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In vitro Susceptibilities of Linezolid and Tigecycline against Drug-Resistant *Mycobacterium tuberculosis* Isolates

ORIGINAL ARTICLE

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ABSTRACT

Objective: The treatment of multidrug-resistant tuberculosis (MDR-TB) is extremely difficult, and there is a need for new and effective drugs. The aim of this study was to determine the minimum inhibitory concentration (MIC) of linezolid (LZD) and tigecycline (TGC) against DR Mycobacterium tuberculosis isolates.

Materials and Methods: The study was conducted at a referral chest diseases and chest surgery hospital. Isolates were obtained from consecutive patients. Susceptibilities to LZD and TGC were studied using the agar dilution method.

Results: A total of 26 strains resistant to any drug against M. tuberculosis were tested. Ten isolates (38.5%) had an MDR pattern. MIC50 and MIC90 values of LZD were detected to be 0.05 and 1 µg/mL, respectively. Among all isolates, 96% M. tuberculosis strains were found to be susceptible to LZD. An approved breakpoint for TGC does not exist. MIC50 and MIC90 values of TGC were found to be 16 and 64 µg/mL, respectively.

Conclusion: LZD seems to be a good alternative for treatment of DR-TB. However, TGC showed high MIC values. Treatment of DR-TB with TGC is not promising according to study results.

Keywords: Linezolid, tigecycline, tuberculosis, agar dilution

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INTRODUCTION

Tuberculosis (TB) is one of the leading causes of death owing to a single infectious agent worldwide. According to the World Health Organization (WHO), 1.5 million people die of TB each year and about 9.6 million new cases are reported each year (1, 2). TB control is challenged by multidrug-resistant TB (MDR-TB) and extensively DR-TB (XDR-TB). Because of the extent of resistance, treatment options for MDR-TB and XDR-TB are limited, and new drugs and alternative treatment regimens are needed (3, 4).

Linezolid (LZD) was approved in 2000 for DR, gram-positive bacterial infections and also has shown good activity against *Mycobacterium tuberculosis* strains, including MDR-TB and XDR-TB strains, both *in vitro* and in animal studies (5, 6). LZD spreads in the well-perfused regions of the body and penetrates well into bronchoalveolar tissues. It inhibits protein synthesis at an early stage of translation by binding the 23S ribosomal RNA (rRNA) portion of the bacterial 50S ribosomal subunit (7).

Tigecycline (TGC) is only commercially available glycylcycline and was approved in 2005 (8). It is a novel drug that is a bacteriostatic agent with a broad antibacterial spectrum (8, 9). It exhibits antimicrobial effect by blocking of protein synthesis; it binds reversibly to the 30S subunit of ribosome, blocking the binding of acyltRNA to region A (8). Although TGC has demonstrated antibacterial activity against several bacteria, there has not been any data regarding its anti-TB effect (9).

The aims of the study are to investigate *in vitro* LZD and TGC effects against clinical isolates of *M. tuberculosis* and review these newly developed drugs for their efficacy against TB bacilli.

MATERIALS and METHODS

Clinical strains were isolated from respiratory samples from patients hospitalized in the TB service of the Pulmonary Disease and Chest Surgery Training Hospital in 2012. Every strain was obtained from unique patient.

Clinical respiratory specimens were decontaminated and homogenized using the N-Asetil L-sistein-sodyumhidroksit (NALC-NAOH) method. MGIT 960 automated culture system (BD, Sparks MD, USA) and Lowenstein–

Jensen (LJ) (Gül Biyoloji, İstanbul, Turkey) medium were used for the isolation of mycobacteria. Strains were identified via the ARB stain characterization and ProbeTech System (BD, Sparks MD, USA). The susceptibility patterns of all isolates were studied with regard to first-line anti-TB drugs (isoniazid, rifampin, streptomycin, ethambutol, and pyrazinamide) using the MGIT 960 AST system (BD, Sparks MD, USA) according to manufacturer's instructions. Drug resistance patterns of isolates are given in Table 1. Standard identification and susceptibility testing were applied according to Clinical and Laboratory Standards Institute (CLSI) standards (10). Resistance to any first-line anti-TB drugs was classified as follows:

MDR: Resistance to both rifampicin and isoniazid

XDR: Resistance to both isoniazid and rifampicin, a fluoroquinolone, and one or more of the following injectable drugs: amikacin, capreomycin, or kanamycin. Of the strains that were previously investigated for the antibiotic resistance patterns of the four first-line drugs, at least one strain that was found to be DR was studied. All strains were subcultured on Middlebrook 7H10 agar media (BD, Heidelberg, Germany) supplemented with oleic acid, albumin, dextrose, and catalase (OADC) for testing minimum inhibitory concentrations (MICs) of LZD and TGC. Internal quality control was performed using *M. tuberculosis* H37 Ra and H37Rv strains for daily culture and drug susceptibility testing. Since 2004, an external quality (EQ) control system has been used by UK National External Quality Assessment System for smear and culture confirmation. EQ for drug susceptibility testing was started in 2005 by "Lot Quality Assurance Sampling" in a national third-level TB laboratory. MIC was defined as the lowest antimicrobial concentration that inhibited more than 99% of bacterial growth. Antibiotic preparations used in this study were obtained as pure substances from their manufacturers. Susceptibilities to LZD and TGC were studied using the agar dilution method according to CLSI standards (11). MIC was determined by serially diluting each compound 2-fold on Middlebrook 7H10 agar media supplemented with OADC. The stock solutions of LZD (Pfizer Inc.) and TGC were prepared in distilled water at a concentration of 2 mg/mL. Aliquots of these solutions were frozen at −20°C until used. H37Rv (ATCC 27294) strain was used as the control bacteria. For the agar dilution method, an MIC equal to the breakpoint concentration is reported as susceptible; plates were read after 4, 7, 10, and 14 days of incubation and on each working day thereafter. For each strain, one agar medium without drug

Table 1. Resistance patterns of test isolates against first-line anti-TB drugs

Drug	Number	%
Isoniazid (INH)	4	15.4
Ethambutol (ETB)	2	7.7
Streptomycin (SM)	3	11.5
INH-Rifampicin (RM)	3	7.7
INH-SM	7	26.9
INH-RM-SM	1	3.8
INH-SM-RM-ETB	4	15.4
INH-SM-RM-ETB-Pyrazinamid (PZA)	2	7.7
Total	26	100

INH: Isoniazid, ETB: Ethambutol, SM: Streptomycin: RM: Rifampicin, PZA: Pyrazinamid

Table 2. MIC values of LZD and TGC against *M. tuberculosis* strains

Resistance pattern	No. of isolates	MIC LZD (µg/ml)	MIC TGC (µg/ml)
INH	4		
		0.25	16
		<0.06	16
		0.5	64
		1	32
SM	3		
		0.5	32
		2	16
		0.25	16
ETB	2		
		<0.06	16
		1	16
INH-SM	7		
		0.5	16
		1	32
		0.5	16
		1	16
		1	<8
		1	16
		0.5	32
Total	16		
INH-RF	3		
		<0.06	<8
		0.25	16
		<0.06	32
INH-RF-SM	1		
		0.5	<8
INH-RF-ETB-SM	3		
		0.5	64
		0.25	32
		0.5	32
INH-RF-SM-ETB-PZA	3		
		0.5	64
		0.25	32
		0.5	32
MDR total	10		
Gross total	26		

INH: Isoniazid, ETB: Ethambutol, SM: Streptomycin: RM: Rifampicin, PZA: Pyrazinamid

was also prepared as the growth control. The standard inoculum of each isolate was adjusted to an equal density of 1 McFarland standard by diluting the initial inoculum with Middlebrook 7H9 broth (BD & Difco, New Jersey, USA)). Final suspensions were prepared using 10^{-2} and 10^{-4} dilutions of the standardized suspensions with MB 7H9 broth. On each part of the agar plates, 100 µL of the diluted inoculum was placed as spots.

All plates were sealed and incubated at 37°C in 5% to 10% CO_2 for 3 weeks. The MIC of each isolate was determined to be the lowest concentration of an antibiotic that inhibited more than 99% of the colonies growing on the drug-free control. Appropriate volumes of diluted TGC solutions were incorporated into aliquots of 7H10 agar medium to achieve the desired final concentrations of LZD (0.1–8 mg/L) and TGC (8–64 mg/L). Once the drugs were introduced to the media, the solutions were dispensed quickly into sterile plastic petri dishes, allowed to solidify, and either used immediately or stored at 4°C until use. This study has been approved by the official scientific publication committee of the hospital.

RESULTS

A total of 26 *M. tuberculosis* strains resistant to any drug were tested. Strains were isolated from 24 sputum (92.4%), one bronchoscobic (3.8%), and one gastric lavage (3.8%) specimens. Ten isolates (38.5%) had an MDR pattern. MIC50 and MIC90 values of LZD were detected to be 0.05 and 1 µg/mL, respectively. Among all isolates, 96% *M. tuberculosis* strains were found susceptible to LZD. Approved breakpoint for TGC does not exist. MIC50 and MIC90 values of TGC were found to be 16 and 64 µg/mL, respectively. Results are presented in Table 2.

DISCUSSION

The treatment of MDR-TB is extremely difficult, and a well-equipped laboratory is needed to determine drug resistance in these strains. Second-line anti-TB drugs are used in the treatment of infections caused by strains that are resistant to first-line drugs. However, the effectiveness of these drugs is low, they are not cost-effective, and they may have toxic side effects. Therefore, TB chemotherapy requires new therapy options that have both different action mechanisms and bactericidal effects (12).

Since 2006, the WHO has considered LZD for the treatment of MDR-TB, acknowledging that its efficacy is unclear and recommending against its routine use (13). The findings by Lee et al. (14) add to the evidence on the safety profile and efficacy of LZD for salvage treatment of XDR-TB and reinforce the recommendations of WHO (15). Most recent studies indicate very susceptible results *in vitro*. Yang et al. (16) reported the MIC90 value of LZD in susceptible, MDR, and XDR strains to be 0.25 µg/mL. Similarly, Tato et al. (17) found that MIC value of LZD to be 0.5 µg/mL in their 42 susceptible, 3 isoniazid-resistant, and 10 isoniazid- and rifampicin-resistant isolates. No differences were observed between susceptible and resistant isolates, with an MIC range of 0.12–0.5 µg/mL.

M. tuberculosis (33 MDR, 34 non-MDR) isolates showed MIC50 and 90 values of 0.5 µg/mL in a study by Ermertcan et al. (18). LZD was found to be very effective against DR-TB isolates in several studies. Bektore et al. (19) reported zero resistance in 81 MDR isolates. Also, Ingen et al. (20) found no resistance in their 29 MDR isolates. Some recent studies reported higher MIC values of LZD. Huang et al. (21) had found a decline in LZD susceptibility in Taiwan. MIC90 increases in 94.2% (114/121) of the *M. tuberculosis* isolates were inhibited by LZD at concentrations ≤1 µg/mL. However, they reported no statistical difference in the MIC values of sensitive and resistant strains (21). Likewise, Cabrera et al. (22) found the antimicrobial concentrations at which MIC50 and MIC90 of the *M. tuberculosis* isolates were 1 and 2 µg/mL, respectively. Erturan et al. (23) tested the *in vitro* activity of this drug against 39 MDR *M. tuberculosis* strains isolated from clinical specimens. They remarked that all strains were inhibited at and under 8 mg/L (MIC50 = µg/ml, MIC90 = µg/ml). MIC values were higher compared with other studies (23). Richter et al. (24) found LZD resistance in four (1.9%) of the 210 MDR *M. tuberculosis* strains. The MIC values of LZD were 4 µg/mL (one strain) and 8 µg/mL (three strains). Because no mutations were detected in the potential target genes, the mechanism of resistance remained unclear (24). However, 1 year later, Hilleman et al. (25) declared LZD mutants in their study. Resistance to other anti-TB drugs did not affect susceptibility to LZD. Further investigations to prove its usefulness in the treatment of MDR-TB should be performed. Studies on TGC susceptibility against *M. tuberculosis* are less in number than those on LZD susceptibility. It is demonstrated that TGC is effective against rapidly growing mycobacteria, but it is not effective against slowly growing mycobacteria (26). We found just one study that investigates *in vitro* effects of TGC against clinical isolates of *M. tuberculosis* in the literature. Coban et al. (9) studied 50 *M. tuberculosis* strains (20 MDR isolates) for the detection of the TGC breakpoint. MIC values of TGC for *M. tuberculosis* isolates had been found to vary between 8 and 64 µg/mL. Although there is no defined breakpoint value in the study, none of the isolates have been observed to be susceptible to TGC when a resistance break point value of 8 µg/mL for non-TB mycobacteria is considered (9). Alsaad et al. (27) had reviewed the *in vitro* and *in vivo* and clinical anti-TB activities of some antimicrobial drugs not listed in the WHO guidelines on MDR-TB treatment but could offer potential for TB treatment in addition to pharmacokinetics and side effects. They reported that TGC was effective against rapid-growing mycobacteria (*M. fortuitum, M. chelonae,* and *M. abscessus*) but showed no activity against more slow-growing mycobacteria (*M. tuberculosis*). They indicated that *in vitro* MIC values of TGC against clinical isolates of *M. tuberculosis* were high, and no publications mentioned the antimicrobial activity of TGC *in vivo* (27).

CONCLUSION

Local drug resistance patterns are very important for national and international TB programs. We planned this study because the data on LZD resistance is very rare and that on TGC resistance is absent in our country and geographic area. In conclusion, LZD is effective against *M. tuberculosis* isolates *in vitro*. MIC values were not affected by resistance to other anti-TB drugs. LZD is promising for MDR-TB cases. Furthermore, TGC is not promising as a treatment option at least according to actual data. However, experimental *in vivo* studies are needed on this aspect of TGC.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İzmir Dr. Suat Seren Cheast Diseases and Surgery Training and Research Hospital (No: 5840).

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