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In vitro activity of bedaquiline against slow-growing nontuberculous mycobacteria

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Abstract

Bedaquiline (BDQ) is a recently approved antibiotic for the treatment of multidrug-resistant tuberculosis, but its potential against slow-growing mycobacteria (SGM) is still unknown. The objective of this study was to determine the *in vitro* activity of BDQ on SGM by assessing their MIC and minimal bactericidal concentration (MBC). The MIC of BDQ against 17 clinical isolates including *Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium chimaera, Mycobacterium kansasii* and *Mycobacterium simiae* species was determined by the resazurin microtitre assay and the MBC by the c.f.u. determination on 7H10 agar plates. BDQ has a bacteriostatic activity on all SGM tested with a MIC range from 0.03 to 0.007 µg ml^{-1} and surprisingly a good bactericidal activity on the majority of the isolates tested with an MBC of 1–2 µg ml^{-1} . Based on these preliminary results BDQ seems to be very promising for treatment of diseases caused by SGM.

Nontuberculous mycobacteria (NTM) include all *Mycobacterium* species other than those belonging to the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae* [1]. NTM are generally classified into 'slow' and 'rapid' growers (SGM and RGM). *Mycobacterium avium* (SGM) and *Mycobacterium abscessus* (RGM) are the most prevalent sources of infection in humans, both in immunocompromised individuals and in other susceptible populations, such as patients with cystic fibrosis and with other pulmonary conditions [2]. The other most clinically relevant species among the SGM include *Mycobacterium chimaera, Mycobacterium intracellulare, Mycobacterium kansasii, Mycobacterium malmoense, Mycobacterium marinum, Mycobacterium simiae* and *Mycobacterium xenopi*. *M. chimaera* has recently been reported in outbreaks attributed to heating–cooling devices in surgery operating rooms [3]. Most NTM are intrinsically resistant to many of the antimicrobial drugs currently available and treatment regimens, often based on combination chemotherapy, differ depending on the mycobacterial species involved [4]. Treatment options, however, remain extremely limited [5]. In the context of the efforts to combat antimicrobial drug resistance, particularly for multidrug-resistant (MDR)-TB, a new antibiotic was developed and approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [6]. Bedaquiline (BDQ) is a diarylquinoline antibiotic with a broad antimycobacterial spectrum and a novel mode of action, inhibiting the ATP synthase and interfering with the energy generation needed by the bacteria [7, 8]. BDQ has recently been used off-label as salvage treatment in patients with *M. intracellulare* lung disease, with promising results; however, not enough data in the literature are yet available on the activity of BDQ on SGM [9]. This study aimed to determine the *in vitro* MIC and minimum bactericidal concentration (MBC) of BDQ against a panel of representative SGM of clinical significance using the resazurin microplate assay (REMA), which to our knowledge has not been previously reported.

Seventeen SGM clinical isolates were used in this study (Table 1). Isolates were cultured on Löwenstein–Jensen (L–J) medium and the inoculum was prepared in distilled water, adjusted to McFarland 1 and diluted 1 : 10 in 7H9 broth medium. *M. xenopi* UCL R8 was used as the control

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Abbreviations: BDQ, bedaquiline; CFU, colony-forming unit; EMA, European Medicines Agency; FDA, Food and Drug Administration; MBC, minimal bactericidal concentration; MIC, minimal inhibitory concentration; NTM, nontuberculous mycobacteria; REMA, resazurin microtiter assay; RGM, rapid growing mycobacteria; SGM, slow growing mycobacteria.

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Table 1. MIC and MBC determined on the 17 SGM tested

Species (*n*=)	UCL number	Bedaquiline	
		MIC (µg ml–1)	MBC (µg ml^{-1})
M. xenopi	R8	>2	ND
M. avium	AG10	0.007	>2
M. avium	ISP6	0.007	1
M. avium	ISP11	0.015	>2
M. intracellulare	IT5	0.015	2
M. intracellulare	AG11	0.015	1
M. intracellulare	R10	0.015	1
M. intracellulare	ISP 7	0.007	1
M. intracellulare	AG8	0.015	>2
M. chimaera	IT1	0.007	1
M. chimaera	IT2	0.015	2
M. chimaera	ISP4	0.015	1
M. chimaera	AG3	0.015	1
M. kansasii	IT10	0.015	2
M. kansasii	AG2	0.015	0.03
M. simiae	ISP13	0.015	1
M. simiae	R12	0.03	>2

ND: not determined (control strain).

(*M. xenopi* is resistant to BDQ). The MIC was performed using REMA [10–12]. MIC50 and MIC90 values were defined as the concentration of the antibiotic at which 50 and 90 % of the isolates were inhibited (Table 2). Briefly, twofold serial dilutions of BDQ were prepared in 7H9 broth in 96-well polystyrene plates in a concentration range of 2.0–0.0035 µg ml^{-1}. An inoculum equal to McFarland 1 diluted 1 : 10 was prepared. Growth controls without drug (positive control), a drug control and a sterile control (negative control) were also prepared for each test. Then, 200 µl of sterile distilled water was added to all perimeter wells to prevent evaporation during incubation. Plates were sealed and incubated at 37 °C for 7 days before adding 30 µl of 0.01 % resazurin to all wells and incubating for a further 24 h. The MIC was defined as the lowest drug concentration that prevented growth reflected in a colour change from blue (oxidized state) to pink (reduced state). Each test was performed in triplicate. The same plates were used for MBC determination. At day 8 of incubation and after the MIC determination, blue wells were chosen to test the viability of the mycobacteria. Then, 100 µl from each well at the MIC, and all the concentrations higher, were transferred to a tube and tenfold dilutions were prepared in sterile distilled water and plated in duplicate on 7H10 agar for c.f.u. counting. The plates were incubated at 37 °C for 21 days. The percentage of killed bacteria was calculated against the control and the MBC was defined as the lowest drug concentration that killed 99.9 % of bacteria. To investigate the presence of mutations in the target gene *atpE*, DNA was extracted according to Aguilar-Ayala *et al.* [10] and the *atpE* gene was amplified according to Huitric *et al.* [8] and sequenced using the ABI Prism 3130 XL Genetic Analyzer (Applied Biosystems). Fastq data were imported into Geneious 9.0.4 software and aligned to the wild-type H37Rv *atpE* gene (GenBank accession number NC_000962.3 and GeneID: 886937) and then translated into protein sequences.

Table 2. MIC50 and MIC90 of the isolates tested

Drug	MIC (µg ml^{-1})		
	Range	MIC50	MIC90
Bedaquiline	0.007–0.03	0.015	0.03

Our data show a strong inhibitory effect (MIC) of BDQ against the SGM mycobacterial tested. BDQ MICs ranged from 0.03 to 0.007 µg ml^{-1}. The majority of isolates tested showed BDQ MICs of 0.015; only one isolate (*M. simiae*) showed MICs of 0.03 µg ml^{-1} (Table 1). Quality control was performed using *M. xenopi* (naturally resistant to BDQ) and showed an MIC >2 µg ml^{-1}. The MBC was found between 1–2 µg ml^{-1} and for some isolates>2 µg ml^{-1} (Table 1). The *atpE* gene was sequenced in all strains and no mutations were detected.

BDQ, being the only TB drug approved by the FDA during the past 40 years, has low MIC values against *M. avium* and *M. abscessus* clinical isolates [8, 13–15]. However, despite being an excellent growth inhibitor at low doses, it lacks bactericidal activity against NTM *in vitro* [13, 14, 16]. Our study shows a strong inhibitory effect of BDQ against SGM. Surprisingly, our study shows a bactericidal activity on the majority of the SGM tested. The MBC for *M. intracellulare, M. chimaera, M. kansasii* were found to be between 1 or 2 µg ml^{-1}. Nevertheless, some of them had an MIC >2 µg ml^{-1}. Interestingly, we observed a difference in MBC between different strains of the same species. We recently evaluated the *in vitro* activity of BDQ against a panel of RGM[10] and found also a strong inhibitory effect. However, the majority of the RGM tested in that study had an MIC >2 µg ml^{-1}. In a previous study by Huitric *et al.* [8] the only *M. kansasii* strain tested had an MIC of 0.03 µg ml^{-1} for BDQ but MBC was not reported. In another recent study by Pang *et al.* [15] the MIC_{50} for *M. kansasii* was reported as 0.06 µg ml^{-1}. Soni *et al.*, reported an MIC of BDQ against different mycobacteria done by the agar dilution method [17]. They found a MIC for BDQ against *M. intracellulare* of 0.010 µg ml^{-1}, for *M. kansasii* 0.03 µg ml^{-1} and for *M. simiae* 0.03 µg ml^{-1}. Ruth *et al.*, [18] reported an MIC of BDQ in 7H9 broth for *M. avium* of 0.125 µg ml^{-1}, for *M. intracellulare* 0.03 µg ml^{-1}, for *M. chimaera* 0.03 µg ml^{-1}, for *M. simiae* 0.3–0.125 µg ml^{-1} and for *M. kansasii* 0.125–0.25 µg ml^{-1}. To our knowledge, no MBC data have been published on BDQ for SGM. However, the exact reasons for the bactericidal

activity of BDQ on SGM are still not known and should be explored. The present study adds new evidence into the possible usefulness of BDQ in the treatment of infections caused by SGM.

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Conflicts of interest
N.L. has conducted research experiments on bedaquiline activity against *M. tuberculosis* in Janssen Laboratory. All other authors report no potential conflicts.

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