European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:1293–1296
https://doi.org/10.1007/s10096-019-03551-w

ORIGINAL ARTICLE

Comparison of in vitro activity of the nitroimidazoles delamanid and pretomanid against multidrug-resistant and extensively drug-resistant tuberculosis

Shu'an Wen[1] · Wei Jing[2] · Tingting Zhang[1] · Zhaojing Zong[1] · Yi Xue[1] · Yuanyuan Shang[1] · Fen Wang[1] · Hairong Huang[1] · Naihui Chu[2] · Yu Pang[1]

Received: 3 February 2019 / Accepted: 26 March 2019 / Published online: 5 April 2019

Abstract
Delamanid exhibited greater in vitro potency than pretomanid against multidrug-resistant (MDR-) and extensively drug-resistant tuberculosis (XDR-TB) isolates. The pretomanid minimum inhibitory concentration (MIC) values of four MDR-TB isolates were found to be resistant to delamanid ranging from 0.031 to 0.063 mg/L. A novel nonsynonymous mutation within the *fbiA* gene (Glu249Lys) may be contributing to high-level resistance to delamanid and pretomanid in *Mycobacterium tuberculosis*.

Keywords *Mycobacterium tuberculosis* · Delamanid · Pretomanid · Susceptibility · Minimum inhibitory concentration

Introduction

Nitroimidazoles have been widely used in the treatment of anaerobic bacterial infections [1]. Recently, several new generations of nitroimidazoles in clinical development have demonstrated potent in vitro and in vivo activities against *Mycobacterium tuberculosis* (MTB), especially multidrug-resistant (MDR-) and extensively drug-resistant tuberculosis (XDR-TB) [9]. Of these agents, pretomanid has entered the phase III clinical trial for TB treatment, and the phase III clinical trial of delamanid has recently been completed [3]. Considering that these two compounds belong to the same class of antimicrobials, the comparative in vitro activity of delamanid and pretomanid will provide new insights in the clinical usage of the potent nitroimidazoles against tuberculosis, while the data on this aspect were limited to our knowledge. In the present study, we parallelly compared in vitro activity of delamanid and pretomanid against MDR- and XDR-TB isolates. In addition, the genetic mutations were analyzed to explore the potential mechanism conferring nitroimidazole resistance in MTB.

Shu'an Wen, Wei Jing and Tingting Zhang contributed equally to this work.

✉ Hairong Huang
huanghairong@tb123.org

✉ Naihui Chu
chunaihui1994@sina.com

✉ Yu Pang
pangyupound@163.com

[1] National Clinical Laboratory on Tuberculosis, Beijing Key Laboratory on Drug-Resistant Tuberculosis Research, Beijing Chest Hospital, Beijing Tuberculosis and Thoracic Tumor Institute, Capital Medical University, Beijing, China

[2] Department of Tuberculosis, Beijing Chest Hospital affiliated to Capital Medical University, BeijingTuberculosis and Thoracic Tumor Research Institute, Beijing, China

Materials and methods

Bacterial strains

A total of 220 *Mycobacterium tuberculosis* strains, including 110 MDR- and 110 XDR-TB strains, were randomly selected from Tuberculosis Biobank in National Clinical Laboratory on Tuberculosis as previously described [7].

Determination of the minimum inhibitory concentration

For delamanid and pretomanid, the microplate alamar blue assay (MABA) was performed to identify the minimal inhibitory concentrations (MICs) of MDR- and XDR-TB strains [7]. Pure delamanid and pretomanid powder was synthesized

and purified by Hanxiang Biotech Co. Ltd. (Shanghai, China), and the quality characteristics of drugs were confirmed using nuclear magnetic resonance. The drug concentrations used ranged from 0.016 to 16 mg/L. The MIC breakpoint concentration was defined as 0.2 mg/L for delamanid according to a previous report [8]. The reference H37Rv (ATCC 27294) MTB strain was used as a quality control isolate and was tested with each batch.

DNA sequencing

Genomic DNA was isolated from freshly cultured bacteria as described previously [7]. The crude DNA was used as a template to amplify and sequence the gene fragments associated with nitroimidazole resistance [6]. The amplicons were sequenced by the Sanger method in Tsingke Biotech Company (Beijing, China).

The sequencing results were analyzed by the alignment with the corresponding reference H37Rv MTB strain, which was amplified and sequenced within each batch of tests.

Statistical analysis

Comparisons of the resistance rate of MTB isolates were conducted using Pearson's chi-squared test with the SPSS version 17.0 software (SPSS Inc., Chicago, IL). The difference was declared as significant if P was less than 0.05.

Results

Minimal inhibitory concentrations

Overall, delamanid revealed more active potency against MDR- and XDR-TB isolates than pretomanid, the MIC_{90} of which (less than or equal to 0.016 mg/L) was fourfold lower than that of pretomanid (0.063 mg/L). When setting 0.2 mg/L as a critical concentration to distinguish susceptible and resistant isolates, the delamanid resistance was noted in 4 (3.6%, 4/110) and 3 (2.7%, 3/110) isolates for MDR- and XDR-TB, respectively (Table 1). Statistical analysis revealed that there was no significant difference in the resistance rate between two groups (P = 1.00). The pretomanid MIC values of four MDR-TB isolates were found to be resistant to delamanid ranging from 0.031 to 0.063 mg/L. For XDR-TB, two of three delamanid-resistant isolates had higher pretomanid MIC values, one at 8.0 mg/L and another with an MIC value of > 16 mg/L, while the remaining one XDR-TB isolate had an MIC value of less than or equal to 0.016 mg/L. In addition, one XDR-TB isolate with a pretomanid MIC value of > 16 mg/L harbored a low delamanid MIC value at ≤ 0.016 mg/L.

Mutations conferring nitroimidazole resistance

The six genes contributing to nitroimidazole resistance were firstly sequenced in 11 strains with MIC values higher than 0.2 mg/L for either pretomanid or delamanid (Table 2). Of the 12 isolates, one isolate carried nonsynonymous mutation within codon 249 of the *fbiA* gene (Glu249Lys). We also found that 9 isolates harbored a synonymous mutation in the *fgd1* gene (Phe320Phe).

Discussion

In this in vitro study, approximately 97% MTB isolates tested harbored MIC values no more than 0.016 mg/L, which was similar to a recent report [4]. Our data also demonstrated that delamanid is more potent than pretomanid against MDR- and XDR-TB isolates. Consistent to our observations, a recent study from Upton and colleagues confirmed that delamanid is at least eightfold more active against MTB than pretomanid [10]. In addition to the greater potency of delamanid compared with pretomanid, there is strong evidence that these two compounds have similar frequency of resistance and comparable plasma concentration [10], indicating that delamanid may be a more compromising choice to be included in the drug combinations against MDR-TB.

Table 1 Comparison of MICs for MDR- and XDR-TB isolates against delamanid and pretomanid

No. of isolates with different MIC values against delamanid (mg/L)	No. of isolates with different MIC values against pretomanid (mg/L)													
	Multidrug-resistant TB							Extensively drug-resistant TB						
	≤ 0.016	0.031	0.063	0.13	0.25	0.5	Total	≤ 0.016	0.031	0.063	0.13	0.25	8.0	> 16.0	Total
≤ 0.016	39	42	22	1	1	1	106	36	42	21	6	1		1	107
0.5			1				1								0
16.0			1				1								0
> 16.0		1	1				2	1					1	1	3
Total	39	43	25	1	1	1	110	37	42	21	6	1	1	2	110

Table 2 MICs and mutations located in *ddn*, *fgd1*, *fbiA*, *fbiB*, and *fbiC* in the 12 MDR- and XDR-TB strains

ID	MIC (mg/L)		Resistance genotype				
	Pretomanid	Delamanid	*ddn*	*fgd1*	*fbiA*	*fbiB*	*fbiC*
MDR008	0.063	16	WT	Phe320Phe (TTT → TTC)	WT	WT	WT
MDR023	0.031	> 16	WT	WT	WT	WT	WT
MDR024	0.13	≤ 0.016	WT	Phe320Phe (TTT → TTC)	WT	WT	WT
MDR053	0.25	≤ 0.016	WT	Phe320Phe (TTT → TTC)	WT	WT	WT
MDR057	0.063	0.5	WT	Phe320Phe (TTT → TTC)	WT	WT	WT
MDR071	0.063	> 16	WT	Phe320Phe (TTT → TTC)	WT	WT	WT
MDR107	0.5	≤ 0.016	WT	Phe320Phe (TTT → TTC)	WT	WT	WT
XDR007	8	> 16	WT	WT	WT	WT	WT
XDR008	0.25	≤ 0.016	WT	Phe320Phe (TTT → TTC)	WT	WT	WT
XDR054	> 16	≤ 0.016	WT	WT	WT	WT	WT
XDR060	≤ 0.016	> 16	WT	Phe320Phe (TTT → TTC)	WT	WT	WT
XDR095	> 16	> 16	WT	Phe320Phe (TTT → TTC)	Glu249Lys (GAA → AAA)	WT	WT

The nucleotide positions of the mutations are listed according to *Escherichia coli* numbering

MIC, minimum inhibitory concentration; *MDR*, multidrug resistance; *XDR*, extensive drug-resistance; *WT*, wild type; *Phe*, Phenylalanine; *Glu*, Glutamicacid; *Lys*, Lysine

Despite exhibiting potent anti-TB properties, the delamanid resistance is noted in seven clinical MTB isolates. We only identified that one isolate with high MIC values for both two nitroimidazoles carries nonsynonymous mutation in the codon 249 of the *fbiA* gene involved in synthetization of the F_{420} cofactor [2]. An in vitro investigation found that the greatest diversity in SNP insertions and deletions is identified in the *fbiA* and *fbiC* genes among the spontaneously generated nitroimidazole-resistant mutant strains, and multiple SNPs in *fbiA* conferring nitroimidazole resistance are firstly identified by Haver and colleagues [2]. Similarly, the genetic substitution of *fbiA* reported in this study has also never been reported before. Its high diversity reflects the fact that the enzyme encoded by the *fbiA* gene is nonessential for survival in vivo for tubercle bacilli.

More importantly, five out of seven delamanid-resistant isolates show low MIC values between 0.016 and 0.063 mg/L for pretomanid. In addition, we also found that two isolates with MIC values higher than 0.25 mg/L for pretomanid are fully susceptible to delamanid. These results show very little cross-resistance to delamanid and pretomanid, which cannot be easily explained by the assumed same mechanism of action between these two nitroimidazoles [5]. We hypothesize that the intrinsic level of resistance can serve as a compensator to explain the emergence of nitroimidazole resistance, such as efflux pumps, while the poor cross-resistance indicates that these two compounds may be recognized by distinct efflux pumps conferring nitroimidazole resistance.

We also acknowledged an obvious limitation of this study. Previous publications have revealed that the MIC values of some MTB isolates were lower than 0.002 mg/L, which were not covered by the drug concentrations tested. Therefore, it may result in the overestimation of MIC values of nitroimidazoles for these isolates. Nevertheless, the inclusion of their breakpoint in our experiment is sufficient to evaluate the resistance to two nitroimidazoles and the cross-resistance between them.

In conclusion, our results demonstrate that delamanid exhibits greater in vitro potency than pretomanid against MDR- and XDR-TB isolates. A novel nonsynonymous mutation within the *fbiA* gene may be contributing to high-level resistance to delamanid and pretomanid in MTB. The exceptional cases concerning the absent cross-resistance between these two drugs highlight the urgent need to explore the new mechanisms that have not yet been described.

Acknowledgements We thank the staff from the National Clinical Laboratory on Tuberculosis for their technical assistance.

Author contributions SW, WJ, NC, YP, and HH designed this study. SW, WJ, TZ, ZZ, and YX performed the experiments. SW, WJ, YS, FW, and YP interpreted the data. SW, TZ, NC, YP, and HH wrote the manuscript. All authors approved the final version of the paper.

Funding information This study was supported by the Beijing Talents Foundation (2017000021223ZK39), the Beijing Municipal Administration of Hospitals' Youth Programme (QML20171601), the Beijing Municipal Administration of Hospitals' Ascent Plan (DFL20151501 and DFL20181602), and the Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZYLX201809).

References

1. Denny WA, Palmer BD (2010) The nitroimidazooxazines (PA-824 and analogs): structure-activity relationship and mechanistic studies. Future Med Chem 2:1295–1304
2. Haver HL, Chua A, Ghode P, Lakshminarayana SB, Singhal A, Mathema B, Wintjens R, Bifani P (2015) Mutations in genes for the F420 biosynthetic pathway and a nitroreductase enzyme are the primary resistance determinants in spontaneous in vitro-selected PA-824-resistant mutants of Mycobacterium tuberculosis. Antimicrob Agents Chemother 59:5316–5323
3. Lee SFK, Laughon BE, Mchugh TD, Lipman M (2019) New drugs to treat difficult tuberculous and nontuberculous mycobacterial pulmonary disease. Curr Opin Pulm Med
4. Liu Y, Matsumoto M, Ishida H, Ohguro K, Yoshitake M, Gupta R, Geiter L, Hafkin J (2018) Delamanid: from discovery to its use for pulmonary multidrug-resistant tuberculosis (MDR-TB). Tuberculosis (Edinb) 111:20–30
5. Manjunatha U, Boshoff HI, Barry CE (2009) The mechanism of action of PA-824: novel insights from transcriptional profiling. Commun Integr Biol 2:215–218
6. Pang Y, Zong Z, Huo F, Jing W, Ma Y, Dong L, Li Y, Zhao L, Fu Y, Huang H (2017) In vitro drug susceptibility of bedaquiline, delamanid, linezolid, clofazimine, moxifloxacin, and gatifloxacin against extensively drug-resistant tuberculosis in Beijing, China. Antimicrob Agents Chemother 61
7. Shi J, Lu J, Wen S, Zong Z, Huo F, Luo J, Liang Q, Li Y, Huang H, Pang Y (2018) In vitro activity of PBTZ169 against multiple Mycobacterium species. Antimicrob Agents Chemother 62
8. Stinson K, Kurepina N, Venter A, Fujiwara M, Kawasaki M, Timm J, Shashkina E, Kreiswirth BN, Liu Y, Matsumoto M, Geiter L (2016) MIC of delamanid (OPC-67683) against Mycobacterium tuberculosis clinical isolates and a proposed critical concentration. Antimicrob Agents Chemother 60:3316–3322
9. Stover CK, Warrener P, Vandevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, Mcmurray DN, Kreiswirth BN, Barry CE, Baker WR (2000) A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 405:962–966
10. Upton AM, Cho S, Yang TJ, Kim Y, Wang Y, Lu Y, Wang B, Xu J, Mdluli K, Ma Z, Franzblau SG (2015) In vitro and in vivo activities of the nitroimidazole TBA-354 against Mycobacterium tuberculosis. Antimicrob Agents Chemother 59:136–144