ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2003, p. 1736–1738 Vol. 47, No. 5
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.5.1736–1738.2003

NOTES

In Vitro Activity of Linezolid against Slowly Growing Nontuberculous Mycobacteria

Barbara A. Brown-Elliott,* Christopher J. Crist, Linda B. Mann, Rebecca W. Wilson, and Richard J. Wallace, Jr.

Department of Microbiology, The University of Texas Health Center, Tyler, Texas

Received 2 December 2002/Returned for modification 13 January 2003/Accepted 29 January 2003

MICs of linezolid in broth microdilutions were tested against 341 slowly growing nontuberculous mycobacteria (NTM) belonging to 15 species. The proposed linezolid susceptibility MICs for all *Mycobacterium marinum*, *Mycobacterium szulgai*, *Mycobacterium kansasii*, *Mycobacterium malmoense*, and *Mycobacterium xenopi* isolates and for 90% of *Mycobacterium gordonae* and *Mycobacterium triplex* isolates were ≤8 μg/ml. Linezolid has excellent therapeutic potential against most species of NTM.

Treatment of infections due to slowly growing nontuberculous mycobacteria (NTM) remains difficult for many species. In some cases only a few drugs are available for therapy, and in most situations combination therapy is necessary. Previous in vitro studies with linezolid have shown it to be active against most species of rapidly growing mycobacteria (RGM) (11) and *Nocardia* (4) and recently against *Mycobacterium tuberculosis* (1) at readily achievable levels in serum. Thus, we undertook a study of the in vitro activity of linezolid against species of slowly growing NTM.

(This work was presented in part at the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, Calif., 27 to 30 September 2002.)

We tested linezolid against 341 isolates of slowly growing NTM belonging to 15 species. This number included 335 clinical strains submitted for susceptibility testing and/or identification and six reference isolates kindly provided by the American Type Culture Collection (ATTC). The isolates included those of the *Mycobacterium avium* complex (MAC) (189 isolates), *Mycobacterium marinum* (47 isolates), *Mycobacterium szulgai* (10 isolates), *Mycobacterium gordonae* (21 isolates), *Mycobacterium kansasii* (19 isolates), *Mycobacterium simiae* complex (15 isolates), *Mycobacterium terrae* complex (11 isolates), *Mycobacterium triplex* (10 isolates), *Mycobacterium lentiflavum* (5 isolates), *Mycobacterium xenopi* (5 isolates), *Mycobacterium malmoense* (5 isolates), and one isolate each of *Mycobacterium interjectum*, *Mycobacterium asiaticum*, *Mycobacterium scrofulaceum*, and *Mycobacterium branderi*. Susceptibility was determined once for each isolate except for some reference strains.

The isolates were identified by standard methods, including a combination of traditional biochemicals and high-performance liquid chromatography (2, 6; S. H. Chiu, K. C. Jost, Jr., D. F. Dunbar, and L. B. Elliott, Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998, abstr. U-76, p. 508, 1998), nucleic acid probes, (5) or PCR-restriction fragment length polymorphism analysis of the 439-bp Telenti fragment of the 65-kDa *hsp* gene (9, 10). Most isolates of the MAC were identified by high-performance liquid chromatography and/or nucleic acid probes. All species other than those of the MAC, *M. kansasii*, *M. marinum*, and *M. gordonae* were confirmed by PCR-restriction fragment length polymorphism analysis.

MICs were determined by using NCCLS-recommended serial twofold broth microdilutions in cation-adjusted Mueller-Hinton broth (12). MICs were tested with multiple lots of plates with linezolid concentrations ranging from ≤0.5 to 128 μg/ml. The MIC breakpoints were those proposed by the NCCLS for testing linezolid against RGM (12). Results were determined after 7 days of incubation at 35°C. The end point was complete (100%) inhibition of visible growth.

Quality control assays were performed with *Staphylococcus aureus* ATCC 29213; the linezolid MIC range for this strain is 1 to 4 μg/ml (after 18 to 24 h of incubation) (8). Additional quality control assays were performed with *M. marinum* ATCC 927^T, *M. avium* ATCC 700898, *M. avium* ATCC 35712, *M. avium* ATCC 35718, *M. triplex* ATCC 700071^T, and *M. malmoense* ATCC 29571^T.

Generally, linezolid showed excellent activity, with most MICs in the proposed ranges for susceptible (MIC, ≤8 μg/ml) or intermediate (MIC, ≤16 μg/ml) organisms (Tables 1 and 2). The MICs for five isolates of *M. lentiflavum* were 8 to 16 μg/ml, and the MICs for five isolates of *M. xenopi* were 4 to 8 μg/ml (data not shown). The MICs for the single isolates of *M. interjectum* and *M. asiaticum* were 16 μg/ml (data not shown).

In contrast, linezolid was less active in vitro against isolates of the MAC, the *M. terrae* complex, and the *M. simiae* complex. The MICs for the single isolates of *M. scrofulaceum* and *Mycobacterium branderi* were >32 and 32 μg/ml, respectively (data not shown).

The MICs for the *S. aureus* quality control strain were within the expected range. *M. marinum* ATCC 927^T was tested 58 times; the MIC for this strain was ≤2 μg/ml (58 values). *M.*

* Corresponding author. Mailing address: University of Texas Health Center, Department of Microbiology, 11937 US Hwy. 271, Tyler, TX 75708. Phone: (903) 877-7685. Fax: (903) 877-7652. E-mail: barbara.elliott@uthct.edu.

TABLE 1. Inhibition of slowly growing NTM by linezolid at specified MICs

Mycobacterial species[a]	No. of isolates tested[c]	No. of isolates susceptible at indicated MIC[b] (μg/ml) (cumulative % susceptible)									
		≤0.5	1	≤2	2	≤4	4	8	16	32	>32
MAC	189			2 (1)	2 (2)		3 (4)	17 (13)	50 (39)	63 (72)	52 (100)
M. marinum	47		7 (17)	28 (74)	12 (100)						
M. szulgai	10			2 (20)	3 (50)		5 (100)				
M. gordonae	21	2 (10)	1 (14)	15 (86)			1 (90)		2 (100)		
M. kansasii	19	2 (11)	2 (21)	15 (100)							
M. simiae	15							3 (20)	4 (47)	6 (87)	2 (100)
M. terrae or *M. nonchromogenicum*	11			2 (18)			1 (27)	1 (36)	4 (73)	2 (91)	1 (100)
M. triplex	10			2 (20)	1 (30)	4 (70)	1 (80)	1 (90)	1 (100)		

[a] Only species with 10 or more isolates are included.
[b] The three lowest MICs reflect different lot numbers of panels used.
[c] Total, 322.

avium ATCC 700898, which is the NCCLS-recommended strain for susceptibility testing of clarithromycin with the MAC, was also tested 23 times, with a resulting modal MIC of 32 μg/ml (range, 8 to 32 μg/ml). *M. triplex* ATCC 700071 was tested twice, with a resulting MIC of 16 μg/ml both times. Other quality control organisms were tested only once. The MICs for *M. avium* ATCC 35712, *M. avium* ATCC 35718, *M. triplex* ATCC 700071^T, and *M. malmoense* ATCC 29571^T were 64, 16, 16, and ≤2 μg/ml, respectively.

Previous studies have shown that 90% of the isolates of the RGM species *Mycobacterium fortuitum* and *Mycobacterium chelonae* were inhibited by ≤16 μg of linezolid per ml, with modal MICs of ≤8 μg/ml (11). Two immunosuppressed patients with macrolide-resistant disseminated *M. chelonae* infections as well as small numbers of other, nonimmunosuppressed patients have been successfully treated with linezolid (3; B. A. Brown-Elliott, R. J. Wallace, Jr., D. E. Griffith, D. Lakey, E. Moylett, M. Gareca, T. R. Perry, R. Blinkhorn, and D. Hopper, Abstr. 40th Annu. Meet. Infect. Dis. Soc. Am. 2002, abstr. 609, p. 151, 2002; M. G. Gareca, B. A. Brown-Elliott, and R. J. Wallace, Jr., Abstr. 40th Annu. Meet. Infect. Dis. Soc. Am. 2002, abstr. 265, p. 91, 2002). In the present study, linezolid demonstrated similar activities against most commonly encountered, slowly growing NTM, with only isolates of the MAC, the *M. terrae* complex, and *M. simiae* requiring ≥32 μg/ml to inhibit 90% of the isolates tested. *M. marinum*, *M. kansasii*, *M. gordonae*, *M. malmoense*, and *M. szulgai* were the most susceptible species, with 100% of the strains inhibited by ≤8 μg of linezolid per ml.

The peak levels of linezolid in serum after oral doses of 600 mg twice daily are 21.2 ± 5.8 μg/ml with a half-life of 5.4 h (package insert for Zyvox, Pharmacia and The Upjohn Co., Kalamazoo, Mich.). This suggests that 16 μg/ml may be a reasonable intermediate value for other organisms such as the NTM. Pending further clinical experience, we propose the following MIC breakpoints for the slowly growing NTM species: for susceptible isolates, ≤8 μg/ml; for intermediate isolates, 16 μg/ml; and for resistant isolates, ≥32 μg/ml. These same breakpoints have also been proposed by the NCCLS for antimycobacterial susceptibility testing (11, 12) for the RGM. For quality control in susceptibility testing, we recommend the use of *M. avium* ATCC 700898, with an acceptable linezolid MIC range of 8 to 32 μg/ml. We also recommend linezolid concentrations of 2 to 32 μg/ml for testing isolates of slowly growing NTM.

The use of linezolid for treatment of NTM infections has been very limited. This in part reflects the lack of long-term safety data, concern over limiting adverse hematologic events, and the cost of the drug (Brown-Elliott et al., Abstr. Annu. Meet. Infect. Dis. Soc. Am., 2002). One study suggested that once-daily adult dosing (600 mg) rather than the standard 600-mg twice-daily dosing that is used for bacterial species may be adequate for the treatment of mycobacterial infections and may also help to limit bone marrow suppression (Brown-Elliott

TABLE 2. In vitro activity of linezolid against eight species of slowly growing NTM

Mycobacterial species	No. of isolates[b]	MIC (μg/ml)[a] in broth				
		Range[c]	50%	90%	Mode	% Susceptible or intermediate
MAC	189	≤2–>32	32	64	32	39
M. marinum	47	1–2	≤2	2	≤2	100
M. szulgai	10	≤2–4	≤2	4	4	100
M. gordonae	21	≤0.5–16	≤2	4	≤2	100
M. kansasii	19	≤0.5–≤2	≤2	≤2	≤2	100
M. simiae	15	8–>32	32	>32	32	46
M. terrae or *M. nonchromogenicum*	11	≤2–>32	16	32	16	73
M. triplex	10	2–16	≤4	8	≤4, ≤2	100

[a] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.
[b] Total, 322.
[c] Total range, ≤0.5 to >32 μg/ml.

et al., Abstr. Annu. Meet. Infect. Dis. Soc. Am., 2002). Linezolid has been used successfully to treat small numbers of patients with *Nocardia* (7) and RGM (3; Brown-Elliott et al., Abstr. Annu. Meet. Infect. Dis. Soc. Am., 2002; Gareca et al., Abstr. 40th Annu. Meet. Infect. Dis. Soc. Am., 2002), infections, which suggests that the drug may work for infections with slowly growing NTM.

We thank Pharmacia Inc. for support of this research.

We also thank Joanne Woodring for preparation of the manuscript and the mycobacteriology section of the Texas Department of Health for assistance with the identification of some of the isolates.

REFERENCES

1. **Alcalá, L., M. J. Ruiz-Serrano, C. Pérez-Fernández Turégano, D. García de Viedma, M. Díaz-Infantes, M. Marín-Arriaza, and E. Bouza.** 2003. In vitro activities of linezolid against clinical isolates of *Mycobacterium tuberculosis* that are susceptible or resistant to first-line antituberculous drugs. Antimicrob. Agents Chemother. **47:**416–417.
2. **Brown, B. A., B. Springer, V. A. Steingrube, R. W. Wilson, G. E. Pfyffer, M. J. Garcia, M. C. Menendez, B. Rodriguez-Salgado, K. C. Jost, S. H. Chiu, G. O. Onyi, E. C. Böttger, and R. J. Wallace, Jr.** 1999. Description of *Mycobacterium wolinskyi* and *Mycobacterium goodii*, two new rapidly growing species related to *Mycobacterium smegmatis* and associated with human wound infections: a cooperative study from the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. **49:**1493–1511.
3. **Brown-Elliott, B. A., R. J. Wallace, Jr., R. Blinkhorn, C. J. Crist, and L. B. Mann.** 2001. Successful treatment of disseminated *Mycobacterium chelonae* infection with linezolid. Clin. Infect. Dis. **33:**1433–1434.
4. **4. Brown-Elliott, B. A., S. C. Ward, C. J. Crist, L. B. Mann, R. W. Wilson, and R. J. Wallace, Jr.** 2001. In vitro activities of linezolid against multiple *Nocardia* species. Antimicrob. Agents Chemother. **45:**1295–1297.
5. **LeBrun, L., F. Espinasse, J. D. Poveda, and V. Vincent-Levy-Frebault.** 1992. Evaluation of nonradioactive DNA probes for identification of mycobacteria. J. Clin. Microbiol. **30:**2476–2478.
6. **Metchock, B. G., F. S. Nolte, and R. J. Wallace, Jr.** 1999. *Mycobacterium*, p. 399–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
7. **Moylett, E. H., S. E. Pacheco, B. A. Brown-Elliott, T. R. Perry, E. S. Buescher, M. C. Birmingham, J. J. Schentag, J. F. Gimbel, A. Apodaca, M. A. Schwartz, R. M. Rakita, and R. J. Wallace, Jr.** 2003. Clinical experience with linezolid for the treatment of *Nocardia* infection. Clin. Infect. Dis. **36:**313–318.
8. **NCCLS.** 2002. Performance standards for antimicrobial susceptibility testing: 11th informational supplement. Document M100-S11. National Committee for Clinical Laboratory Standards, Wayne, Pa.
9. **Steingrube, V. A., J. L. Gibson, B. A. Brown, Y. Zhang, R. W. Wilson, M. Rajagopalan, and R. J. Wallace, Jr.** 1995. PCR amplification and restriction endonuclease analysis of a 65-kilodalton heat shock protein gene sequence for taxonomic separation of rapidly growing mycobacteria. J. Clin. Microbiol. **33:**149–153.
10. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Böttger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
11. **Wallace, R. J., Jr., B. A. Brown-Elliott, S. C. Ward, C. J. Crist, L. B. Mann, and R. W. Wilson.** 2001. Activities of linezolid against rapidly growing mycobacteria. Antimicrob. Agents Chemother. **45:**764–767.
12. **Woods, G. L., B. A. Brown-Elliott, E. P. Desmond, G. S. Hall, L. Heifets, G. E. Pfyffer, M. R. Plaunt, J. C. Ridderhof, R. J. Wallace, Jr., N. G. Warren, and F. G. Witebsky.** 2000. Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes, 2nd ed. Tentative standard M24-T2. National Committee for Clinical Laboratory Standards, Wayne, Pa.