In Vitro Activities of the Novel Oxazolidinones DA-7867 and DA-7157 against Rapidly and Slowly Growing Mycobacteria[▽]

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DA-7867 and DA-7157 are oxazolidinones active against pathogenic aerobic actinomycetes including *Nocardia* spp. and *Mycobacterium tuberculosis*. However, the activity of these drugs against nontuberculous mycobacterium (NTM) species is not known. In this work, we compared the susceptibilities of 122 clinical isolates and 29 reference species of both rapidly growing and slowly growing mycobacteria to linezolid, DA-7867, and DA-7157 by the broth microdilution method. The MICs for 50 and 90% of the strains tested (MIC₅₀s and MIC₉₀s, respectively) of DA-7867 and DA-7157 were lower than those of linezolid. In all of the cases, a MIC₉₀ of <8 µg/ml was observed for all of the species tested in both groups of NTM. For *M. kansasii* and *M. marinum* isolates, the MIC₉₀s of both DA-7867 and DA-7157 were less than 0.5 µg/ml. These results demonstrate the potential of these compounds to treat NTM infections.

Nontuberculous mycobacteria (NTM) are associated with a number of clinical diseases, including lymphadenitis, skin abscesses, disseminated infections, and pulmonary infections, in immunocompetent and immunocompromised patients (4). The most commonly isolated species include the *Mycobacterium avium* complex, *M. kansasii*, *M. marinum*, *M. chelonae*, *M. abscessus*, and the *M. fortuitum* group. Because of the increasing numbers of immunocompromised patients, e.g., organ transplant recipients, patients receiving corticosteroid therapy, and patients with human immunodeficiency virus, together with the availability of better technology, the number of species identified is expanding (13). Therapy of these infections is complicated because they are resistant to conventional antitubercular antimicrobials and only a few agents are active.

Oxazolidinones are recently developed antimicrobials that inhibit protein synthesis at a site not targeted by other antimicrobials (11). Resistance by mutations or the presence of a resistance determinant has been rarely reported. The first of these compounds to be approved by the Food and Drug Administration, linezolid, is active in vitro against many gram-positive actinomycetes, including *Nocardia*, *Actinomyces*, and *M. tuberculosis* (14, 15, 16), and has been given with successful results to patients infected with *Nocardia* spp. or *M. tuberculosis* (6, 7).

These preliminary findings are promising for the development of new oxazolidinones. Several such chemically modified compounds have been produced, and two of them, DA-7867 [(*S*)-[*N*-3-(4-(2-(1-methyl-5-tetrazolyl)-pyridin-5-yl)-3-fluorophenyl)-2-oxo-5-oxazolidinyl] methyl acetamide} and DA-7157 [(*R*)-3-(4-(2-(2-methyltetrazol-5-yl)pyridin-5-yl)-3-fluorophenyl)-5-hydroxymethyl oxazolidin-2-on], have been observed to be ac-

tive against *M. tuberculosis*, including drug-resistant isolates (15). In the present work, we undertook a comparative study of the in vitro susceptibilities of rapidly growing mycobacteria (RGM) and slowly growing NTM to the two new oxazolidinones DA-7867 and DA-7157 versus the already approved drug linezolid.

MATERIALS AND METHODS

Microorganisms. A total of 151 NTM isolates, corresponding to 15 species, were analyzed. This number included 122 clinical isolates and 29 reference isolates that were obtained from the culture collection of the University of Texas Health Center at Tyler Mycobacteria/Nocardia Laboratory. Isolates were identified to the species level with a commercial DNA-RNA probe (*M. avium* complex, *M. kansasii*) or by PCR restriction analysis of a 441-bp sequence (Telenti fragment) of the *hsp65* gene as previously described (12). The reference isolates had been provided by or obtained from the American Type Culture Collection (ATCC). The isolates tested were *Mycobacterium abscessus* (14 isolates), *M. avium* complex (13 isolates), *M. chelonae* complex (17 isolates), *M. fortuitum* third biovariant complex (24 isolates), *M. fortuitum* group (17 isolates), *M. goodii* (6 isolates), *M. kansasii* (8 isolates), *M. marinum* (7 isolates), *M. mucogenicum* (4 isolates), *M. peregrinum* (16 isolates), *M. porcinum* (2 isolates), *M. senegalense* (1 isolate), *M. simiae* (6 isolates), *M. smegmatis* group (8 isolates), *M. terrae* complex (1 isolate), *M. triplex* (3 isolates), and *M. wolinskyi* (4 isolates).

Broth microdilution technique. Linezolid, DA-7867, and DA-7157 were provided by the Dong-A Pharmaceutical Company, Ltd., Yongin, Korea. Stock solutions of these drugs (linezolid, DA-7157, and DA-7867) were prepared in 100% dimethyl sulfoxide and then diluted in cation-adjusted Mueller-Hinton broth (CA-MHB; Becton Dickinson, Sparks, MD). MICs were determined as recommended by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical and Laboratory Standards) by a broth microdilution method in CA-MHB for RGM and CA-MHB with the addition of an oleic acid-albumin-dextrose-catalase supplement (Becton Dickinson, Sparks, MD) at a final concentration of 10% for slowly growing mycobacteria (9). The final drug concentration range was 0.25 to 64 µg/ml. The MIC was read for the RGM after 72 h of incubation at 30°C and for the slowly growing mycobacteria after 7 days of incubation at 35°C. Some strains of *M. chelonae* and *M. abscessus* required extended incubation because of their poor growth.

Quality control. For quality control testing, *Staphylococcus aureus* ATCC 29213 and *M. peregrinum* ATCC 700686 were used. Acceptable linezolid MIC ranges for those strains are 1 to 4 µg/ml and ≤2 to 4 µg/ml, respectively (3, 17).

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TABLE 1. Comparison of MICs of linezolid, DA-7157, and DA-7867 for *M. avium* complex and RGM

Species (no. of isolates tested) and test agent	MIC ($\mu\text{g/ml}$)		
	Range	For 50% of isolates	For 90% of isolates
<i>M. avium</i> complex (13)			
Linezolid	2–32	32	32
DA-7157	1–8	8	8
DA-7867	0.5–8	4	8
<i>M. abscessus</i> (14)			
Linezolid	8–64	64	64
DA-7157	0.5–4	4	4
DA-7867	0.5–8	4	8
<i>M. chelonae</i> complex (17)			
Linezolid	1–32	8	16
DA-7157	≤ 0.25 –4	1	4
DA-7867	0.5–8	2	8
<i>M. fortuitum</i> 3rd biovariant complex (24)			
Linezolid	1–8	8	8
DA-7157	≤ 0.25 –4	2	4
DA-7867	≤ 0.25 –8	2	4
<i>M. fortuitum</i> group ^a (33)			
Linezolid	0.5– ≥ 64	4	16
DA-7157	≤ 0.25 –64	1	4
DA-7867	≤ 0.25 –32	1	4

^a Includes *M. fortuitum* and *M. peregrinum*.

RESULTS

Broth microdilution assays. The in vitro antibacterial activities of DA-7157, DA-7867, and linezolid against NTM isolates are presented in Table 1. In both cases (*M. avium* complex isolates and RGM), the linezolid MIC ranges, as well as the MICs for 50 and 90% of the isolates tested (MIC₅₀s and MIC₉₀s, respectively), were very similar to those previously reported (3, 17).

For isolates of the *M. avium* complex, the MICs of linezolid were high (MIC₉₀, 32 $\mu\text{g/ml}$) (Table 1). The in vitro activities of DA-7157 and DA-7867 were greater than that of linezolid, particularly that of the latter compound, which was four times more active than linezolid. DA-7867 and DA-7157 had lower MICs than did linezolid for *M. kansasii* and *M. marinum*. The MICs of DA-7157 for eight isolates of *M. kansasii* were ≤ 0.25 to 0.5 $\mu\text{g/ml}$, and those of DA-7867 were ≤ 0.25 $\mu\text{g/ml}$. For seven isolates of *M. marinum*, the MICs of DA-7157 were ≤ 0.25 $\mu\text{g/ml}$ and those of DA-7867 were ≤ 0.25 to 0.5 $\mu\text{g/ml}$. The MICs of DA-7157 and DA-7867 for six *M. simiae* isolates tested were 1 to 8 $\mu\text{g/ml}$ and 2 to 8 $\mu\text{g/ml}$, respectively. For the *M. triplex* isolates ($n = 3$), the MICs of DA-7157 were ≤ 0.25 to 4 $\mu\text{g/ml}$ and those of DA-7867 were ≤ 0.25 to 4 $\mu\text{g/ml}$. For the single isolate of *M. terrae* complex, the MIC of DA-7157 was 1 $\mu\text{g/ml}$ and that of DA-7867 was 2 $\mu\text{g/ml}$. Linezolid MICs were 0.5 to 1 $\mu\text{g/ml}$ for *M. kansasii*, 1 $\mu\text{g/ml}$ for *M. marinum*, 8 to 32 $\mu\text{g/ml}$ for *M. simiae*, 0.5 to 16 $\mu\text{g/ml}$ for *M. triplex*, and 16 $\mu\text{g/ml}$ for the single isolate of *M. terrae* complex.

For all of the isolates of *M. abscessus*, *M. chelonae*, and *M. fortuitum* (including the third biovar) tested, the MIC₉₀ of DA-7157 was 4 $\mu\text{g/ml}$ and both new oxazolidinones were more

active than linezolid (Table 1). DA-7867 and DA-7157 were also two- to eightfold more active than linezolid against the remainder of the RGM. The MICs of both DA-7157 and DA-7867 for six isolates of *M. goodii* were ≤ 0.25 $\mu\text{g/ml}$. For the four isolates of *M. mucogenicum*, the MICs of both DA-7157 and DA-7867 were ≤ 0.25 to 1 $\mu\text{g/ml}$. For the two *M. porcinum* isolates, the MIC of DA-7157 was 2 $\mu\text{g/ml}$ and that of DA-7867 was 4 to 8 $\mu\text{g/ml}$. The MICs of both compounds for eight *M. smegmatis* isolates tested were ≤ 0.25 $\mu\text{g/ml}$. The MICs of both DA-7157 and DA-7867 for four isolates of *M. wolinskyi* were ≤ 0.25 to 4 $\mu\text{g/ml}$. The MIC of DA-7157 and DA-7867 for the single isolate of *M. senegalense* was 1 $\mu\text{g/ml}$. The linezolid MICs were ≤ 0.25 to 1 $\mu\text{g/ml}$ for *M. goodii*, 0.5 to 2 $\mu\text{g/ml}$ for *M. mucogenicum*, 8 to 16 $\mu\text{g/ml}$ for *M. porcinum*, ≤ 0.25 to 0.5 $\mu\text{g/ml}$ for *M. smegmatis*, 1 to 8 $\mu\text{g/ml}$ for *M. wolinskyi*, and 4 $\mu\text{g/ml}$ for *M. senegalense*.

Quality control. The MIC for quality control strains was determined with each lot of microtiter plates prepared, and the results for linezolid were within the expected range. The MICs of DA-7867 were < 0.25 $\mu\text{g/ml}$ for *S. aureus* ATCC 29213 and 0.5 $\mu\text{g/ml}$ for *M. peregrinum*. The MICs of DA-7157 were < 0.25 $\mu\text{g/ml}$ for *S. aureus* and 1 $\mu\text{g/ml}$ for *M. peregrinum*.

DISCUSSION

Antimicrobial therapy for NTM infections includes the use of aminoglycosides, macrolides, fluoroquinolones, and antituberculosis agents such as rifampin and ethambutol, depending upon the species identified (5, 18). However, the cure rates obtained are variable, especially in pulmonary infections due to species such as *M. avium* complex, *M. abscessus*, and *M. simiae*. This is in part explained by the natural resistance of these latter mycobacterial species to most currently available drugs. Linezolid has shown in vitro activity against many species of *Mycobacterium*, and successful therapy has been observed in patients with tuberculosis (including those infected with drug-resistant isolates) and *M. chelonae* and *M. avium* infections (2, 6, 8). The principal disadvantage of the chronic use of linezolid has been the appearance of side effects such as bone marrow suppression and peripheral neuropathy.

Many chemical derivatives of the basic oxazolidinone ring have been synthesized and analyzed for their antimicrobial activity. Among them, two compounds, DA-7867 and DA-7157, have exhibited activity against many gram-positive bacteria, including methicillin-resistant *S. aureus*, *Enterococcus*, and *Streptococcus pneumoniae* isolates (K. Lee, J. H. Yum, D. Yong, Y. Chong, S. H. Choi, and J. K. Rhee, Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1419, 2005). These new agents also have activity against susceptible and drug-resistant isolates of *M. tuberculosis*, showing good activity against both (15). Although breakpoints for these new oxazolidinones have not been established, in the present study we considered all of the isolates tested to be susceptible to DA-7867 and DA-7157, since all MICs were less than the breakpoint established for linezolid by the Clinical and Laboratory Standards Institute. However, it will be important to determine the pharmacokinetics of these oxazolidinones in humans in order to establish breakpoints and be able to better interpret in vitro susceptibility tests.

To date, only a few new drugs have been observed to be

active against *Mycobacterium* spp., particularly *M. tuberculosis*. These include new quinolones, a recently described ATPase inhibitor (R207910), and ethambutol derivatives such as SQ109 (1, 10). However, the main obstacles to their use in human infections are their toxicity and poor solubility in water. DA-7157 is produced by the metabolism of a prodrug named DA-7218 {(R)-[3-(4-(2-(2-methyltetrazol-5-yl)pyridin-5-yl)-3-fluorophenyl)-2-oxo-5-oxazolidinyl]methyl disodium phosphate}, which is highly hydrophilic and does not require the use of dimethyl sulfoxide or hydrophobic substances to be dissolved. In preliminary assays, we have observed that DA-7218 shows greater in vivo activity than linezolid in an experimental model of *Nocardia brasiliensis* infection of BALB/c mice (N. A. Espinoza-Gonzalez, O. Welsh, G. Lozano, S. Said-Fernandez, J. Ocampo-Candiani, J. Castro-Garza, and L. Vera-Cabrera, unpublished data). Although studies of the in vivo activity of DA-7218 (DA-7157) against *Mycobacterium* spp. are still pending, the in vitro results shown in this paper are promising.

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