

Randomized Dose-Ranging Study of the 14-Day Early Bactericidal Activity of Bedaquiline (TMC207) in Patients with Sputum Microscopy Smear-Positive Pulmonary Tuberculosis

Andreas H. Diacon,^{a,b} Rodney Dawson,^c Florian Von Groote-Bidingmaier,^b Gregory Symons,^c Amour Venter,^d Peter R. Donald,^e Almari Conradie,^f Ngozi Erondu,^f Ann M. Ginsberg,^f Erica Egizi,^f Helen Winter,^g Piet Becker,^h Carl M. Mendel^f

Division of Physiology, Department of Medical Biochemistry, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa^a; Task Applied Science, Karl Bremer Hospital, Bellville, Cape Town, South Africa^b; Division of Pulmonology, Department of Medicine, University of Cape Town Lung Institute, Cape Town, South Africa^c; MRC Centre for Molecular and Cellular Biology, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa^d; Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa^e; Global Alliance for TB Drug Development, New York, New York, USA, and Pretoria, South Africa^f; School of Pharmacy, University of Otago, Dunedin, New Zealand^g; Medical Research Council, Pretoria, South Africa^h

Bedaquiline is a new antituberculosis agent targeting ATP synthase. This randomized, double-blinded study enrolling 68 sputum smear-positive pulmonary tuberculosis patients evaluated the 14-day early bactericidal activity of daily doses of 100 mg, 200 mg, 300 mg, and 400 mg bedaquiline, preceded by loading doses of 200 mg, 400 mg, 500 mg, and 700 mg, respectively, on the first treatment day and 100 mg, 300 mg, 400 mg, and 500 mg on the second treatment day. All groups showed activity with a mean (standard deviation) daily fall in log₁₀ CFU over 14 days of 0.040 (0.068), 0.056 (0.051), 0.077 (0.064), and 0.104 (0.077) in the 100-mg, 200-mg, 300-mg, and 400-mg groups, respectively. The linear trend for dose was significant ($P = 0.001$), and activity in the 400-mg dose group was greater than that in the 100-mg group ($P = 0.014$). All of the bedaquiline groups showed significant bactericidal activity that was continued to the end of the 14-day evaluation period. The finding of a linear trend for dose suggests that the highest dose compatible with safety considerations should be taken forward to longer-term clinical studies.

Bedaquiline (BDQ), previously known as TMC207, is a new antituberculosis agent with a unique mechanism of action targeting ATP synthase (1). The U.S. Food and Drug Administration recently approved BDQ as part of combination therapy to treat adults with multidrug-resistant (MDR) pulmonary tuberculosis (TB). In a previous study of its early bactericidal activity (EBA) during the first 7 days of treatment, a dose of 400 mg once daily reduced counts of CFU of *Mycobacterium tuberculosis* in sputum, with a delayed onset of action. At doses of 25 mg daily and 100 mg daily, no significant effect was found (2). In a later study, BDQ in a dose of 400 mg daily for the first 2 weeks, followed thereafter by 200 mg three times per week, added to an optimized regimen for MDR TB, significantly increased the proportion of patients achieving sputum culture negativity after 2 months of treatment compared to patients receiving an optimized background regimen with a placebo (48% versus 9%, respectively) (3). Furthermore, significantly fewer patients acquired additional resistance to companion drugs after receiving BDQ (4). Because of the slow rise to steady state, suboptimal BDQ plasma concentrations early in treatment were possibly responsible for the delayed onset of action in the initial EBA study and for the lack of any detectable early action at lower doses. As it is important that the optimal BDQ dosing regimen be taken forward in further clinical development, the present study evaluated the 14-day EBA of four daily doses ranging from 100 mg to 400 mg, preceded by loading doses on the first two treatment days.

MATERIALS AND METHODS

Patients and setting. This was a two-center, double-blinded, centrally randomized phase II trial investigating the EBA, safety, tolerability, and pharmacokinetics (PK) of BDQ given at one of four possible daily doses in matching 100-mg tablets: 100 mg, 200 mg, 300 mg, and 400 mg from treatment days 3 to 14, preceded by single daily loading doses of 200 mg, 400 mg, 500 mg, and 700 mg, respectively, on treatment day one and 100

mg, 300 mg, 400 mg, and 500 mg, respectively, on treatment day two. Loading doses were designed for each dose level to bring BDQ plasma levels to the desired target concentration quickly. A smaller patient group received the South African standard treatment of weight-adjusted Rifampin e-275 (Sanofi, Midrand, South Africa), a fixed-dose combination of rifampin (RMP), isoniazid (INH), pyrazinamide, and ethambutol, as a positive control to assess mycobacteriological laboratory procedures. Clinical staff were fully blinded within the BDQ arms, but the standard-treatment group was not blinded due to the inevitable discoloration of urine by RMP. Laboratory personnel were fully blinded while assessing bacteriologic endpoints.

Sixty-eight treatment-naïve sputum microscopy smear-positive (at least 1+ on the International Union against Tuberculosis and Lung Disease scale) (5) adult patients aged 18 to 65 years with a body weight of 40 to 90 kg and with uncomplicated pulmonary tuberculosis sensitive to INH and RMP (GenoType MTBDR; Hain Lifescience, Nehren, Germany) were recruited from outpatient clinics in Cape Town, South Africa, and were hospitalized for the study duration at one of two centers (Task Applied Science, Intercare, and the Centre for Tuberculosis Research Innovation, UCT Lung Institute). HIV-infected patients participated if their CD4 count was >300 cells/ μ l and they had not received antiretroviral therapy within the previous 90 days. Diabetics and patients with any QT/QTc interval abnormalities on electrocardiogram (ECG) were excluded.

Microbiological methods. Microbiological testing was done centrally in the Department of Medical Biochemistry, Faculty of Medicine and

Received 6 November 2012 Returned for modification 8 December 2012

Accepted 23 February 2013

Published ahead of print 4 March 2013

Address correspondence to Andreas H. Diacon, ahd@sun.ac.za.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.02243-12

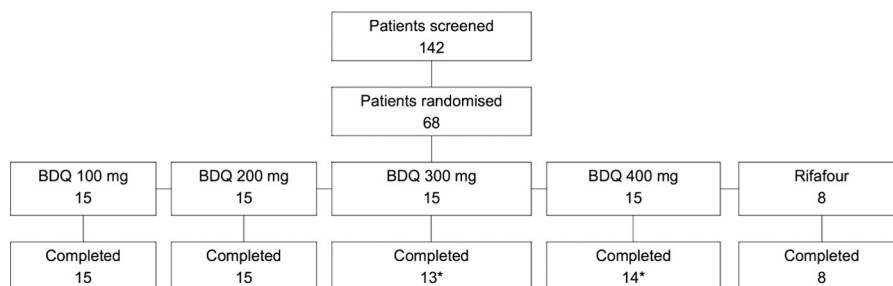


FIG 1 Disposition of patients. *, one patient randomized to 300 mg BDQ was withdrawn before drug treatment was initiated and one patient following two doses of 400 mg BDQ, both for laboratory abnormalities at baseline that only became available after randomization. One patient in the 300 mg group was withdrawn in error.

Health Sciences, Stellenbosch University, Cape Town, South Africa. Sputum for CFU counts of *M. tuberculosis* and determination of time to a positive signal (TTP) in liquid culture medium (Bactec Mycobacteria Growth Indicator Tube; MGIT 960; BD, Franklin Lakes, NJ) were collected for a 16-hour period overnight for 2 days prior to treatment initiation and on days 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 14 after starting treatment and subjected to laboratory processing as described previously (2, 6). Briefly, sputum was homogenized with magnetic stirring. Dithiothreitol (1:20 dilution; Sputasol; Oxoid, Cambridge, United Kingdom) was added to a maximum of 10 ml of homogenized sputum in equal volume, vortexed for 20 s, and left to digest at room temperature for 20 min. For CFU counting, 1 ml of this digested sputum was used to prepare a range of 10-fold dilutions from 10^0 to 10^{-5} . From each dilution, 100 μ l was plated in quadruplicate on 7H11 agar plates (BD) that contained 200 units/ml of polymyxin B, 10 μ g/ml of amphotericin B, 100 μ g/ml of ticarcillin, and 10 μ g/ml of trimethoprim (Selectatab; Mast, Merseyside, United Kingdom). Numbers of CFU were counted after 3 to 4 weeks of incubation at 37°C at the dilution yielding 20 to 200 visible colonies. For TTP measurement, we used a standardized liquid culture system (Bactec MGIT 960; BD). Briefly, homogenized sputum was decontaminated (MycPrep; BD), centrifuged, and resuspended, and 0.5 ml of the resulting 2 ml was used for incubation in duplicate (6). Cultures from baseline and the last overnight sputum collection were used for susceptibility testing for first-line drugs (MGIT SIRE kit; BD); susceptibility to BDQ was analyzed by a resazurin microtiter assay (Institute of Tropical Medicine, Antwerp, Belgium). *M. tuberculosis* identification to the species level was by PCR (7).

Safety, tolerability, and pharmacokinetics. Patients were hospitalized for the duration of BDQ treatment and visited daily to capture vital signs and adverse events. Clinical laboratory tests and 12-lead ECGs were carried out at baseline and at regular intervals during treatment. Blood for PK studies of BDQ and its M2 metabolite using a previously described methodology (PRA International, Assen, The Netherlands) was drawn predose each day during treatment and at 1, 3, 5, 6, 8, 12, and 24 h after

dosing on treatment days 1, 8, and 14 (3). Noncompartmental PK analysis of plasma TMC207 concentration-time data was performed (Aeras, Rockville, MD). Following discharge, patients were referred for a full course of standard antituberculosis treatment and seen for follow-up 5 weeks after discharge for a clinical evaluation, including a 12-lead ECG and drawing of a blood sample.

Statistical methods. This was a descriptive proof-of-concept study of exploratory character; the sample size (15 patients per group) was similar to those of previous phase II studies, allowing for up to 3 dropouts per arm. The data from all randomized participants who received any study medication were included in the analysis. Quantitative variables are expressed as means and standard deviations (SD), and PK and pharmacodynamic (PD) data are expressed as medians and ranges. Briefly, bactericidal efficacy was assessed by the decrease in \log_{10} CFU/ml sputum as EBA_{CFU} or the prolongation of TTP in hours as EBA_{TTP} in liquid medium (MGIT 960) during the periods of 0 to 2, 0 to 14, 2 to 14, and 7 to 14 days after treatment commencement. Activities were described by bilinear regression of \log_{10} CFU over time, fitted by least squares with an inflection point at 2.5 days for the Rifafour group and 3.5 days (CFU) or 7.5 days (TTP) for the BDQ groups. The inflection points for each group were set at the midpoint between the 2 days for which the mean squared error of the bilinear regression was the smallest. The fit to all data in the entire curve was used to calculate the EBA as the weighted mean of the slopes over each time interval. EBA was calculated for each patient individually and then averaged to obtain the group mean EBA. The mean of two baseline determinations was recorded as the baseline. Although the study was not powered for statistical significance between any two groups, an exploratory analysis was done and the presence of a linear-dose-related trend over the BDQ groups was evaluated by testing the contrast (-3, -1, 1, 3) following one-way analysis of variance (ANOVA); Hochberg's step-up approach, dealing with multiplicity, was used to compare the BDQ dose groups.

TABLE 1 Demographic characteristics of sputum CFU counts and time to positivity of patients prior to commencement of chemotherapy

Characteristic ^a	Value for:					
	Bedaquiline					All
	100 mg	200 mg	300 mg	400 mg	Rifafour	
<i>n</i>	15	15	15	15	8	68
Age (yrs) (SD)	29.5 (9.4)	34.7 (18.1)	31.4 (7.4)	31.8 (10.9)	26.1 (4.9)	27 (11.45)
No. of males (%)	9 (60)	11 (73.3)	8 (53.3)	10 (66.7)	6 (75)	44 (64.7)
No. HIV positive (%)	1 (7)	1 (7)	1 (7)	2 (13)	1 (12.5)	6 (9)
Weight (kg) (SD)	53.0 (7.74)	52.5 (7.55)	52.7 (8.20)	50.6 (5.96)	49.1 (6.69)	51.8 (7.23)
BMI (kg/m ²) (SD)	19.80 (2.988)	18.63 (1.955)	18.80 (2.754)	18.36 (2.174)	17.83 (2.018)	18.77 (2.459)
Baseline \log_{10} CFU/ml (SD)	6.302 (0.697)	6.001 (0.903)	6.071 (1.087)	6.625 (0.756)	5.995 (1.018)	6.199 (0.892)
Baseline TTP (h) (SD)	104.8 (23.5)	111.1 (30.6)	115.7 (40.8)	88.5 (10.8)	106.7 (28.8)	105.3 (26.9)

^a BMI, body mass index; TTP, time to positivity.

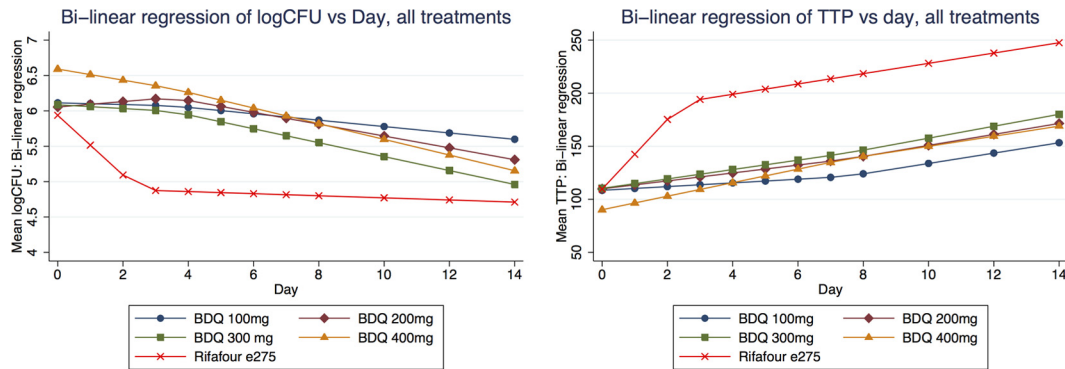


FIG 2 Early bactericidal activity of BDQ administered in doses of 100 mg, 200 mg, 300 mg, and 400 mg on treatment days 3 to 14, preceded by single daily loading doses of 200 mg, 400 mg, 500 mg, and 700 mg, respectively, on treatment day 1 and 100 mg, 300 mg, 400 mg, and 500 mg, respectively, on treatment day 2. Rifafour (isoniazid, rifampin, pyrazinamide, and ethambutol) was used as a positive control. Early bactericidal activity was measured as the daily fall in CFU of *M. tuberculosis* and the daily prolongation of time to positive signal (TTP) in hours. The slopes are modeled using bilinear regression with slope changes at 2.5 days for Rifafour, at 3.5 days for BDQ measured with CFU, and at 7.5 days for BDQ measured with TTP.

RESULTS

Patients. The patient disposition is summarized in Fig. 1, and demographic characteristics are given in Table 1. There were no significant differences for any characteristic between groups. One patient each from the 300-mg and 400-mg groups was withdrawn owing to pretreatment laboratory abnormalities, which became known only after drug administration had started. One patient in the 300-mg group was withdrawn in error. Sixty-five patients completed drug treatment.

Microbiological outcomes. All patients were infected with *M. tuberculosis* susceptible to the treatment given, and all MICs of BDQ before and after treatment were <0.1 µg/ml. Because of insufficient data for bilinear regression, four patients were omitted from EBA_{CFU} calculations and nine were omitted from EBA_{TTP} calculations, leaving 64 and 59 participants for EBA_{CFU} and EBA_{TTP} calculations, respectively. Time trends using bilinear regression of log₁₀ CFU/ml sputum on treatment day and TTP (hours) on treatment day are shown in Fig. 2, and numeric EBA_{CFU} and EBA_{TTP} results are displayed in Tables 2 and 3. *Post hoc* estimation, following analysis of variance comparing BDQ

groups with respect to EBA_{CFU} for the time intervals of interest, showed a significant linear trend over doses for the period of 0 to 14 days and 2 to 14 days for EBA_{CFU}. Furthermore, mean EBA_{CFU} between days 0 and 14 and EBA_{CFU} between days 2 and 14 at the 400-mg dosage were significantly greater than those at the 100-mg dosage. The EBA_{TTP} of the BDQ groups did not differ from one another except EBA_{TTP} for days 0 to 2, for which a significant linear trend over the dose groups was found and over which period the result of the 400-mg dosage differed significantly from that of the 100-mg dosage. Specific differences were detected using Hochberg’s step-up approach that takes care of multiplicity during multiple pairwise comparisons. None of the covariates, age, sex, or ethnicity, was associated with any efficacy variable.

Safety. All subjects, barring the three withdrawn patients, completed drug intake. At least one adverse event considered to be related to the study medication was reported in 8 (13.3%) patients on BDQ and in 2 (25%) patients on Rifafour. These were generally mild to moderate events, such as skin abnormalities, headache, nausea, or vomiting, routinely observed in similar studies. The only event rated severe was reported in a patient on 100 mg BDQ

TABLE 2 Extended early bactericidal activity determined by the fall in CFU/ml of sputum of *M. tuberculosis*^a

Days	Mean EBA _{CFU} (SD) for each treatment regimen (n)				Standard treatment (8)
	Bedaquiline				
	100 mg (14)	200 mg (15)	300 mg (13)	400 mg (14)	
0–2	0.004 (0.168)	−0.033 (0.128)	0.015 (0.149)	0.093 (0.136)	0.413 (0.291)
0–14	0.040 (0.068)	0.056 (0.051)	0.077 (0.064)	0.104 (0.077)	0.112 (0.077)
2–14	0.047 (0.074)	0.071 (0.047)	0.088 (0.071)	0.106 (0.078)	0.046 (0.101)
7–14	0.053 (0.088)	0.086 (0.048)	0.098 (0.084)	0.107 (0.082)	0.046 (0.101)

^a Determined by the fall in CFU/ml of sputum of *M. tuberculosis* (EBA_{CFU}) over days 0 to 2, 2 to 14, 0 to 14, and 7 to 14 in pulmonary tuberculosis patients receiving BDQ in daily doses of 100 mg, 200 mg, 300 mg, or 400 mg or standard treatment (Rifafour) consisting of isoniazid, rifampin, pyrazinamide, and ethambutol, derived with bilinear regression with a node at day 3.5 fitted by least squares for all BDQ groups and 2.5 days for standard treatment. The BDQ groups do not differ from one another for the mean efficacy variables EBA_{CFU} for days 0 to 2 (*P* = 0.143), EBA_{CFU} for days 0 to 14 (*P* = 0.073), EBA_{CFU} for days 2 to 14 (*P* = 0.141), and EBA_{CFU} for days 7 to 14 (*P* = 0.269); however, the linear trend for dose was significant for the periods of days 0 to 14 (*P* = 0.001) and 2 to 14 (*P* = 0.021) but not for days 0 to 2 (*P* = 0.076). For EBA_{CFU} for days 0 to 14 and 2 to 14, the 400-mg dose group was significantly better than the 100-mg group (*P* = 0.014 and *P* = 0.026, respectively).

TABLE 3 Extended early bactericidal activity determined by the prolongation in time to positivity^a

Days	Mean EBA _{TTP} (SD) for each treatment regimen (n)				Standard treatment (8)
	Bedaquiline				
	100 mg (13)	200 mg (13)	300 mg (13)	400 mg (12)	
0–2	1.5 (2.3)	3.7 (4.3)	4.1 (4.7)	6.2 (3.3)	27.3 (13.8)
0–14	4.0 (5.1)	4.2 (3.1)	4.9 (5.1)	5.4 (3.4)	14.3 (11.4)
2–14	4.4 (5.9)	4.3 (3.1)	5.1 (5.3)	5.3 (3.5)	11.5 (13.9)
7–14	6.9 (11.2)	4.8 (4.4)	5.9 (7.2)	4.4 (5.0)	11.5 (13.9)

^a Determined over days 0 to 2, 2 to 14, 0 to 14, and 7 to 14 by the prolongation in time to positivity (hours) in liquid medium (EBA_{TTP}) in pulmonary tuberculosis patients receiving BDQ in daily doses of 100 mg, 200 mg, 300 mg, or 400 mg or standard treatment (Rifafour) consisting of isoniazid, rifampin, pyrazinamide, and ethambutol, derived with bilinear regression with a node at day 7.5 fitted by least squares for all BDQ groups and 2.5 days for standard treatment. The BDQ groups do not differ from one another for the mean efficacy variables EBA_{TTP} for days 0 to 14 (*P* = 0.831), EBA_{TTP} for days 2 to 14 (*P* = 0.937), and EBA_{TTP} for days 7 to 14 (*P* = 0.835), but dose groups did differ significantly for EBA_{TTP} for days 0 to 2 (*P* = 0.027). The linear trend over dose groups was not significant for the periods of days 0 to 14 (*P* = 0.366), 2 to 14 (*P* = 0.575), or 7 to 14 (*P* = 0.508) but was significant for days 0 to 2 (*P* = 0.003). The 400-mg dose group differed significantly from the 100-mg group (*P* = 0.003).

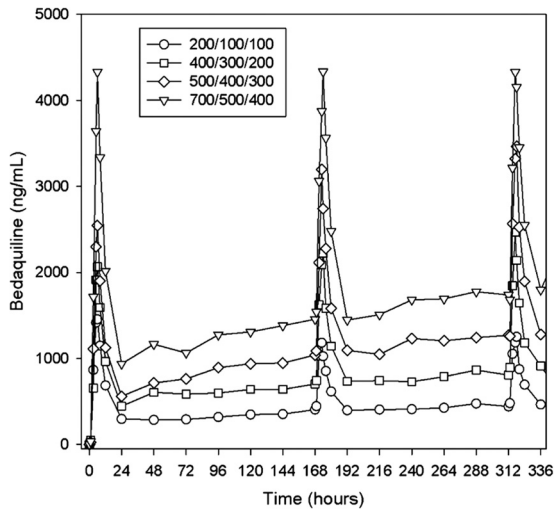


FIG 3 Geometric mean plasma concentrations of BDQ following administration of BDQ in doses of 100 mg, 200 mg, 300 mg, and 400 mg on treatment days 3 to 14, preceded by single daily loading doses of 200 mg, 400 mg, 500 mg, and 700 mg, respectively, on treatment day 1 and 100 mg, 300 mg, 400 mg, and 500 mg, respectively, on treatment day 2. Full pharmacokinetic profiles are provided for doses administered on days 1 (0 to 24 h), 8 (168 to 192 h), and 14 (312 to 336 h), with trough plasma concentrations provided for the other days.

who exhibited asymptomatic transient elevation of aspartate aminotransferase (AST) from 78 U/liter at baseline to a maximum of 125 U/liter that resolved by completion of drug intake. No patients recorded a change from baseline of >60 ms or a QT time of >500 ms corrected by Bazett's method (QTcB) or Fridericia's method (QTcF).

Pharmacokinetics and pharmacodynamics. Absorption of BDQ was moderately rapid, with a median time to maximum plasma concentration of 5 to 6 h after dosing in each of the BDQ treatment groups. BDQ plasma concentrations appeared to increase proportionally with dose level over the range studied (Fig. 3). Table 4 shows the BDQ PK parameters after the first loading dose on treatment day one and on treatment day 14. Steady-state BDQ trough concentrations were reached by treatment day 11,

but steady-state peak plasma concentrations were already reached after the first dose, indicating that the dose-loading scheme was successful. Steady-state plasma concentrations for the M2 metabolite were not reached during the 14 days of dosing (data not shown). Plasma concentrations of both BDQ and M2 were quantifiable at follow-up 5 weeks following the last drug intake for all but 1 patient. Figures 4A and B illustrate the relationship between areas under the curve (AUC) for each of the BDQ groups determined on day 1 and day 14, respectively, and the EBA_{CFU} and EBA_{TTP} for days 0 to 2 and 0 to 14 for the same groups; over both periods, the rise in AUC is accompanied by a rise in EBA_{CFU} and EBA_{TTP} .

DISCUSSION

In this dose-ranging EBA study over 14 days, BDQ plasma concentrations and bactericidal activity appeared to increase with dose up to 400 mg daily. This is consistent with the recent conclusion based on mouse data that AUC is the primary PK/PD driver of BDQ activity *in vivo* (8). BDQ was safe and well tolerated in this study. In contrast to the earlier EBA study, conducted over only 7 days (2), BDQ following two loading doses and given for 14 days had demonstrable EBA also at the lowest dose level of 100 mg daily and this activity continued to the end of the 14-day evaluation.

Despite the loading dose applied in this study and the rapid achievement of near-steady-state plasma levels, there was still some delay in the onset of action, particularly at the lower doses. This may be due to BDQ's underlying mechanism of action (interference with ATP synthesis) and the need to deplete intracellular ATP stores before its action becomes clinically evident. The slow rise to effective exposures in humans can be largely compensated for by augmented early dosing. Figures 4A and B suggest that dose loading for the 400-mg dose group and the ensuing higher BDQ concentrations led to a 0-to-2-day EBA that was similar to the 0-to-14-day EBA. A measurable 0-to-2-day EBA_{CFU} was not observed in the earlier study in which no dose loading was used (2). In the same context, it is intriguing to compare the activity of the 400-mg dosage in this study with that in a recent similar study with single drugs and combinations of novel and established antituberculosis agents (9). That study included a group of 15 patients treated with BDQ in the same augmented dosing scheme as

TABLE 4 Pharmacokinetic parameters for BDQ^a

Day and parameter	Value with each dose of BDQ			
	100 mg	200 mg	300 mg	400 mg
Day 1				
C_{max} (ng/ml)	1,770 (743–3,190)	2,770 (811–3,880)	3,270 (1,280–5,380)	4,510 (2,330–13,400)
T_{max} (h)	5.0 (3.0–8.0)	5.0 (3.0–8.0)	5.0 (5.0–8.0)	6.0 (3.0–8.0)
AUC_{0-24h} (ng · h/ml)	19,587 (8,885–39,294)	25,714 (8,375–37,656)	32,069 (13,629–49,487)	52,992 (21,005–92,595)
C_{min} (ng/ml)	296 (126–549)	494 (158–991)	621 (180–1,850)	897 (497–1,940)
Day 14				
C_{max} (ng/ml)	1,550 (878–3,620)	2,770 (1,730–4,340)	3,940 (2,060–4,740)	4,540 (2,450–13,600)
T_{max} (h)	5.0 (3.0–8.0)	5.0 (3.0–6.0)	5.0 (3.0–8.0)	5.0 (3.0–8.0)
AUC_{0-24h} (ng · h/ml)	17,576 (13,988–29,975)	33,543 (22,881–42,315)	51,120 (28,561–74,260)	64,302 (37,957–144,050)
C_{min} (ng/ml)	486 (228–681)	1,010 (491–1,340)	1,240 (640–2,290)	1,830 (1,090–3,430)

^a Following administration of BDQ in doses of 100 mg, 200 mg, 300 mg, and 400 mg on treatment days 3 to 14, preceded by single daily loading doses of 200 mg, 400 mg, 500 mg, and 700 mg, respectively, on treatment day 1 and 100 mg, 300 mg, 400 mg, and 500 mg, respectively, on treatment day 2. Values are medians (ranges). C_{max} , maximum plasma concentration following dosing; T_{max} , time of maximum plasma concentration; AUC_{0-24h} , area under the concentration-time curve over the dose interval of 0 to 24 h; C_{min} , plasma concentration 24 h after dosing.

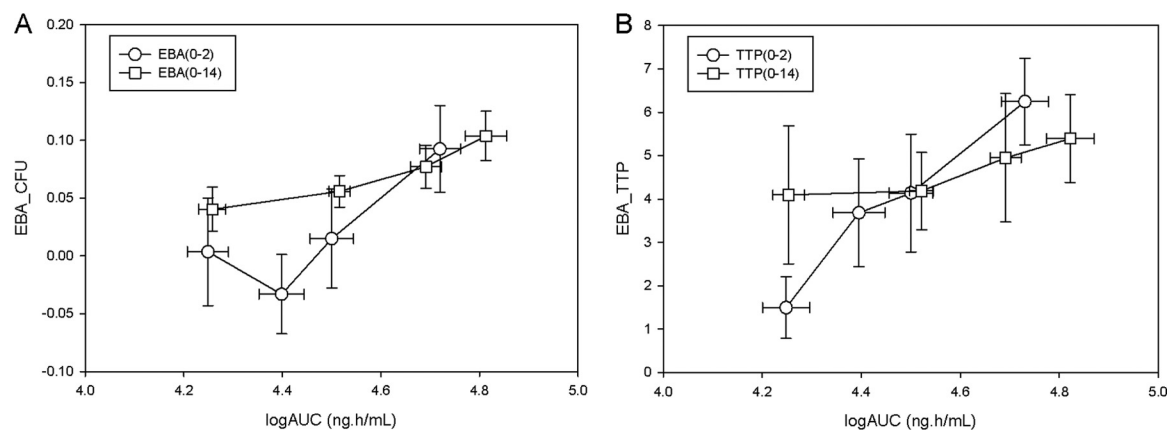


FIG 4 Relationship of the early bactericidal activity (EBA) of BDQ to the area under the concentration-time curve (AUC) of BDQ for the different dosage groups. BDQ was administered in doses of 100 mg, 200 mg, 300 mg, and 400 mg on days 3 to 14, preceded by single daily loading doses of 200 mg, 400 mg, 500 mg, and 700 mg, respectively, on treatment day 1 and 100 mg, 300 mg, 400 mg, and 500 mg, respectively, on treatment day 2. EBA over treatment days 0 to 2 and 0 to 14 was measured by the daily fall in CFU of *M. tuberculosis* (A) or the daily prolongation of time (hours) to positive signal (TTP) (B) and plotted against AUC on treatment days 1 and 14, respectively. EBA and AUC are means. Error bars indicate standard errors.

in the present study, but with lower initial counts of 5.956 ± 1.060 log CFU versus 6.625 ± 0.956 log CFU in the present study. Bactericidal activities measured for the periods of 0 to 14 days, 2 to 14 days, and 7 to 14 days were similar, but the activity for the period of 0 to 2 days was much greater in the present study than in the previous study (0.093 ± 0.136 log CFU versus -0.022 ± 0.121 log CFU, respectively). The measurement with TTP shows a similar trend. This might indicate that a higher baseline bacillary sputum load may independently result in a greater 0-to-2-day activity, possibly due to the presence of more highly active mycobacteria particularly vulnerable to chemotherapy; furthermore, due to the more rapid reduction in numbers of actively dividing bacilli, this might be important for the prevention of drug resistance (4, 10).

Two-week EBA studies allow supervised treatment and standardized measurements in a relatively small number of hospitalized, highly selected individuals; however, such studies cannot explore long-term toxicity (or the sterilizing activity) of a compound or regimen. Nonetheless, we did not observe specific toxicity of BDQ or its M2 metabolite in this study. Exposure to M2 is expected to reach about 25 to 30% of BDQ upon repeated dosing. M2 is 3- to 6-fold less active against *M. tuberculosis* and shares the high volume of distribution and long terminal elimination half-life of 5 to 6 months with the parent compound. The significance of the M2 metabolite for toxicity is not known, but two trials with 6 months of exposure in MDR TB have recently been completed and await analysis and publication.

In conclusion, this study found bactericidal activity at each of the BDQ doses evaluated that continued until the end of the 14-day study period, suggesting that the highest of these doses compatible with safety considerations should be taken forward to future long-term clinical studies.

REFERENCES

- Andries K, Verhasselt P, Guillemont J, Gohlmann HW, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V. 2005. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 307:223–227.
- Rustomjee R, Diacon AH, Allen J, Venter A, Reddy C, Patientia RF, Mthiyane TC, De Marez T, van Heeswijk R, Kerstens R, Koul A, De Beule K, Donald PR, McNeeley DF. 2008. Early bactericidal activity and pharmacokinetics of the diarylquinoline TMC207 in treatment of pulmonary tuberculosis. *Antimicrob. Agents Chemother.* 52:2831–2835.
- Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R, Page-Shipp L, Pistorius C, Krause R, Bogoshi M, Churchyard G, Venter A, Allen J, Palomino JC, De Marez T, van Heeswijk RP, Lounis N, Meyvisch P, Verbeeck J, Parys W, de Beule K, Andries K, McNeeley DF. 2009. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N. Engl. J. Med.* 360:2397–2405.
- Diacon AH, Donald PR, Pym A, Grobusch M, Patientia RF, Mahanyele R, Bantubani N, Narasimooloo R, De Marez T, van Heeswijk R, Lounis N, Meyvisch P, Andries K, McNeeley DF. 2012. Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. *Antimicrob. Agents Chemother.* 56:3271–3276.
- Rieder HL, Van Deun A, Kam KM, Kim SJ, Chonde TM, Trébuq A, Urbanczik R. 2007. Priorities for tuberculosis bacteriology services in low-income countries, 2nd ed. International Union Against Tuberculosis and Lung Disease (The Union), Paris, France.
- Donald PR, Sirgel FA, Venter A, Parkin DP, Seifart HI, van de Wal BW, Maritz JS, Fourie PB. 2003. Early bactericidal activity of antituberculosis agents. *Expert Rev. Anti Infect. Ther.* 1:141–155.
- Warren RM, Gey van Pittius NC, Barnard M, Hesselting A, Engelke E, de Kock M, Gutierrez MC, Chege GK, Victor TC, Hoal EG, van Helden PD. 2006. Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference. *Int. J. Tuberc. Lung Dis.* 10:818–822.
- Rouan MC, Lounis N, Gevers T, Dillen L, Gilissen R, Raoof A, Andries K. 2012. Pharmacokinetics and pharmacodynamics of TMC207 and its *N*-desmethyl metabolite in a murine model of tuberculosis. *Antimicrob. Agents Chemother.* 56:1444–1451.
- Diacon AH, Dawson R, von Groote-Bidlingmaier F, Symons G, Venter A, Donald PR, van Niekerk C, Everitt D, Winter H, Becker P, Mendel CM, Spigelman MK. 2012. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. *Lancet* 380:986–993.
- Mitchison DA. 1998. Basic concepts in the chemotherapy of tuberculosis, p 15–50. In Gangadharam PRJ, Jenkins PA (ed), *Mycobacteria II—chemotherapy*. Chapman & Hall, New York, NY.