ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1999, p. 2059–2062 Vol. 43, No. 8
0066-4804/99/$04.00+0

In Vitro Activities of Linezolid against Important Gram-Positive Bacterial Pathogens Including Vancomycin-Resistant Enterococci

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Received 21 December 1998/Returned for modification 29 April 1999/Accepted 18 May 1999

The emergence of resistance in gram-positive bacteria has necessitated a search for new antimicrobial agents. Linezolid is an oxazolidinone, a new class of antibacterial agents with enhanced activity against pathogens. We compared the activity of linezolid to those of other antimicrobial agents against 3,945 clinical isolates. Linezolid demonstrated potent activity against all isolates tested. For all vancomycin-susceptible enterococci, staphylococci, and streptococci, the activity of linezolid was comparable to that of vancomycin. Against oxacillin-resistant staphylococci and vancomycin-resistant enterococci, linezolid was the most active agent tested. In summary, linezolid appears to be a promising new antimicrobial agent for the treatment of gram-positive infections.

In recent years, there has been a dramatic increase in the number of infections caused by gram-positive bacteria (13). This is compounded by the emergence of resistance in enterococci, staphylococci, and pneumococci. For many patients infected with these resistant organisms, there may not be effective antimicrobial therapy.

Linezolid is a member of a new class of antibacterial agents called oxazolidinones, which are chemically unrelated to currently available agents. This agent selectively binds to the 50S ribosomal subunit, thereby resulting in selective inhibition of bacterial protein synthesis (7). These compounds inhibit the formation of the initiation complex constructed with 30S ribosomes, mRNA, initiation factors IF2 and IF3, and fMet-tRNA (3, 4, 7, 14). In addition, linezolid is bioavailable both orally and parenterally, is highly active against gram-positive organisms, and is difficult to select for resistance in vitro (6, 15). In this study, we investigated the in vitro activity of linezolid against fresh clinical isolates of streptococci, enterococci, and staphylococci.

(This study was presented in part at the 36th Annual Meeting of the Infectious Diseases Society of America, Denver, Colo., 12 to 15 November 1998.)

All clinical isolates of gram-positive cocci submitted to the clinical microbiology laboratory at Northwestern Memorial Hospital (Chicago, Ill.) for bacterial susceptibility testing from 1 April 1997 to 4 March 1998 were tested against linezolid.

The following antimicrobial agents were obtained from their manufacturers for use in this investigation: linezolid (Pharmacia & Upjohn, Kalamazoo, Mich.), vancomycin (Eli Lilly & Co., Indianapolis, Ind.), teicoplanin (Marion Merrell Dow, Cincinnati, Ohio), ampicillin-sulbactam (Pfizer Inc., New York, N.Y.), piperacillin and piperacillin-tazobactam (Wyeth-Ayerst, Philadelphia, Pa.), levofloxacin (Ortho-McNeil Pharmaceuticals, Raritan, N.J.), imipenem (Merck Inc., Wilmington, Del.), and trovafloxacin (Pfizer, Inc.). Ampicillin, chloramphenicol, clindamycin, erythromycin, oxacillin, and penicillin were obtained from the Sigma Chemical Co. (St. Louis, Mo.). Agar dilution testing was performed according to the guidelines established by the National Committee for Clinical Laboratory Standards (8). Using a Steers replicator, an organism density of 10^4 CFU/spot was inoculated onto Mueller-Hinton plates (Difco) with various concentrations of antimicrobial agents. For *Streptococcus pneumoniae*, susceptibility testing was performed by using in-house prepared microtiter panels. Isolates were grown in tryptic soy broth (Difco) to reach log-phase growth and were then diluted in sterile tryptic soy broth to achieve a final inoculum density in the microtiter wells of approximately 5×10^5 CFU/ml. The microtiter trays contain an enrichment medium consisting of Mueller-Hinton broth supplemented with 3 to 5% lysed horse blood.

Linezolid was tested at concentrations of 0.5, 1, 2, 4, 8, and 16 μg/ml, and teicoplanin was tested at a concentration of 10 μg/ml. The other antimicrobial agents were tested at concentrations determined by National Committee for Clinical Laboratory Standards guidelines. Plates were incubated at 35°C for 18 to 24 h and examined for visible growth. The MIC was defined as the lowest dilution at which growth of ≤1 colony occurred or at which only a faint haze caused by the inoculum occurred. Along with the clinical isolates, the reference quality control strains *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were tested on a daily basis.

The MICs of linezolid and the comparison antimicrobial agents for 3,945 bacterial clinical isolates of streptococci, enterococci, and staphylococci are summarized in Table 1. Linezolid demonstrated in vitro activity against all isolates tested. The activity of linezolid was comparable to that of vancomycin for all vancomycin-susceptible bacterial groups. Among enterococci, linezolid had an activity similar to those of vancomycin and the β-lactam agents (ampicillin, imipenem, and piperacillin) against *E. faecalis*. For *Enterococcus faecium*, lin-

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TABLE 1. Activity of linezolid and other antimicrobial agents against gram-positive bacteria

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
S. pneumoniae (79)	Penicillin	0.06–2	0.06	0.5
	Ampicillin	0.125–8	0.125	2
	Ampicillin-sulbactam	0.125–8	0.125	2
	Imipenem	0.125–1	0.125	1
	Piperacillin	8–32	8	8
	Piperacillin-tazobactam	8–32	8	8
	Chloramphenicol	2–16	2	8
	Clindamycin	0.125–>8	0.25	1
	Erythromycin	0.25–16	0.25	4
	Linezolid	0.25–2	1	1
	Levofloxacin	1–2	2	2
	Trovafloxacin	0.06–4	0.25	0.25
	Vancomycin	0.25–2	1	1
E. faecalis (1,137)	Penicillin	2–>8	2	8
	Ampicillin-sulbactam	0.25–8	2	2
	Imipenem	1–>8	4	8
	Piperacillin	8–128	8	16
	Piperacillin-tazobactam	8–128	8	16
	Chloramphenicol	8–>16	8	16
	Linezolid	1–4	2	4
	Levofloxacin	1–>4	2	>4
	Trovafloxacin	0.5–>4	0.5	>4
	Vancomycin	2–>64	2	8
	Teicoplanin	10–>10	10	10
E. faecium (452)	Penicillin	2–>8	>8	>8
	Ampicillin	0.25–>128	128	>128
	Ampicillin-sulbactam	0.25–>128	128	>128
	Imipenem	1–>8	>8	>8
	Piperacillin	8–128	128	128
	Piperacillin-tazobactam	8–128	128	128
	Chloramphenicol	8–>16	8	>16
	Linezolid	0.5–4	2	4
	Levofloxacin	1–>4	>4	>4
	Trovafloxacin	0.5–>4	>4	>4
	Vancomycin	2–>64	>64	>64
	Teicoplanin	10–>10	>10	>10
S. aureus				
Oxacillin-susceptible (1,020)	Penicillin	0.125–>8	2	8
	Ampicillin-sulbactam	0.25–16	2	2
	Imipenem	1–8	1	1
	Oxacillin	0.5–8	0.5	1
	Piperacillin-tazobactam	8–64	8	16
	Chloramphenicol	8–16	8	16
	Clindamycin	1–>4	1	1
	Erythromycin	0.5–>4	0.5	>4
	Linezolid	1–4	2	4
	Levofloxacin	0.5–>4	1	1
	Trovafloxacin	0.5–>4	0.5	0.5
	Vancomycin	2	2	2
Oxacillin-resistant (451)	Penicillin	>8	>8	>8
	Ampicillin-sulbactam	2–128	8	16
	Imipenem	1–>8	8	8
	Oxacillin	>8	>8	>8
	Piperacillin-tazobactam	8–128	128	128
	Chloramphenicol	8–>16	8	>16
	Clindamycin	2–>8	>8	>8
	Erythromycin	0.5–>4	>4	>4
	Linezolid	0.5–4	2	4
	Levofloxacin	1–>4	>4	>4
	Trovafloxacin	0.5–>4	2	>4
	Vancomycin	2	2	2
S. epidermidis				
Oxacillin-susceptible (365)	Penicillin	0.06–>8	2	>8
	Ampicillin-sulbactam	0.25–8	0.25	2
	Imipenem	1–>8	1	1

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TABLE 1—*Continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
	Oxacillin	0.5–>8	0.5	2
	Piperacillin-tazobactam	8–16	8	8
	Chloramphenicol	8–>16	8	16
	Clindamycin	1–>8	1	>8
	Erythromycin	0.5–>4	>4	>4
	Linezolid	0.5–4	2	4
	Levofloxacin	1–>4	1	>4
	Trovafloxacin	0.5–>4	0.5	4
	Vancomycin	2	2	2
Oxacillin resistant (441)	Penicillin	0.06–>8	>8	>8
	Ampicillin-sulbactam	0.25–128	2	8
	Imipenem	1–>8	8	>8
	Oxacillin	>8	>8	>8
	Piperacillin-tazobactam	8–>64	8	8
	Chloramphenicol	8–>16	8	16
	Clindamycin	1–>8	>8	>8
	Erythromycin	0.5–>4	>4	>4
	Linezolid	0.5–4	2	4
	Levofloxacin	1–>4	>4	>4
	Trovafloxacin	0.5–>4	4	>4
	Vancomycin	2	2	2

[a] 50% and 90%, MICs at which 50 and 90% of the isolates are inhibited, respectively.

ezolid was the most active agent tested, although two-thirds of these isolates were vancomycin resistant. Against *Enterococcus avium* and *Enterococcus durans*, the activities of linezolid and vancomycin were comparable (data not shown). In addition, vancomycin-resistant enterococci (VRE) of either the VanA or VanB phenotype were inhibited by linezolid at a MIC of 2 to 4 μg/ml. Both vancomycin and linezolid demonstrated activity against staphylococci. Against oxacillin-susceptible *S. aureus*, there were many agents with in vitro activity, including the new fluoroquinolones levofloxacin and trovafloxacin as well as clindamycin and the antistaphylococcal β-lactams. For oxacillin-resistant *S. aureus*, the potency of linezolid was comparable to that of vancomycin. Against the coagulase-negative staphylococci (both oxacillin susceptible and oxacillin resistant), the activity of linezolid was within one twofold dilution of vancomycin for all of the species tested.

Against streptococci, there were many active agents and the activity of linezolid was comparable or superior to that of vancomycin. There was no difference in activity between linezolid and vancomycin against pneumococci, including penicillin-resistant strains. We did not test linezolid against gram-negative bacteria or anaerobes, although the oxazolidones are known to possess activity against anaerobes (15).

Emerging antimicrobial resistance is a significant problem among both nosocomially and community-acquired gram-positive bacteria. While VRE have become endemic at many medical centers (1, 5), the recent identification of glycopeptide-insensitive *S. aureus* represents a formidable therapeutic challenge. As resistance among gram-positive bacteria continues to spread, therapeutic options have become increasingly limited. Thus, the oxazolidinones represent a novel class of investigational antibacterial agents for the treatment of these multidrug-resistant infections.

The primary antimicrobial activity of linezolid is against gram-positive bacteria. This investigation describes the largest in vitro experience to date with linezolid and confirms that this antibacterial has excellent activity against all of the organisms tested, including staphylococci, streptococci, and enterococci. These tests were performed on fresh clinical isolates concurrently with other testing performed in the clinical microbiology laboratory. Against oxacillin-resistant strains of both *S. aureus* and *Staphylococcus epidermidis*, the activity of linezolid was comparable to that of vancomycin. This novel antimicrobial agent also demonstrated excellent activity against enterococci, including both vancomycin-susceptible and vancomycin-resistant isolates of *E. faecalis* and *E. faecium*. For *E. faecalis*, the activity of linezolid was similar to that of other agents tested; however, linezolid was the most active of all agents tested against *E. faecium*. There was no difference in activity for VRE. Significant in vitro activity was also observed for streptococci, including penicillin-resistant pneumococci. Our results confirm those previously reported by others (2, 11, 15); however, they represent a significant increase in the number of isolates tested. Based on data from pharmacokinetic studies (6), the preliminary susceptibility breakpoint for linezolid is ≤8 μg/ml. If this remains as the breakpoint, all of the gram-positive organisms that we tested were fully susceptible.

Previous reports have indicated that linezolid is bacteriostatic against VRE. This is consistent with the results reported from phase I trials with linezolid (12). While we did not perform time-kill kinetics analyses with staphylococci and streptococci, other investigators have demonstrated bacteriostatic activity against *S. aureus* and *S. epidermidis* (10, 11) and bactericidal activity against pneumococci (15). Despite the reported lack of in vitro bactericidal activity, we successfully treated a neutropenic patient who developed persistent VRE bacteremia with the combination of linezolid and gentamicin (9). This would suggest that the current in vitro testing method may not be able to fully assess the clinical activity of linezolid.

In summary, linezolid was the most active antimicrobial agent tested against oxacillin-resistant *S. aureus*, oxacillin-resistant *S. epidermidis*, and VRE (both *E. faecalis* and *E. faecium*). Based on our in vitro results, linezolid appears to be a promising new antimicrobial agent for the treatment of gram-positive infections.

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