In Vitro Activities of DA-7157 and DA-7218 against *Mycobacterium tuberculosis* and *Nocardia brasiliensis*

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The in vitro activities of DA-7157, a novel oxazolidinone, against clinical isolates of *Nocardia brasiliensis* and *Mycobacterium tuberculosis* were determined. Equal MIC₅₀s and MIC₉₀s (0.25 and 0.5 µg/ml, respectively) were found for susceptible and multidrug-resistant isolates of *M. tuberculosis*. The *N. brasiliensis* isolates showed an MIC₉₀ of 1 µg/ml and an MIC₅₀ of 1 µg/ml. The DA-7157 prodrug, DA-7218, exhibited similar MICs for *M. tuberculosis* but fivefold-higher MICs for *N. brasiliensis*.

Oxazolidinones are a new, unique class of synthetic antibacterial agents effective against many gram-positive bacteria, including aerobic pathogenic actinomycetes of the genera *Mycobacterium*, *Nocardia*, and *Actinomyces* (3, 7, 8, 9). Linezolid, the first oxazolidinone on the market, has been proven to be active in animal models (2), as well as in clinical trials with patients infected with *Nocardia* spp. (5) or *Mycobacterium tuberculosis* (1). Oxazolidinones selectively inhibit bacterial protein synthesis by binding to bacterial 23S rRNA of the 50S subunit and preventing the formation of a functional 70S initiation complex (6). The antibacterial activity of oxazolidinones depends on the affinity for the site of action on the ribosome; therefore, by modifying their chemical structure, new compounds with improved antimicrobial activity can be obtained.

Recently, a new oxazolidinone, DA-7157, was shown to be active in vitro against several gram-positive species, with the observation of a level of activity superior to that of linezolid (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). The DA-7157 compound is produced by the metabolism of a highly hydrophilic prodrug, DA-7218 (Fig. 1). In the present work, we report the in vitro susceptibilities of clinical isolates of *M. tuberculosis*, including isolates of drug-resistant *M. tuberculosis*, and isolates of *Nocardia brasiliensis* obtained from patients suffering from mycetoma to both DA-7157 and its prodrug.

Both oxazolidinones DA-7157 and DA-7218 were obtained from Dong-A Pharmaceutical Company, Ltd., Yongin, Korea. Stock solutions of DA-7157 were prepared in 100% dimethyl sulfoxide and 7H9GC broth (4.7 g of Middlebrook 7H9 broth base [Difco, Detroit, Mich.], 20 ml of 10% [vol/vol] glycerol, 1 g of Bacto Casitone [Difco], 880 ml of distilled water, 100 ml of oleic acid-albumindextrose-catalase [Becton Dickinson, Maryland] [8]). The stock solution of DA-7218 was prepared in distilled water. In order to compare our results, we also per-

formed susceptibility assays with linezolid, a drug previously tested in vitro against *M. tuberculosis* with good results. The latter was obtained from Pharmacia and Upjohn (Kalamazoo, Mich.), and the stock was prepared in 7H9GC broth. All working solutions were diluted in 7H9GC broth to a 2× concentration prior to their addition to the microplates. Dimethyl sulfoxide controls were also run to determine its possible toxicity on the microorganisms.

A total of 95 *M. tuberculosis* isolates were tested, including 8 isolates resistant to isoniazid, 1 isolate resistant to rifampin, and 25 clinical isolates resistant to both isoniazid and rifampin. The susceptibility assays were performed by the proportion method. For controls, we ran *M. tuberculosis* H37Rv and H37Ra. In order to determine the susceptibilities to linezolid, DA-7157, and DA-7218, the broth microdilution method with Alamar Blue was utilized (8). In brief, mycobacterial suspensions were prepared in 0.04% (vol/vol) Tween 80–0.2% bovine serum albumin so their turbidities equaled a McFarland turbidity standard of 1. Suspensions were further diluted 1:25 in 7H9GC broth. The rest of the technique was performed as published before (8). The MIC was defined as the lowest drug concentration which prevented a color change of blue to pink. *M. tuberculosis* H37Rv was run as a susceptible-strain control.

For the *N. brasiliensis* isolates ($n = 31$), we utilized the broth microdilution method previously described (9). Briefly, we prepared a cellular suspension from fresh colonies grown on Sabouraud agar (7 days old) in 1 ml of saline solution and diluted it with cation-adjusted Mueller-Hinton broth until the turbidity equaled a 0.5 McFarland standard. The suspension was diluted with Mueller-Hinton broth to obtain a final concentration of 1×10^4 to 5×10^4 CFU per well in 0.1 ml and then added to microplate wells (Microtest Primaria; Becton Dickinson and Co., Franklin Lakes, NJ) containing an equal volume of broth (0.1 ml) with serial dilutions of the drugs tested. After 3 days of incubation at 35°C, the plates were read and the MIC (the lowest concentration of drug totally inhibiting nocardial growth) was determined. For external controls, we utilized *Escherichia coli* ATCC 25922 and *Staphy-*

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FIG. 1. Chemical structures of the oxazolidinones linezolid, DA-7867, DA-7157, and DA-7218.

lococcus aureus ATCC 29213. All the antimicrobials were tested at concentrations of 64 to 0.015 $\mu\text{g/ml}$.

The range of MICs, the MIC₅₀s, and the MIC₉₀s for the *M. tuberculosis* isolates are shown in Table 1. All the isolates tested were inhibited at ≤ 0.5 $\mu\text{g/ml}$, including the drug-resistant isolates. The ranges, MIC₅₀s, and MIC₉₀s for the multi-drug-resistant strains were exactly the same. Comparatively, DA-7157 was slightly more active than linezolid (the MIC₅₀ and MIC₉₀ were 1 and 2 $\mu\text{g/ml}$, respectively) (8) and much less active than DA-7867 (the MIC₅₀ and MIC₉₀ were 0.0625 and 0.125 $\mu\text{g/ml}$, respectively) (8). The prodrug of DA-7157, DA-7218, is theoretically inactive in vitro; in order to corroborate this, we tested 10 susceptible and 10 drug-resistant *M. tuberculosis* isolates. We observed similar MICs for all isolates, with an MIC₉₀ and MIC₅₀ of 0.5 $\mu\text{g/ml}$.

In Table 2, the susceptibilities of the *N. brasiliensis* isolates to DA-7157 are compared with those of the isolates to linezolid. The MIC₅₀ and MIC₉₀ were both 1 $\mu\text{g/ml}$, very similar to the values obtained for linezolid, although much higher than those reported for DA-7867 (9). When testing the prodrug, DA-7218, we observed fivefold-higher MICs.

Mycetoma is a chronic infectious disease caused mainly by *N. brasiliensis* in Mexico (4). Therapy is given for several months, and the best schemes have a cure rate ranging from 70%, with trimethoprim-sulfamethoxazole (SXT), to 95%, with SXT-amikacin (10), and although several drugs have been used in isolated cases, there are not many alternatives for the cases of drug resistance and cases in which side effects to these drugs develop. Linezolid has been used in a

few cases of nocardial infections, and good results have been obtained (5), which opens the possibility for new oxazolidinones to be used. In a previous work, we observed the exquisite susceptibility of *N. brasiliensis* to a new oxazolidinone, DA-7867; however, the in vivo assays are complicated because of the drug's poor solubility in water. As we showed, the new compound tested, DA-7157, is not as potent in vitro as DA-7867; however, its prodrug is highly soluble in water, and that might facilitate its deposition in tissues. In initial in vivo assays with DA-7218, using an experimental model of infection with *N. brasiliensis* in BALB/c mice, we observed that this drug is more active than linezolid (N. A. Espinoza-Gonzalez, O. Welsh, G. Lozano, S. Said-Fernandez, J. Ocampo-Candiani, J. Castro-Garza, and L. Vera-Cabrera, unpublished data). These results make DA-7157 an excellent candidate to be used in human cases, perhaps at lower doses than linezolid. However, this drug is experimental, and its toxicological properties and pharmacokinetics in humans are yet to be determined.

Many chemical derivatives of the common structure of oxazolidinones have been assayed in vitro, with various results being obtained. Structurally, DA-7157 and DA-7218 are identical, with the exception of a Na₂PO₄ group instead of an OH in the oxazolidinone ring (Fig. 1), which makes DA-7218 more hydrophilic. In the present work, we also observed that *M. tuberculosis* isolates, even the drug-resistant ones, are susceptible to low concentrations of DA-7157. Although there is no cutoff point to define susceptibility or resistance for these oxazolidinones in the case of *M. tuberculosis*, since the MICs are lower than those of linezolid and this drug has been proven to be active clinically, we consider them of potential clinical use. As

TABLE 1. In vitro susceptibilities of 95 clinical isolates of *M. tuberculosis* to DA-7157

Type of <i>M. tuberculosis</i> isolate (n)	MIC of DA-7157 ($\mu\text{g/ml}$)		
	Range	50%	90%
Susceptible to isoniazid and rifampin (61)	0.125–0.5	0.25	0.5
Resistant to isoniazid or rifampin (9)	0.125–0.5	0.25	0.25
Resistant to both (25)	0.125–0.5	0.25	0.5
Total (95)	0.125–0.5	0.25	0.5

TABLE 2. Comparison of MICs of several oxazolidinones for isolates of *N. brasiliensis* (n = 31)

Drug	MIC for <i>N. brasiliensis</i> ($\mu\text{g/ml}$)		
	Range	50%	90%
Linezolid	0.12–2	0.5	1
DA-7157	0.5–2	1	1
DA-7218	8–>32	16	>32

we mentioned above, the advantage of DA-7157 is the high solubility of the prodrug DA-7182, permitting a higher availability and perfusion in the tissues and eliminating the infection, as we have seen with experimental infections with *N. brasiliensis*, better than linezolid, although the in vivo effect in tuberculosis is still to be determined.

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