (SAS Institute, Cary, NC).

Within-treatment group comparisons between day 1 and day 29 values for individual time point plasma concentrations, and for pharmacokinetic summary parameters, were performed using paired *t* tests on the logarithmically transformed values. All statistical tests of hypothesis were two sided and used a 0.05 level of significance.

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