In Vitro and In Vivo Activities of DA-7867, a New Oxazolidinone, against Aerobic Gram-Positive Bacteria

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In vitro and in vivo activities of DA-7867 were assessed against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and penicillin-resistant *Streptococcus pneumoniae*. All isolates were inhibited by DA-7867 at ≤ 0.78 $\mu\text{g/ml}$, a four-times-lower concentration than that of inhibition by linezolid. For murine infection models, DA-7867 also exhibited greater efficacy than linezolid against all isolates tested.

Infections due to gram-positive cocci resistant to multiple antimicrobial agents have become a problem in recent years (4, 5, 11, 16). Since Dup-721 was introduced (7, 13) as the first developed oxazolidinone, linezolid has been approved for the treatment of infections caused by susceptible strains including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (3, 10). The success of linezolid and resistance to linezolid reported in clinical isolates of *Enterococcus faecium* (8, 9) and *S. aureus* (15) have inspired further efforts towards expanded-spectrum molecules with improved potency and spectrum.

DA-7867, (S)-[N-3-(4-[2-(1-methyl-5-tetrazolyl)-pyridin-5-yl]-3-fluorophenyl)-2-oxo-5-oxazolidinyl] methyl acetamide, is a new oxazolidinone derivative containing a 1-methyl-tetrazolyl-substituted pyridine ring. Preliminary reports indicate that this agent would be more potent than linezolid against aerobic and anaerobic gram-positive bacteria, including multi-drug-resistant bacteria (17), and the pharmacokinetic characteristics in rats seem to be superior to those of linezolid (2). In this study, we compared the activities of DA-7867 and linezolid against aerobic gram-positive bacteria both in vitro, using MIC and time-kill studies, and in vivo, using murine infection models.

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DA-7867 and linezolid were synthesized at the Research Laboratories, Dong-A Pharm. Co., Yongin, Korea. Reference antibiotics were obtained from Sigma-Aldrich, Inc. (St. Louis, Mo.). Clinical isolates were obtained from hospitals in Seoul, Korea, between 1998 and 2002. MICs were determined by the agar dilution method according to the National Committee for Clinical Laboratory Standards (12). Inhibition of protein synthesis was evaluated in a coupled transcription-translation assay that uses pBEST coding for luciferase and an *Escherichia*

coli S30 system (Promega, Madison, Wis.) according to methods provided by the manufacturer. The concentrations at which luciferase synthesis was inhibited by 50% were determined by plotting concentration versus percent inhibition of luciferase synthesis.

Time-kill analyses were done as follows: DA-7867 and linezolid stock solution were added to logarithmic-phase broth cultures of MRSA, VRE, and penicillin-resistant *Streptococcus pneumoniae* (PRSP) (approximately 10^6 to 10^7 CFU/ml) to give concentrations equivalent to 1, 2, and $4\times$ MIC. Viable

TABLE 1. In vitro antibacterial activities of DA-7867 and linezolid against aerobic gram-positive clinical isolates

Organism (no. of isolates) and antimicrobial agent	MIC ($\mu\text{g/ml}$)		
	Range	MIC ₅₀	MIC ₉₀
Methicillin-susceptible <i>Staphylococcus aureus</i> (37)			
DA-7867	0.1–0.39	0.2	0.2
Linezolid	0.78–3.13	1.56	3.13
Methicillin-resistant <i>S. aureus</i> (35)			
DA-7867	0.2–0.78	0.39	0.78
Linezolid	3.13–3.13	3.13	3.13
Vancomycin-susceptible <i>Enterococcus faecalis</i> (36)			
DA-7867	0.2–0.2	0.2	0.2
Linezolid	1.56–1.56	1.56	1.56
Vancomycin-susceptible <i>E. faecium</i> (34)			
DA-7867	0.2–0.39	0.2	0.2
Linezolid	1.56–3.13	1.56	1.56
Vancomycin-resistant <i>E. faecium</i> (36)			
DA-7867	0.05–0.39	0.2	0.2
Linezolid	1.56–3.13	1.56	1.56
Penicillin-resistant <i>Streptococcus pneumoniae</i> (33)			
DA-7867	0.05–0.39	0.2	0.39
Linezolid	0.39–6.25	1.56	1.56
<i>Enterococcus</i> spp. ^a (8)			
DA-7867	0.12–2	0.5	2
Linezolid	4–128	16	128

^a The mutants resistant to linezolid have been selected in laboratory experiments.

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TABLE 2. Time-kill study for DA-7867 and linezolid against MRSA, VRE, and PRSP after 24-h exposure to four times the MIC

Organism	MIC ($\mu\text{g/ml}$)		Log ₁₀ CFU/ml change at 4× MIC		
	DA-7867	Linezolid	DA-7867	Linezolid	Control
Methicillin-resistant					
<i>S. aureus</i> M126	0.39	3.13	-1.73	-1.44	2.34
<i>S. aureus</i> R12	0.39	3.13	-1.45	-1.03	2.27
<i>S. aureus</i> R782	0.39	3.13	-1.88	-1.60	2.37
Vancomycin-resistant					
<i>E. faecalis</i> ^b P442	0.2	1.56	-0.21	0.21	2.19
<i>E. faecium</i> U1211	0.2	1.56	-2.58	-1.76	1.23
<i>E. faecium</i> C327	0.2	1.56	-0.79	0.13	1.49
Penicillin-resistant					
<i>S. pneumoniae</i> R456	0.2	1.56	-3.96 ^a	-3.12 ^a	1.37 ^a
<i>S. pneumoniae</i> R748	0.2	1.56	-2.02 ^a	-1.32 ^a	1.24 ^a
<i>S. pneumoniae</i> 3588	0.2	1.56	-1.87 ^a	-0.78 ^a	0.89 ^a

^a The ΔLog_{10} CFU/ml values reported for PRSP were measured after 7 h of exposure to four times the MIC. Data were reported as the means from two experiments.

^b *E. faecalis*, *Enterococcus faecalis*.

bacterial counts were determined at 0, 1, 3, 5, 7, and 24 h after addition of the antimicrobial agents. The bactericidal activity is defined as a 3-log₁₀ decrease in CFU/ml.

In vivo efficacy of DA-7867 was determined for three murine models. For the systemic (6) and respiratory tract (1) infection models, ICR male mice (3 to 4 weeks old; BioGenomics, Korea) were infected intraperitoneally and intranasally, respectively, with each challenge strain suspended in 5% mucin (MP

TABLE 3. In vivo protective efficacy (ED₅₀s) and MICs of DA-7867 and linezolid against aerobic gram-positive clinical isolates

Animal model and organism (CFU/mouse)	Compound ^a	MIC ($\mu\text{g/ml}$)	ED ₅₀ (mg/kg) ^b
Systemic infection	DA-7867	0.39	3.4 (2.1–5.7)
		Linezolid	8.0 (4.8–13.0)
	DA-7867	0.39	2.6 (1.9–3.6)
		Linezolid	8.3 (6.1–11.2)
	DA-7867	0.39	1.4 (0.9–2.2)
		Linezolid	3.6 (1.6–5.4)
	DA-7867	0.39	11.6 (8.3–16.2)
		Linezolid	66.0 (46.9–95.6)
	DA-7867	0.2	2.4 (1.2–4.4)
		Linezolid	5.9 (3.5–10.1)
	DA-7867	0.2	4.5 (1.5–9.9)
		Linezolid	28.4 (16.1–58.8)
Respiratory infection	DA-7867	0.2	4.9 (3.2–7.7)
		Linezolid	22.7 (7.2–85.4)
	DA-7867	0.2	8.9 (5.9–13.5)
		Linezolid	46.8 (30.9–71.4)

^a Each compound was administered 1 h after systemic infection and 2 h and 6 h after respiratory infection.

^b Numbers in parentheses are 95% confidence ranges.

^c Methicillin-resistant strain.

^d Penicillin-resistant strain.

^e Vancomycin-resistant strain.

^f Infections with these strains were performed in neutropenic mice. Thus, cyclophosphamide, 200 mg/kg, was administered intraperitoneally to mice 4 days and 1 day before infection.

FIG. 1. Survival of mice infected with penicillin-resistant *S. pneumoniae* 3588 and given no treatment (×) or treated with 60 mg/kg (●), 20 mg/kg (■), 6.6 mg/kg (◆), and 2.2 mg/kg (▼) of DA-7867 or 60 mg/kg (○), 20 mg/kg (□), and 6.6 mg/kg (◇) of linezolid in a respiratory infection model. Groups of eight mice were used for each dosage.

Biomedicals, Irvine, Calif.), which contained sufficient bacteria to kill 100% of the untreated control mice. DA-7867 and linezolid were administered orally 1 h after systemic infection and 2 h and 6 h after respiratory infection. There were four or five dosage groups per infection organism, and each group comprised eight animals. Mortality was observed for 10 days. The 50% effective dose (ED₅₀), the dose required to protect 50% of the infected mice from death, and 95% confidence limit determinations were determined by Probit analysis within each test. The efficacy in soft-tissue infection was examined by a modification of a previously described method (14). Pouches were generated by injecting 3 ml of sterile air subcutaneously into the back of ICR male mice. After 24 h, the challenge strain, MRSA M126 or *E. faecium* U1211, in 5% mucin was injected into the air pouches. Immediately after bacterial infection, DA-7867 was administered at doses of 1, 3, and 10 mg/kg of body weight and linezolid was given at doses of 3, 10, and 30 mg/kg. There were six to eight mice/group. Twenty-four hours later 5 ml of saline was injected into the pouch. The viable bacterial count reductions in the pouch fluid were determined and compared to control by *t* tests. A *P* value of <0.05 was considered to be significant.

The in vitro antibacterial activity of DA-7867 against gram-positive clinical isolates is presented in Table 1. It inhibited the growth of all *S. aureus* strains at 0.78 $\mu\text{g/ml}$, and the MIC₉₀ (MIC at which 90% of the isolates tested were inhibited) for MRSA was fourfold lower than that of linezolid. All enterococci, including VRE, were inhibited at 0.39 $\mu\text{g/ml}$ by DA-7867. Its MIC₉₀ for VRE was 16-fold lower than that of linezolid. For PRSP, the MIC range of DA-7867 was 0.05 to 0.39 $\mu\text{g/ml}$ and the MIC₉₀ was four times lower than that of linezolid. DA-7867 also showed strong activity against several linezolid-resistant mutants selected in laboratory experiments. Moreover, DA-7867 presented comparatively higher inhibition activity of protein synthesis than linezolid did. The 50% inhib-

TABLE 4. In vivo efficacy of DA-7867 against MRSA M126 and VRE U1211 in the murine air pouch model

Organism (CFU/mouse)	Compound ^a	Dose (mg/kg)	No. of animals	Log ₁₀ CFU/ml ± SD	ΔLog ₁₀ CFU/ml
MRSA M126 ^b (1.0×10^5)	Control		8	6.80 ± 0.63	
	DA-7867	1	6	5.90 ± 1.05	-0.90
		3	8	2.84 ± 0.44	-3.96 ^d
		10	8	2.80 ± 0.46	-4.00 ^d
	Linezolid	3	8	6.39 ± 0.41	-0.41
		10	8	4.74 ± 0.17	-2.06 ^d
		30	8	3.46 ± 0.82	-3.34 ^d
	Vancomycin	3	6	6.03 ± 1.18	-0.77
		10	7	5.13 ± 0.34	-1.67 ^d
		30	7	5.04 ± 0.11	-1.76 ^d
<i>E. faecium</i> U1211 ^c (9.0×10^4)	Control		8	9.30 ± 0.34	
	DA-7867	1	7	7.76 ± 1.41	-1.53
		3	7	6.42 ± 1.03	-2.88 ^d
		10	7	3.87 ± 0.91	-5.43 ^d
	Linezolid	3	7	8.38 ± 0.31	-0.92
		10	7	6.58 ± 1.00	-2.72 ^d
		30	7	4.70 ± 1.36	-4.60 ^d

^a DA-7867 and linezolid were administered by the oral route, and vancomycin was administered by subcutaneous injection immediately after bacterial infection.

^b Methicillin-resistant strain.

^c Vancomycin-resistant strain.

^d The *P* value for a comparison with the control group was <0.001.

itory concentrations of DA-7867 and linezolid were 0.157 μM and 1.270 μM, respectively. This considerable discrepancy between 50% inhibitory concentrations corresponded to DA-7867 having much more potent antibacterial activity than that of linezolid. Time-kill studies showed that both DA-7867 and linezolid had bacteriostatic activity against MRSA and VRE, but they exhibited bactericidal activity at four times the MIC against one of the three PRSP strains (Table 2).

In the mouse systemic infection model, DA-7867 showed good in vivo protective efficacy against gram-positive pathogens such as *S. aureus*, including MRSA; *S. pneumoniae*, including PRSP; and VRE. Its ED₅₀s were in the range of 1.4 to 11.6 mg/kg, and efficacy was two to six times better than that of linezolid (Table 3). For the respiratory tract infection, DA-7867 was found to have efficacy against PRSP five times more potent than that of linezolid (Table 3; Fig. 1). DA-7867 was shown to be more effective in reducing the CFU against MRSA and VRE than linezolid in the pouch model (Table 4). The doses required for 2-log killing in the pouch fluid were 1.5 mg/kg for DA-7867 and 10.1 and 6.0 mg/kg for linezolid. These ED₅₀ results for DA-7867 were correlated with the in vitro MICs.

Conclusions. Based on these potent in vitro and in vivo activities against gram-positive bacteria such as MRSA, VRE, and PRSP, DA-7867 should be considered as a possible therapeutic agent for the treatment of infections by gram-positive organisms resistant to multiple antimicrobial agents. Our findings need to be confirmed in further preclinical and clinical studies.

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REFERENCES

1. Azoulay-Dupuis, E., and P. Moine. 1999. Mouse pneumococcal pneumonia models, p. 481–493. In O. Zak and M. Sande (ed.), *Handbook of animal models of infection*. Academic Press, Inc., San Diego, Calif.
2. Bae, S. K., W. Chung, E. J. Kim, J. K. Rhee, J. W. Kwon, W. B. Kim, and M. G. Lee. 2004. Pharmacokinetics of DA-7867, a new oxazolidinone, after intravenous or oral administration to rats: intestinal first-pass effect. *Antimicrob. Agents Chemother.* **48**:659–662.
3. Barrett, J. F. 2000. Linezolid Pharmacia Corp. *Curr. Opin. Investig. Drugs* **1**:181–187.
4. Biedenbach, D. J., G. J. Moet, and R. N. Jones. 2004. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997–2002). *Diagn. Microbiol. Infect. Dis.* **50**:59–69.
5. Clark, N. M., E. Hershberger, M. J. Zervos, and J. P. Lynch III. 2003. Antimicrobial resistance among gram-positive organisms in the intensive care unit. *Curr. Opin. Crit. Care* **9**:403–412.
6. Cleeland, R., and E. Squires. 1991. Evaluation of new antimicrobials in vitro and in experimental animal infections, p. 747–762. In V. Lorian (ed.), *Antibiotics in laboratory medicine*, Williams & Wilkins, Baltimore, Md.
7. Daly, J. S., G. M. Eliopoulos, E. Reizner, and R. C. Moellering, Jr. 1988. Activity and mechanism of action of DuP 105 and DuP 721, new oxazolidinone compounds. *J. Antimicrob. Chemother.* **21**:721–730.
8. Gonzales, R. D., P. C. Schreckenberger, M. B. Graham, S. Kelkar, K. DenBesten, and J. P. Quinn. 2001. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. *Lancet* **357**:1179.
9. Herrero, I. A., N. C. Issa, and R. Patel. 2002. Nosocomial spread of linezolid-resistant, vancomycin-resistant *Enterococcus faecium*. *N. Engl. J. Med.* **346**:867–869.
10. Hilliard, J. 2002. Linezolid (Zyvox). *Prim. Care Update Ob/Gyns.* **9**:178–180.
11. Livermore, D. M. 2003. Linezolid in vitro: mechanism and antibacterial spectrum. *J. Antimicrob. Chemother.* **51**:9–16.
12. National Committee for Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
13. Neu, H. C., A. Novelli, G. Saha, and N. X. Chin. 1988. In vitro activities of two oxazolidinone antimicrobial agents, DuP 721 and DuP 105. *Antimicrob. Agents Chemother.* **32**:580–583.
14. Tsao, N., T. Luh, C. Chou, J. Wu, Y. Lin, and H. Lei. 2001. Inhibition of group A streptococcus infection by carboxyfullerene. *Antimicrob. Agents Chemother.* **45**:1788–1793.
15. Tsiodras, S., H. S. Gold, G. Sakoulas, G. M. Eliopoulos, C. Wennersten, L. Venkataraman, R. C. Moellering, and M. J. Ferraro. 2001. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. *Lancet* **358**:207–208.
16. Wisplinghoff, H., T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond. 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin. Infect. Dis.* **39**:309–317.
17. Yong, D., J. H. Yum, K. Lee, Y. Chong, S. H. Choi, and J. K. Rhee. 2004. In vitro activities of DA-7867, a novel oxazolidinone, against recent clinical isolates of aerobic and anaerobic bacteria. *Antimicrob. Agents Chemother.* **48**:352–357.