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In vitro interaction profiles of the new antitubercular drugs bedaquiline and delamanid with moxifloxacin against clinical *Mycobacterium tuberculosis* isolates

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ABSTRACT

Objectives: The emergence of drug-resistant tuberculosis (TB) poses a serious challenge to existing anti-TB therapies. Hence, there is a direct need for identification of new drugs and effective combination regimens.

Methods: In this study, minimum inhibitory concentrations (MICs) of the anti-TB drugs bedaquiline (BDQ), delamanid (DEL) and moxifloxacin (MFX) were evaluated using a resazurin microtiter assay (REMA) against five drug-resistant clinical*Mycobacterium tuberculosis* (MTB) isolates as well as the drug-susceptible reference strain H37Rv. In addition, their fractional inhibitory concentration indices (FICIs) were evaluated using a REMA-based calorimetric chequerboard assay to assess their interaction profiles against the MTB isolates.

Results: The FICI indicated that BDQ acted synergistically with DEL against isoniazid (INH)-monoresistant, rifampicin (RIF)-monoresistant and extensively drug-resistant (XDR) clinical MTB isolates. In addition, the combination of DEL acted synergistically with MFX against INH-monoresistant, RIF-monoresistant and XDR clinical MTB isolates. Moreover, the combination of BDQ and MFX showed a synergistic effect against RIF-monoresistant and pre-XDR clinical MTB isolates. DEL at 0.125 × MIC (i.e. 0.015 μg/mL) used in combination with BDQ at 0.25 × MIC (i.e. 0.015 μg/mL) had a stronger bactericidal effect against the XDR-TB clinical isolate than DEL alone at 1 × MIC (i.e. 0.125 μg/mL).

Conclusion: Synergistic and additive effects between these two-drug combinations offer an attractive chemotherapeutic regimen against drug-resistant clinical MTB isolates.

1. Introduction

Tuberculosis (TB) is an airborne infectious disease caused by the bacterium *Mycobacterium tuberculosis* (MTB). In 2017, 6.3 million cases of the disease were reported worldwide, among which 1.3 million deaths occurred [1]. Treatment with existing anti-TB drugs does not lead to the eradication of multidrug-resistant (MDR) and extensively drug-resistant (XDR)-TB. The long duration of treatment, ineffective regimens, serious adverse events, centralised drug-resistant TB services and lack of patient-centred care are strong contributors to patient poor compliance that lead to the development of drug-resistant TB [2,3]. Hence, there is a direct need for identification of new drugs and effective combinations that can shorten the treatment duration against drug-resistant TB.

The TB drug development pipeline has several lead molecules undergoing clinical trials [4,5]. The Global Alliance for Drug TB Development (TB Alliance) is dedicated to discovering new combination regimens with potent molecules to decrease the risk of emerging drug-resistant TB strains [6]. Approved drugs that show promising results in clinical trials owing to their novel mode of action against drug-susceptible and -resistant TB are bedaquiline (BDQ) [approved by the US Food and Drug Administration (FDA)] and delamanid (DEL) [approved by the European Medicines Agency (EMA)].

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BDQ targets the proton pump of mycobacterial adenosine triphosphate (ATP) synthase encoded by the *atpE* gene [7]. Previous reports have shown that BDQ potently inhibits drug-susceptible and -resistant mycobacteria at minimum inhibitory concentrations (MICs) ranging from 0.03–0.12 μg/mL [7,8]. Current mechanisms of BDQ resistance include mutations in the genes *atpE* and *pepQ* [9]. In addition, mutation in the transcriptional regulator *Rv0678,* with upregulation of the multi-substrate efflux pump Mmpl5, accounts for cross-resistance between clofazimine and BDQ [10]. It has previously been reported that early bactericidal activity of BDQ during the first week of chemotherapy is minimal [11].

DEL is a dihydro-imidazooxazole derivative. It is prodrug that is activated by the enzyme deazaflavin-dependent nitroreductase (Rv3547) [12]. Previous reports have shown that DEL potently inhibits drug-susceptible and -resistant TB at MICs ranging from 0.006–0.024 mg/L [12]. Resistance to DEL is conferred by mutation in one of the five coenzyme F420 genes (Rv3547, *fgd*, *fbiA*, *fbiB* and *fbiC*) [13,14].

A previous study reported that, similar to isoniazid (INH) and prothionamide, the frequency of spontaneous mutations conferring resistance to DEL was high in in vitro conditions, whereas rifampicin (RIF) and moxifloxacin (MFX) had lower rates [13]. There is no cross-resistance between DEL and other existing first-line anti-TB agents [15]. This also suggests that DEL monotherapy would rapidly result in resistance and thus evaluation of in vitro combinations is necessary.

Individually, both BDQ and DEL are bactericidal, effective against MDR-TB and XDR-TB strains. BDQ was found to interact synergistically with BTZ-043 (benzothiazinone) and SQ109 [*N*-geranyl-*N*′-(2-adamantyl)ethane-1,2-diamine], but showed additive effects with other frontline anti-TB drugs [16]. In addition, the antiretroviral coformulation ritonavir-boosted lopinavir (LPV/r) increases BDQ exposure, whereas nevirapine does not show any increase in exposure in human immunodeficiency virus (HIV)/MDR-TB co-infected patients [17]. Similarly, no significant interactions were observed between DEL and other antiretroviral drugs [18]. However, currently there are only limited data available on adverse drug interactions with the combination of BDQ and DEL.

In this study, the MICs of the new anti-TB drugs BDQ and DEL were determined in vitro and the efficacy of their combinations with MFX was systematically investigated in order to elucidate potential synergistic, antagonistic or additive interactions against drug-susceptible and -resistant clinical MTB isolates.

2. Materials and methods

2.1. Antimicrobial agents

Four anti-TB drugs (BDQ, DEL, MFX and INH) were used in the drug susceptibility testing assays. The diarylquinoline compound BDQ was a kind gift from Janssen and Janssen (Raritan, NJ, USA). MFX and INH were from Sigma-Aldrich (St Louis, MO, USA) and DEL was a kind gift from Otsuka Pharmaceutical Co. Ltd. (Japan).

2.2. Bacterial strains and culture conditions

The drug-susceptible reference strain *M. tuberculosis* H37Rv as well as five clinical MTB isolates were included in this study, including two monoresistant strains (one INH-monoresistant and one RIF-monoresistant), one MDR-TB strain (resistant to INH and RIF), one pre-XDR-TB strain [resistant to INH, RIF and kanamycin (KAN)] and one XDR-TB strain [resistant to INH, RIF, KAN and levofloxacin (LVX)]. Strain H37Rv and the clinical MTB isolates from retrospective sample collection from specimens referred to the National Institute for Research in Tuberculosis (NIRT) (Chennai, India) were grown at 37 °C in Middlebrook 7H9 broth (BD Difco™; Becton Dickinson, Oxford, UK) supplemented with oleic acid–albumin–dextrose–catalase (OADC), 0.5% glycerol and 0.05% Tween 80 or on Lowenstein–Jensen medium. This study was approved by the Institutional Ethics Board of NIRT. Mycobacteria Growth Indicator Tube (MGIT) was used according to concentrations recommended by the World Health Organization (WHO) to confirm drug-susceptible and -resistant strains [19].

2.3. Minimum inhibitory concentration determination by the resazurin microtitre assay (REMA)

MICs for BDQ, DEL and MFX were determined three times in triplicate to confirm the reproducibility of results using REMA as described previously [20]. Briefly, bacterial stocks were generated from mid-log cultures for drug-susceptible strain H37Rv and drug-resistant clinical MTB isolates and were frozen at −80 °C to standardise the inoculum size. Drug solutions of BDQ (100 μg/mL), DEL (80 μg/mL), MFX (100 μg/mL) and INH (100 μg/mL) were thawed and were diluted in Middlebrook 7H9-S medium containing Middlebrook 7H9 broth, 0.1% casitone, 0.5% glycerol and OADC (Becton Dickinson). Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well flat-bottom polystyrene plate. Frozen aliquots of drug-susceptible and -resistant MTB isolates were thawed and were diluted at a ratio of 1:10 using 7H9-S medium. Growth controls lacking antibiotic and sterility controls lacking bacterial culture were also included. Plates were sealed with Parafilm and were incubated at 37 °C for 7 days. Following 7 days of incubation, 30 μL of freshly prepared 0.01% resazurin (Sigma-Aldrich) was added to each well. Plates were further incubated overnight at 37 °C and were then assessed for colour formation to determine the MIC [20].

2.4. Minimum inhibitory concentration determination by the 24-well agar dilution method

The drug susceptibility patterns of the clinical isolates were determined using a modified indirect proportion method adapted for 24-well agar plates. This experiment was also repeated three independent times in triplicate to confirm the reproducibility. The 24-well plates were prepared by 4× concentration of each antibiotic as used for the REMA assay mixed with Middlebrook 7H11 medium in a 50-mL Falcon tube and then serial two-fold dilution to achieve the required drug concentrations (i.e. same concentrations as used in REMA). Drug-containing and drug-free medium (2.5 mL) was then manually transferred into the respective wells of the 24-well tissue culture plates. The plates were then left in a safety hood until the agar was completely solidified and were subsequently sealed with Parafilm and were stored at 4 °C. Then, 10 μL of inoculum (1:10 bacterial suspension) was spotted in all wells. Drug susceptibility was determined by visual comparison of growth in drug-containing medium (1:10 bacterial suspension) with the drug-free control on which 1:10 and 1:100 bacterial suspensions were inoculated [21].

2.5. Determination of drug interactions using the REMA chequerboard assay

REMA chequerboard titration was carried out three independent times in triplicate to determine drug interactions between BDQ + DEL, BDQ + MFX and DEL + MFX by a broth microdilution-based assay as described previously [22,23]. Two-drug combinations were used in all experiments. Three-drug combinations were not used in this study as a previous report suggested that addition of a single drug does not improve the synergistic activity of the

drug interactions against MTB [24]. Briefly, drugs were diluted in 100 μL volumes in 96-well microtitre plates. One drug was diluted vertically (rows A–G) and the second drug was diluted horizontally (columns 1–9) to obtain various two-drug combinations. Row H and column 10 contained the individual drugs, column 11 was the drug-free positive control and column 12 was the negative control. A mid-log culture of drug-susceptible strain H37Rv and drug-resistant clinical MTB isolates was inoculated in each well (100 μL). The plates were sealed with Parafilm, were placed in zip-lock bags and were incubated at 37 °C for 2 weeks. Following 2 weeks of incubation, 0.01% resazurin was added to all wells. The following formula was used to calculate the fractional inhibitory concentration (FIC) in order to determine the drug interactions: FIC = MIC of drug in combination/MIC of drug alone. The FIC index (FICI) was then determined, calculated as FIC of drug A + FIC of drug B [23,25].

3. Results

3.1. Evaluation of the activity of new antitubercular drugs against drug-resistant clinical Mycobacterium tuberculosis isolates

The MICs of BDQ, DEL and MFX were evaluated by REMA and were found to be consistent with existing reported MICs. BDQ, DEL and INH were used in the assays in the concentration range 0.015–1 μg/mL and MFX was used in the concentration range 0.03–2 μg/mL, which is relevant to testing for the normal dosage of 400 mg daily recommended for patients.

Bacterial growth viability was markedly inhibited in a concentration-dependent manner, and was 0% at 0.06–0.25 μg/mL for BDQ, 0.03–0.125 μg/mL for DEL and 0.06–0.5 μg/mL for MFX against the various drug-resistant clinical MTB isolates (Table 1). In parallel, INH (MIC = 1 μg/mL) was used as a positive control for the INH-monoresistant, MDR-TB, pre-XDR-TB and XDR-TB strains. Bacterial growth was indicated by a colour change to pink for all clinical MTB isolates except for the RIF-monoresistant strain and drug-susceptible strain H37Rv. This indicates that all clinical isolates tested were resistant to INH as evident by colour change similar to the results obtained in the MGIT analysis.

3.2. Evaluation of the 24-well drug susceptibility testing method for the new antitubercular drugs

Drug susceptibility testing was also carried out by the 24-well plate assay for the clinical MTB isolates as well as the drug-susceptible strain H37Rv. MICs were consistent between experiments for the new anti-TB drugs (Table 2).

The results showed very small MIC variations in comparison with the results from REMA. Overall representative results from drug susceptibility testing are presented in Fig. 1. The highest resistance percentage was shown by INH against various drug-resistant clinical MTB isolates. Bacterial growth viability was markedly inhibited in a concentration-dependent manner, and was 0.06–0.125 μg/mL for BDQ, 0.06–0.25 μg/mL for DEL and 0.063–1 μg/mL for MFX against various drug-resistant clinical MTB isolates (Table 2).

Table 1
Minimum inhibitory concentrations (MICs) of antitubercular drugs determined by REMA against drug-susceptible *Mycobacterium tuberculosis* laboratory strain H37Rv and drug-resistant clinical *M. tuberculosis* isolates.

Drug	Mean MIC (μg/mL)					
	H37Rv	INH-mono	RIF-mono	MDR	Pre-XDR	XDR
Bedaquiline	0.125	0.25	0.125	0.063	0.125	0.063
Delamanid	0.063	0.063	0.063	0.031	0.031	0.125
Moxifloxacin	0.063	0.063	0.063	0.125	0.063	0.5
Isoniazid	0.063	PC[a]	0.063	PC	PC	PC

REMA, resazurin microtiter assay; INH, isoniazid; mono, monoresistant; RIF, rifampicin; MDR, multidrug-resistant; XDR, extensively drug-resistant.
[a] PC, positive control (resistant to INH).

Table 2
Minimum inhibitory concentrations (MICs) of antitubercular drugs determined by the 24-well agar dilution method against drug-susceptible *Mycobacterium tuberculosis* laboratory strain H37Rv and drug-resistant clinical *M. tuberculosis* isolates.

Drug	Mean MIC (μg/mL)					
	H37Rv	INH-mono	RIF-mono	MDR	Pre-XDR	XDR
Bedaquiline	0.06	0.125	0.06	0.06	0.125	0.06
Delamanid	0.125	0.06	0.125	0.06	0.125	0.25
Moxifloxacin	0.125	0.125	0.125	0.125	0.06	1
Isoniazid	0.25	PC[a]	0.25	PC	PC	PC

INH, isoniazid; mono, monoresistant; RIF, rifampicin; MDR, multidrug-resistant; XDR, extensively drug-resistant.
[a] PC, positive control (resistant to INH).

3.3. Drug interactions between bedaquiline, delamanid and moxifloxacin assessed by REMA chequerboard titration assay (two-drug combinations)

The REMA chequerboard titration assay was performed to evaluate the efficacy and activity of new anti-TB drugs in combination against strain H37Rv and various drug-resistant clinical MTB isolates. First, the MIC of the individual compounds was evaluated by REMA and was found to be consistent with previously reported MICs. Second, using the REMA chequerboard titration assay, compound interactions were assessed by considering their individual MICs in REMA against drug-susceptible and -resistant clinical isolates as follows: 0.06–0.5 μg/mL for BDQ, 0.06–0.125 μg/mL for DEL and 0.063–2 μg/mL for MFX. The FICIs of each combination against the drug-susceptible strain H37Rv as well as the five drug-resistant clinical MTB isolates, including two monoresistant strains (one INH-monoresistant and one RIF-monoresistant), one MDR-TB strain (resistant to INH and RIF), one pre-XDR-TB strain (resistant to INH, RIF and KAN) and one XDR-TB strain (resistant to INH, RIF, KAN and LVX) were calculated and are summarised in Table 3.

Data obtained from titration assay against drug-susceptible strain H37Rv indicated that the combination of BDQ + DEL was additive with an FICI of 0.6 (Table 3; Supplementary Table S1). On the other hand, the combination of BDQ + MFX was additive

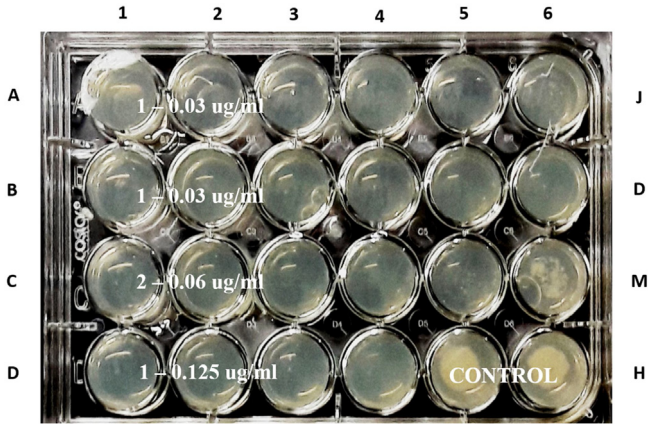

Fig. 1. Representative image of the 24-well plate used for drug susceptibility testing of the drug-susceptible *Mycobacterium tuberculosis* strain H37Rv for bedaquiline (J), delamanid (D), moxifloxacin (M) and isoniazid (H) with a standard inoculum of 10 μL (1.0 McFarland standard). Minimum inhibitory concentrations (MICs) were 0.06 μg/mL for bedaquiline, 0.125 μg/mL for delamanid, 0.125 μg/mL for moxifloxacin and 0.25 μg/mL for isoniazid.

Table 3
Minimum inhibitory concentrations (MICs) of selected antitubercular compounds against drug-susceptible and -resistant clinical *Mycobacterium tuberculosis* isolates and corresponding interaction profiles assessed by REMA chequerboard assay.

Strain	Drug combination	Drug	MIC (μg/mL)		FICI [interaction][a]
			Alone	Combination	
H37Rv	BDQ + DEL	BDQ	0.125	0.015	0.6 [Add]
		DEL	0.063	0.03	
	BDQ + MFX	BDQ	0.125	0.03	0.74 [Add]
		MFX	0.063	0.03	
	DEL + MFX	DEL	0.063	0.015	0.5 [Syn]
		MFX	0.063	0.015	
INH-monoresistant	BDQ + DEL	BDQ	0.25	0.015	0.31 [Syn]
		DEL	0.063	0.015	
	BDQ + MFX	BDQ	0.25	0.125	1.0 [Add]
		MFX	0.063	0.03	
	DEL + MFX	DEL	0.063	0.015	0.5 [Syn]
		MFX	0.063	0.015	
RIF-monoresistant	BDQ + DEL	BDQ	0.125	0.015	0.30 [Syn]
		DEL	0.063	0.015	
	BDQ + MFX	BDQ	0.125	0.015	0.3 [Syn]
		MFX	0.063	0.015	
	DEL + MFX	DEL	0.063	0.015	0.5 [Syn]
		MFX	0.063	0.015	
MDR	BDQ + DEL	BDQ	0.063	0.015	0.75 [Add]
		DEL	0.031	0.015	
	BDQ + MFX	BDQ	0.063	0.03	1.5 [Add]
		MFX	0.125	0.06	
	DEL + MFX	DEL	0.031	0.015	1.0 [Add]
		MFX	0.125	0.03	
Pre-XDR	BDQ + DEL	BDQ	0.125	0.015	0.62 [Add]
		DEL	0.031	0.015	
	BDQ + MFX	BDQ	0.125	0.015	0.37 [Syn]
		MFX	0.063	0.015	
	DEL + MFX	DEL	0.031	0.015	0.75 [Add]
		MFX	0.063	0.015	
XDR	BDQ + DEL	BDQ	0.063	0.015	0.37 [Syn]
		DEL	0.125	0.015	
	BDQ + MFX	BDQ	0.063	0.015	0.75 [Add]
		MFX	0.5	0.25	
	DEL + MFX	DEL	0.125	0.015	0.24 [Syn]
		MFX	0.5	0.06	

REMA, resazurin microtiter assay; FICI, fractional inhibitory concentration index; BDQ, bedaquiline; DEL, delamanid; MFX, moxifloxacin; Add, additive; Syn, synergistic; INH, isoniazid; RIF, rifampicin; MDR, multidrug-resistant; XDR, extensively drug-resistant.
[a] An FICI of ≤0.5 indicates synergy and an FICI of ≥4.0 indicates antagonism; values in between indicate additivity.

(FICI = 0.74): the presence of MFX caused a lowering of the MIC of BDQ by approximately four-fold compared with the MIC for BDQ alone (from 0.125 μg/mL to 0.03 μg/mL); the MIC of MFX in the presence of BDQ was one-half of the MIC of MFX alone (0.03 μg/mL vs. 0.063 μg/mL) (Table 3; Supplementary Table S1). Interestingly, the combination of DEL + MFX showed a synergistic effect against strain H37Rv (FICI = 0.5): the presence of MFX lowered the MIC of DEL by four-fold compared with the MIC of DEL alone (from 0.063 μg/mL to 0.015 μg/mL); and the MIC of MFX in the presence of DEL was also lowered by four-fold compared with the MIC of MFX alone (from 0.063 μg/mL to 0.015 μg/mL) (Table 3; Supplementary Table S1).

The combined effect of BDQ + DEL against the INH-monoresistant clinical MTB isolate was synergistic (FICI = 0.31): addition of DEL lowered the MIC of BDQ by 16-fold from 0.25 μg/mL to 0.015 μg/mL; and addition of BDQ reduced the MIC of DEL by four-fold (from 0.063 μg/mL to 0.015 μg/mL) (Table 3; Supplementary Table S2). The combined activity of BDQ + MFX was additive (FICI = 1.0), with the MICs for BDQ and MFX reduced by one-half of their original values. Furthermore, the combination of DEL + MFX exhibited a synergistic effect (FICI = 0.5), with the MICs reduced by four-fold for both drugs (Table 3; Supplementary Table S2).

In the case of the RIF-monoresistant clinical MTB isolate, all drug combinations showed synergistic activity. The combined activity of BDQ + DEL was synergistic (FICI = 0.30): addition of DEL lowered the MIC of BDQ by eight-fold (from 0.125 μg/mL to 0.015 μg/mL); and the addition of BDQ reduced the MIC of DEL by four-fold compared with when it was used alone (from 0.06 μg/mL to 0.015 μg/mL) (Table 3; Supplementary Table S3). The combined effect of BDQ + MFX was also synergistic (FICI = 0.3) and the MICs changes were similar to the abovementioned combination. Furthermore, the combination of DEL + MFX exhibited a synergistic effect (FICI = 0.5), with the MICs reduced by four-fold for both drugs (Table 3; Supplementary Table S3).

The data obtained from titration assay against the clinical MDR-TB strain (resistant to INH and RIF) indicated that the combination of BDQ + DEL was additive (FICI = 0.75) (Table 3; Supplementary Table S4). The presence of DEL caused a lowering of the MIC of BDQ by four-fold (from 0.063 μg/mL to 0.015 μg/mL); the MIC of DEL was reduced to one-half of its original MIC in the presence of BDQ (from 0.031 μg/mL to 0.015 μg/mL) (Table 3; Supplementary Table S4). On the other hand, the combination of BDQ + MFX was additive (FICI = 1.5); the presence of MFX caused a lowering of the MIC of BDQ by two-fold compared with the MIC obtained with BDQ alone (from 0.063 μg/mL to 0.03 μg/mL); the MIC of MFX in the presence of BDQ was also one-half of the MIC of MFX alone (from 0.125 μg/mL to 0.06 μg/mL) (Table 3; Supplementary Table S4). The combination of DEL + MFX showed an additive effect against MDR-TB (FICI = 1.0); the presence of MFX lowered the MIC of DEL by two-fold in comparison with the MIC of DEL alone (from 0.03 μg/mL to 0.015 μg/mL); and the MIC of MFX in the presence of DEL was lowered by four-fold compared with the MIC

of MFX alone (from 0.125 μg/mL to 0.03 μg/mL) (Table 3; Supplementary Table S4).

The activity of the combination BDQ + DEL against the pre-XDR-TB clinical isolate (resistant to INH, RIF and KAN) was additive (FICI = 0.62): addition of DEL lowered the MIC of BDQ by eight-fold (from 0.125 μg/mL to 0.015 μg/mL); and addition of BDQ to DEL reduced the MIC of DEL by two-fold compared with when it was used alone (from 0.031 μg/mL to 0.015 μg/mL) (Table 3; Supplementary Table S5). The combined activity of DEL + MFX was also additive (FICI = 0.75): addition of MFX lowered the MIC of DEL by two-fold (from 0.03 μg/mL to 0.015 μg/mL); and addition of DEL lowered the MIC of MFX by four-fold (from 0.063 μg/mL to 0.015 μg/mL). However, the combination of BDQ + MFX against the pre-XDR-TB clinical isolate showed a synergistic effect (FICI = 0.37), lowering the MIC of BDQ by eight-fold (from 0.125 μg/mL to 0.015 μg/mL) and the MIC of MFX by four-fold (from 0.063 μg/mL to 0.015 μg/mL).

Further analysis suggested that the interaction profile against the XDR-TB clinical isolate was synergistic in response to combinations of DEL + MFX (FICI = 0.24) and BDQ + DEL (FICI = 0.37). The presence of MFX lowered the MIC of DEL by eight-fold in comparison with the MIC of DEL alone (from 0.125 μg/mL to 0.015 μg/mL); and the MIC of MFX in the presence of DEL was reduced by eight-fold compared with the MIC of MFX alone (from 0.5 μg/mL to 0.06 μg/mL) (Table 3; Supplementary Table S6).

Similarly, for the combination BDQ + DEL, addition of DEL lowered the MIC of BDQ by four-fold (from 0.063 μg/mL to 0.015 μg/mL), and addition of BDQ to DEL reduced the MIC of DEL by eight-fold (from 0.125 μg/mL to 0.015 μg/mL) (Table 3; Supplementary Table S6). The best combination of the different combination tested is BDQ + DEL as they are very significant in the treatment of XDR-TB. However, although the combination of DEL + MFX showed a synergistic effect, it cannot be used for XDR-TB treatment considering it to be resistant to all of the quinolones.

No drug combination of BDQ, DEL and MFX demonstrated any antagonistic interaction profiles against any of the clinical MTB isolates tested in this study.

4. Discussion

TB treatment requires a long duration with multidrug combinations, hence in vitro interaction studies of new anti-TB drugs in combination with currently available or pre-clinical TB drugs are an immediate requirement in order to reduce the duration of treatment [26]. The optimal interaction of standard TB regimens has been already examined in some in vivo studies. A coherent methodology should be developed to assess the interaction of combined drugs in order to achieve the best effect in clinical settings [16].

The present study set out to identify what interactions will be produced by BDQ and DEL in combination with MFX against drug-susceptible and -resistant clinical MTB isolates. This reports clearly shows the sterilising activity of the two-drug regimens BDQ + DEL, DEL + MFX and BDQ + MFX against drug-susceptible and -resistant clinical MTB isolates. Most of the combinations tested were purely additive. No antagonism was observed for any of the combinations tested. Interestingly, a synergistic effect was observed in some combinations tested against the INH-monoresistant strain. However, in the RIF-monoresistant clinical isolate, all combinations such as BDQ + MFX, DEL + MFX and BDQ + DEL showed a synergistic effect.

Recent data from a phase 2b NC-005 clinical trial showed that the novel four-drug regimen of BDQ + pretomanid + MFX + pyrazinamide (PZA) cured 78% of treated mice, whereas RIF + INH + PZA treatment cured only 50% of mice after 4 months. Substitution of MFX into the first-line drug regimen has greater shortening of treatment and it may be a more effective regimen for drug-susceptible TB and MDR-TB [27]. Taking this trial and our data into consideration, we hypothesise that the combinations used in the present study may have similar shortening of treatment against drug-susceptible and monoresistant TB. However, these combinations (BDQ + DEL, BDQ + MFX and DEL + MFX) cannot be used for treating drug-susceptible and monoresistant TB according to the WHO recommendations, which suggest that BDQ can be used only for adults and DEL for adults and children as an add-on agent alongside longer MDR-TB regimens [28]. Taking this into consideration, we further analysed the interaction effects against clinical isolates of pre-XDR-TB (resistant to INH, RIF and KAN) and XDR-TB (resistant to INH, RIF, KAN and LVX).

Interestingly, the current data indicate that combination of BDQ + MFX showed a synergistic effect against the pre-XDR-TB clinical isolate, whereas the combinations of BDQ + DEL and DEL + MFX showed synergistic effects against the XDR-TB clinical isolate. These interaction effects also support the WHO 2016 update policy that both BDQ and DEL can be used as add-on agents for treating MDR-TB and XDR-TB. Unfortunately, the latter combination (DEL + MFX) cannot be used in clinical settings for treating XDR-TB cases because of the high rate of MFX and gatifloxacin resistance [29].

Recent data suggest that synergy arising from the combination of BDQ and BTZ-043 is mediated by inhibition of DprE1, thereby weakening the cell wall and increasing the penetration of BDQ to its target [16,30]. Indeed, to support this statement, another drug, ethylenediamine SQ109 [24], that also weakens the cell wall by targeting the transmembrane protein MmpL3 [31], exhibited a synergistic effect on addition of BDQ. Although the mechanism in this study was not elucidated, a recent study by Xavier et al. found that DEL inhibits mycobacterial cell wall components [32]. We hypothesise that sub-MICs of DEL might weaken bacterial cell wall components and increase the penetration of BDQ to its target ATP synthase, causing a synergistic effect. In the current study, we assume that synergy arising with different combinations of drugs against various clinical isolates may likely be due to improved penetration of the drug to its target, thereby lowering the original MIC. However, three-drug interaction assays were not carried out in this study as the third drug added to the two-drug combination does not improve synergistic activity. A study by Reddy et al. reported the addition of RIF at 0.05× and 0.1 × MIC did not alter the synergy of BDQ and SQ109 [24].

Overall, this study indicated that BDQ, DEL and MFX, even at very low drug concentrations, efficiently enhanced killing of the aerobic laboratory drug-sensitive H37Rv strain and drug-resistant clinical MTB isolates. However, limitations of this study include the relatively small number of samples tested. Inclusion of second-line quality control resistant strains would be an important step in the development of these methods. This methodology should also be developed for three- and four-drug combinations by considering new anti-TB drugs in consideration alongside BDQ + DEL in the future.

In conclusion, we systematically investigated the efficacy of new anti-TB drugs by determining their respective MICs and FICIs against laboratory drug-susceptible and drug-resistant clinical isolates of MTB. BDQ + DEL, BDQ + MFX and DEL + MFX were found to have very good synergistic interaction profiles against various clinical MTB isolates. We suggest that these three different combination, which probably lower the MIC by increasing penetration of the drug to its target, might have an impact in clinical settings in shortening the duration of treatment. These interaction effects also support the WHO 2016 update policy that both BDQ and DEL can be used as add-on agents for treating MDR-TB and XDR-TB. However, BDQ, DEL and MFX have adverse QT prolongation effects and these combination profiles should be reassessed in in vivo conditions for their efficacy, toxicity and safety. In future, coherent methodology should be developed for

other drugs such as clofazimine, linezolid and cycloserine in combination with BDQ and DEL to find the best combination of three- to four-drug regimens that might shorten the duration of treatment, with no QT prolongation or toxicity and greater efficacy and safety.

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Competing interests

None declared.

Ethical approval

This study was approved by the Institutional Ethics Board of the National Institute for Research in Tuberculosis (Chennai, India) [NIRT-IEC ID 2016028].

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.06.013.

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