In Vitro Susceptibility Testing of Tedizolid against Isolates of Nocardia

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ABSTRACT There is a paucity of efficacious antimicrobials (especially oral) against clinically relevant species of Nocardia. To date, all species of Nocardia have been susceptible to linezolid, the first commercially available oxazolidinone. Tedizolid is a new oxazolidinone with previously reported improved in vitro and in vivo (intracellular) potency against multidrug-resistant strains of Mycobacterium sp. and Nocardia brasiliensis. Using the current Clinical and Laboratory Standards Institute-recommended broth microdilution method, 101 isolates of Nocardia spp., including 29 Nocardia cyriacigeorgica, 17 Nocardia farcinica, 13 Nocardia nova complex, 21 Nocardia brasiliensis, 5 Nocardia pseudobrasiliensis, and 5 Nocardia wallacei isolates and 11 isolates of less common species, were tested for susceptibility to tedizolid and linezolid. For the most common clinically significant species of Nocardia, tedizolid MIC50 values were 0.25 g/ml for N. nova complex, N. brasiliensis, N. pseudobrasiliensis, and N. wallacei, compared to linezolid MIC50 values of 1, 2, 0.5, and 1 g/ml, respectively. Tedizolid and linezolid MIC90 values were 2 g/ml for N. nova complex and N. brasiliensis. Tedizolid MIC50 and MIC90 values for both N. cyriacigeorgica and N. farcinica were 0.5 g/ml and 1 g/ml, respectively, compared to linezolid MIC50 and MIC90 values of 2 and 4 g/ml, respectively. Based on MIC 90 values, this study showed that tedizolid was 2- to 3-fold more active than linezolid in vitro against most common species of Nocardia, with the exception of the N. nova complex and N. brasiliensis, for which values were the same. These results may warrant evaluation of tedizolid as a potential treatment option for Nocardia infections.

KEYWORDS Nocardia, susceptibility testing, tedizolid

Nocardia species are Gram-positive branching rods responsible for various types of infections, including respiratory, cutaneous, and systemic infections (1). Some clinically relevant species of Nocardia are multidrug resistant, and there is a paucity of antimicrobials with efficacy against these organisms (1, 2). Thus, new antimicrobials with therapeutic potential are desperately needed.

All strains of Nocardia isolated to date have been susceptible to linezolid (LZD) (1–3), the first commercially available oxazolidinone (3). Tedizolid (TZD) is a new oxazolidinone with previously reported in vitro and in vivo (intracellular) activity against Mycobacterium tuberculosis, including multidrug-resistant strains (4), and Nocardia brasiliensis (5–7). A previously published study by Vera-Cabrera et al. showed in vitro activity of TZD against Nocardia brasiliensis strains (7).

Other investigators have also reported enhanced in vitro activity of TZD against bacterial strains, including LZD-resistant strains of Streptococcus pneumoniae, in addition to methicillin-susceptible and methicillin-resistant coagulase-negative Staphylococcus, Staphylococcus aureus, Streptococcus pyogenes, and Streptococcus agalactiae (8–11). With this superior activity in mind, we undertook an evaluation of the in vitro MICs of TZD, compared with those of LZD and other comparator antimicrobials that are routinely tested in clinical laboratories, for several clinically significant species of Nocardia.
TABLE 1 MIC ranges and MIC_{50} and MIC_{90} values for tedizolid and linezolid against the most commonly encountered clinical isolates of *Nocardia*

<table>
<thead>
<tr>
<th>Species (no. of isolates tested) and antimicrobial</th>
<th>MIC (µg/ml)</th>
<th>Range</th>
<th>MIC_{50}</th>
<th>MIC_{90}</th>
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</thead>
<tbody>
<tr>
<td><em>N. cyriacigeorgica</em> (29 isolates)</td>
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<tr>
<td>Tedizolid</td>
<td></td>
<td>0.12–2</td>
<td>0.5</td>
<td>1</td>
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<tr>
<td>Linezolid</td>
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<td>1–4</td>
<td>2</td>
<td>4</td>
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<td><em>N. farcinica</em> (17 isolates)</td>
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<tr>
<td>Tedizolid</td>
<td></td>
<td>0.25–2</td>
<td>0.5</td>
<td>1</td>
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<tr>
<td>Linezolid</td>
<td></td>
<td>1–4</td>
<td>2</td>
<td>4</td>
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<tr>
<td><em>N. nova complex</em> (13 isolates)</td>
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<tr>
<td>Tedizolid</td>
<td></td>
<td>0.12–2</td>
<td>0.25</td>
<td>2</td>
</tr>
<tr>
<td>Linezolid</td>
<td></td>
<td>0.25–2</td>
<td>1</td>
<td>2</td>
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<tr>
<td><em>N. brasiliensis</em> (21 isolates)</td>
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<tr>
<td>Tedizolid</td>
<td></td>
<td>0.12–0.5</td>
<td>0.25</td>
<td>2</td>
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<tr>
<td>Linezolid</td>
<td></td>
<td>1–4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>N. pseudobrasiliensis</em> (5 isolates)</td>
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<tr>
<td>Tedizolid</td>
<td></td>
<td>0.12–0.5</td>
<td>0.25</td>
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<tr>
<td>Linezolid</td>
<td></td>
<td>0.25–2</td>
<td>0.5</td>
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<tr>
<td><em>N. wallacei</em> (5 isolates)</td>
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<tr>
<td>Tedizolid</td>
<td></td>
<td>0.06–0.5</td>
<td>0.25</td>
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<tr>
<td>Linezolid</td>
<td></td>
<td>0.5–2</td>
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</table>

There are no intermediate or resistance breakpoints for LZD for *Nocardia*. All known species isolated to date have been susceptible to LZD (≤8 µg/ml).

(A portion of this study was presented at the 1st ASM Microbe meeting, Boston, MA, 16 to 20 June 2016.)

RESULTS

A total of 98/101 (97%) of the *Nocardia* isolates showed MIC_{50} of ≤1 µg/ml for TZD and LZD (Table 1). Generally, isolates of *Nocardia* showed either equivalent or 2- to 3-fold lower TZD MIC_{90} values, compared with LZD MIC values. Table 1 shows the MICs for species tested with ≥5 isolates. The TZD MIC_{50} values were 0.5 µg/ml for 29 isolates of *Nocardia cyriacigeorgica* and 17 isolates of *Nocardia farcinica*, compared to 2 µg/ml for LZD for both species. For 13 isolates of *Nocardia nova* complex, 22 isolates of *Nocardia brasiliensis*, and 5 isolates each of *Nocardia pseudobrasiliensis* and *Nocardia wallacei*, the TZD MIC_{50} values were 0.25 µg/ml, with LZD MIC_{50} values of 1, 2, 0.5, and 1 µg/ml, respectively. Two isolates each of *Nocardia abscessus* and *Nocardia* sp. showed TZD MICs of 0.12 µg/ml, compared to 0.25 to 0.5 µg/ml and 0.5 to 1 µg/ml, respectively, for LZD. Single isolates of *Nocardia beijingensis*, *Nocardia niigatensis*, and *Nocardia otitidiscaviarum* had TZD MICs of 0.25 µg/ml, compared to 1, 1, and 2 µg/ml, respectively, for LZD (data not shown). One isolate each of *Nocardia asiatica*, *Nocardia paucivorans*, *Nocardia mexicana*, and *Nocardia testaceae* showed TZD MICs of 0.12 µg/ml, compared to 0.5 µg/ml for *N. asiatica*, *N. paucivorans*, and *N. mexicana* and 1 µg/ml for *N. testaceae* for LZD (data not shown).

MICs for additional antimicrobials were also assessed, in order to determine whether there are intermediate or resistance breakpoints for LZD for *Nocardia*. All known species isolated to date have been susceptible to LZD (≤8 µg/ml).

The manufacturer’s acceptable MIC ranges for *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were 0.25 to 1 µg/ml. All 34 isolates of *S. aureus* ATCC 29213 and 10 isolates of *E. faecalis* had TZD MICs within the acceptable ranges.
DISCUSSION

Tedizolid phosphate is a novel oxazolidinone prodrug (TR-701) that is orally absorbed and converted in the serum to the active drug, tedizolid (TR-700, formerly DA-7157) (8), with a broad range of activity against Gram-positive microorganisms, including mycobacteria and Nocardia (4–7). The mechanism of action of TZD is through inhibition of protein synthesis. TZD binds to the 50S ribosome, apparently at a site near the 30S ribosome, which blocks formation of the 70S initiation complex and in turn prevents protein synthesis (8). The supposition is that the major site of action of oxazolidinones is at the ribosomal peptidyltransferase center and that unique, phylogenetically conserved, structural features of this site are likely responsible for reducing the mutational resistance and cross-resistance with other antimicrobial classes (8, 11). Studies of crystallographic structures and cross-linking have seemed to substantiate this supposition (12).

Linezolid has been an important addition to the armamentarium of antimicrobials used in the treatment of Nocardia infections (1–3). The introduction of TZD provides another potential antimicrobial with efficacy against these organisms, and early studies showed that TZD exhibited 4- to 16-fold greater potency than did LZD against some bacteria, including LZD-resistant strains (10, 11, 13, 14). Ninety-seven percent of all of the isolates of Nocardia species studied, including all of the major clinically relevant species, had MICs of ≤1 µg/ml and ≤4 µg/ml for TZD and LZD, respectively.

TZD has high oral bioavailability and a longer half-life (11 h, compared with 5 h for LZD), which allows clinicians to modify the route of administration from intravenous to oral easily and to use once-daily dosing, encouraging greater patient compliance and outpatient usage (15, 16). Moreover, although long-term usage has not been assessed, TZD appears to be better tolerated than LZD, especially in regard to hematological adverse events, including thrombocytopenia (8, 13). In a recent clinical trial by Kim et al. at the National Institute of Allergy and Infectious Diseases, the safety and tolerability among 25 patients treated with TZD were comparable to those for LZD after a median duration of 91 days of therapy. Except for gastrointestinal intolerance, which was 20% with TZD versus 40% with LZD, adverse events, including peripheral neuropathy and thrombocytopenia, were equivalent in occurrence (17). The incidences of anemia were not compared in this trial (17). However, a single case study in Spain showed that a patient (age given as “in his 70s”) with a pulmonary infection with Mycobacterium avium complex and Mycobacterium kansasii that was treated with TZD had stable hemoglobin levels for 42 days, although a subsequent decrease after 58 days of treatment with TZD necessitated the withdrawal of TZD (18). No apparent dose-related toxicity with TZD has been reported (14).

TZD was shown to be more active than LZD in evaluations of the ability to decrease the CFU of bacterial species, including Staphylococcus aureus, Listeria monocytogenes, and Legionella pneumophila, in cultured macrophages or human umbilical vein endothelial cells (9, 19). Furthermore, Vera-Cabrera et al. showed that TZD was more active than LZD in inhibiting the intracellular growth of Nocardia (5). TZD accumulates more rapidly than LZD, and the intracellular concentration of TZD was at least 10- to 15-fold higher than the extracellular concentration, in contrast to the intracellular concentration of LZD, which is equivalent to the extracellular concentration (13, 19, 20). Moreover, previous studies showed excellent penetration of TZD into the epithelial lining fluid of the lungs, suggesting that TZD may be useful in the setting of pneumonia (20), and other studies showed superior distribution of TZD into the interstitial fluid of adipose and muscle tissue, possibly making TZD an attractive therapeutic option for skin and soft tissue infections with Nocardia (13).

Previous studies in healthy adults showed that TZD half-life values are approximately 2-fold greater than those of LZD and TZD is rapidly absorbed, with nearly complete oral bioavailability following 200-mg doses of tedizolid phosphate. Studies also suggested that the 200-mg dose of tedizolid phosphate (150 mg TZD equivalent) has a favorable pharmacokinetic, safety, and efficacy profile, and thus this dose was
selected for therapeutic dosing (8, 14, 15, 20). The in vitro MICs of TZD obtained in this and previous studies, along with the once-daily lower dosage for TZD and the potential for fewer and less serious adverse events associated with TZD, compared to LZD, emphasize the potential for TZD in the treatment of infections caused by Nocardiа (8, 9, 15, 19, 20).

**MATERIALS AND METHODS**

**Isolates.** A total of 101 isolates of Nocardia spp. that had been submitted to the Mycobacteria/Nocardia Research Laboratory at the University of Texas Health Science Center at Tyler in 2014 and 2015 were tested against TZD, LZD, and other comparative antimicrobials (see Table S1 in the supplemental material). These isolates included 29 N. cyriacigeorgica isolates, 17 N. farcinica isolates, 13 N. nova complex isolates, 21 N. brasilensis isolates, 5 N. pseudobrasiliensis isolates, 5 N. wallacei isolates, 2 N. abscessus isolates, 2 Nocardia sp. isolates (most closely related to Nocardia carnea), and 1 isolate each of N. beijingensis, N. niigatensis, N. paucivorans, N. asiatica, N. otitidiscaviarum, N. mexicana, and N. testaceus.

**Identification.** Isolates of Nocardia were identified by sequencing of the secA1 gene and partial 16S rRNA gene as indicated by species and group, following the interpretation criteria published by the Clinical and Laboratory Standards Institute (CLSI) (21) and by Conville et al., as applicable (22). Partial 16S rRNA gene sequencing was performed for species with no available secA1 sequence or isolates for which secA1 sequences were ambiguous (21–23).

**Antimicrobial susceptibility testing.** Isolates were tested by broth microdilution using customized, frozen, 96-well panels commercially manufactured by Thermo-Fisher Scientific, with doubling dilutions of antimicrobials (TZD concentrations were 0.008 to 32 μg/ml) in cation-adjusted Mueller-Hinton broth, after 3- to 4-day incubations at 35°C, following the CLSI-recommended procedures for Nocardia (24). Comparative antimicrobials included LZD, amikacin, amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, minocycline, moxifloxacin, tigecycline, tobramycin, and TMP-SMX (Table S1).

**Quality control.** Quality control for susceptibility testing was performed weekly using the CLSI-recommended strain Mycobacterium peregrinum ATCC 700686 for comparative antimicrobials (24) and the strains Staphylococcus aureus ATCC 29213 and Enterococcus faecalis ATCC 29212 for TZD (Table 2).

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found athttps://doi.org/10.1128/AAC.01537-17.

**SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.**

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**REFERENCES**


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