Activities of Linezolid against nontuberculous mycobacteria

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SUMMARY

The activity of linezolid (Pfizer, USA) was tested by broth microdilution against 53 clinical isolates of non-tuberculous mycobacteria (NTM), including the common disease producing species *Mycobacterium avium, M.intracellulare, M.fortuitum, M.chelonae* and *M.abscessus*, obtained from western Turkey. The isolates of *M.abscessus* and *M.intracellulare* were the least susceptible, *M.mucogenicum, M.gordonae* and *M.avium* were the most susceptible to linezolid of the common species of NTM. Linezolid showed a variable sensitivity in all strains; therefore, each species and strain must be individually evaluated, and it is always advisable to perform in vitro sensitivity tests before using the drug for human therapy.

KEY WORDS: Linezolid, Nontuberculous mycobacteria, Broth microdilution

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INTRODUCTION

Infections due to non-tuberculous mycobacteria (NTM) have aquired great clinical importance in recent years as they may affect both immunocompromised and immunocompetant patients (Rodriguez Diaz J.C. *et al.*, 2003). Treatment of infections due to NTM remains difficult, in part because they are resistant to many of the first-line tuberculosis agents and in part because so few other agents are available for therapy (Wallace R.J. *et al.*, 2001).

Linezolid is a member of a new class of antibacterial agents called the oxazolidinones, whose mechanisms of action involves inhibition of protein synthesis at a very early stage (Alcalá L. *et al.*, 2003). Previous in vitro studies with linezolid have shown it to be active against most species of rapidly growing mycobacteria (Brown Elliott B.A. *et al.*, 2003).

This study tested the activities of linezolid against 53 clinical isolates of NTM, including the common disease producing species *Mycobacterium avium, M.intracellulare, M.fortuitum, M.chelonae* and *M.abscessus*.

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MATERIALS AND METHODS

Organisms

The 53 clinical isolates of NTM belonging to 10 species were included in the study. Organisms were identified to the species level by PCR-reverse hybridization (INNO LIPA Mycobacteria v2, Innogenetics NV, Ghent, Belgium) and DNA sequencing of the 441 bp of the hsp65 gene.The test species used and the number of clinical test isolates (in parentheses) were as follows: *M.peregrinum* (4); *M.fortuitum* (11); *M.mucogenicum* (4); *M.abscessus* (2); *M.chelonae* (5); *M.intracellulare* (9); *M.avium* (5); *M.gordonae* (10); *M.gilvum* (1); *M.simiae* (2).

Susceptibility testing

Susceptibility testing utilized serial twofold broth microdilution in cation-adjusted Mueller-Hinton broth. MICs were tested multiple lots of plates with linezolid (Pfizer, USA) concentrations ranging from ≤0.25 to 64 µg/ml. Results were determined after 3 (for rapidly growing mycobacteria; RGM) and 7 (slowly growing mycobacteria; SGM) days of incubation at 35°C. The end point was complete (100%) inhibition of visible growth. The following breakpoints were used mostly recommended: susceptible, ≤8 µg/ml; intermediate 16 µg/ml; and resistant ≥32 µg/ml (Wallace R.J. Jr. *et al.*, 2001; Brown Elliott B.A. *et al.*, 2003).

Quality control

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).

RESULTS

Susceptibility to linezolid was determined for 27 clinical isolates of RGM belonging to 6 (*M.peregrinum*, *M.fortuitum*, *M.mucogenicum*, *M.abscessus*, *M.chelonae* and *M.gilvum*) species and 26 clinical isolates of SGM belonging to 4 (*M.avium*, *M.intracellulare*, *M.gordonae* and *M.simiae*) species (Table 1).

Of the most common pathogenic RGM species, the drug was most active against isolates of *M.mucogenicum* and *M.peregrinum*. Of the *M. mucogenicum* isolates, 2 out of 4 were inhibited by linezolid at concentrations of ≤0.25 µg/ml, 100% were inhibited by linezolid concentrations at 8 µg/ml. For *M.peregrinum*, 3 of 4 of the test isolates were inhibited by linezolid at 8 µg/ml, one was inhibited by linezolid at 16 µg/ml. The linezolid was also partly active when tested against isolates of *M. fortuitum* and *M.chelonae*. Five out of 11 *M. fortuitum* isolates were inhibited by linezolid at concentrations of 8 µg/ml or less, and 6 out of 11 were inhibited by linezolid at 16 µg/ml or less.

For *M.chelonae* 2 out of 5 test isolates were inhibited by linezolid at concentrations 16 µg/ml or less, 3 were not inhibited by linezolid at 64 µg/ml. Isolates of *M.abscessus* were the least susceptible to linezolid of common pathogenic RGM species. With testing of 2 out of 2 isolates were

TABLE 1 - *Susceptibility of 53 clinical isolates of NTM to linezolid by broth microdilution.*

	No. of isolates inhibited by MIC (µg/ml)										
Species	*<0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*>64*	*No. of isolates tested*
M.peregrinum	0	0	0	0	0	3	1	0	0	0	4
M.fortuitum	0	0	1	0	2	2	1	0	0	5	11
M.mucogenicum	2	0	1	1	0	0	0	0	0	0	4
M.abscessus	0	0	0	0	0	0	0	0	0	2	2
M.chelonae	0	0	0	0	1	0	1	0	0	3	5
M.gilvum	1	0	0	0	0	0	0	0	0	0	1
M.avium	0	0	0	0	0	3	0	0	0	2	5
M.intracellulare	1	0	0	0	0	0	2	1	0	5	9
M.gordonae	10	0	0	0	0	0	0	0	0	0	10
M.simiae	0	0	0	0	0	2	0	0	0	0	2
Total	14		2	1	3	10	5	1		17	53

TABLE 2 - *In vitro activity of linezolid against 10 species of NTM (n=53).*

		MIC (16 µg/ml)				
Species	*No. of isolates tested*	*Range*	*50%*	*90%*	*Mode*	*% Susceptible or intermediate (MIC ≤16 µg/ml)*
M.peregrinum	4	8-16	8	16	8	100
M.fortuitum	11	1->64	16	>64	16	55
M.mucogenicum	4	<0.25-2	<0.25	2	1	100
M.abscessus	2	>64	>64	>64	>64	-
M.chelonae	5	4->64	>64	>64	>64	40
M.gilvum	1	<0.25	<0.25	<0.25	<0.25	100
M.avium	5	8->64	8	>64	8	60
M.intracellulare	9	<0.25-32	>64	>64	>64	33
M.gordonae	10	<0.25	<0.25	<0.25	<0.25	100
M.simiae	2	2	8	8	8	100

not inhibited by linezolid at concentrations 64 µg/ml (Tables 1 and 2).
With regard to SGM species, the drug was active against 100% of isolates of *M.gordonae* and *M.simiae*. For *M.avium*, 3 out of 5 of the test isolates were inhibited by linezolid at 8 µg/ml, 2 were not inhibited by linezolid at concentrations 64 µg/ml. Isolates of *M.intracellulare* were the least susceptible to linezolid of common pathogenic SGM species. With testing of 9 isolates, only 3 were inhibited by linezolid at concentrations 16 µg/ml or less (Tables 1 and 2).

DISCUSSION

This study found that the MICs were higher for *M.abscessus*, than for *M.mucogenicum*, *M.peregrinum, M. fortuitum* and *M.chelonae,* but the number of *M.abscessus* strains was limited. In agreement with the previous report (Wallace R.J. *et al.*, 2001), *M.abscessus* was the least susceptible, *M.mucogenicum* was the most susceptible to linezolid of the common species of RGM. However, generally, the MICs of our RGM isolates were higher than the MICs reported by others. With regard to SGM species, The MICs were higher for *M.avium* than for *M.gordonae* and *M.intracellulare.* In agreement with the previous reports (Brown Elliott B.A. *et al.*, 2003; Molicotti P. *et al.*, 2003), isolates of *M.gordonae* and *M.avium* were the most susceptible, while *M.intracellulare* was the least susceptible to linezolid of the common species of SGM.
The treatment of serious infections with RGM is a problem and limited by the small number of available drugs with activity at clinically achievable levels in tissue or/and blood. Linezolid is a member of a new class of antibacterial agents called the oxazolidinones, which are chemically unrelated to currently available agents. This agent selectively binds to the 23S of the 50S ribosomal subunit, thereby resulting in inhibition of bacterial protein synthesis (Swaney S.M. *et al.*, 1998). The molecular basis of linezolid resistance in enterococci has been identified as a single G2576T nucleotide polymorphism in multiple alleles encoding 23S rRNA (Marshall S.H. *et al.*, 2002; Ruggero K.A. *et al.*, 2003). However no mutations were detected in potential target genes in linezolid resistant *Mycobacterium tuberculosis* strains. Therefore, the mechanism of linezolid re-

sistance remains unclear in mycobacteria (Richter E. *et al*., 2007)

In conclusion, a variable sensitivity to linezolid exists in all strains. Therefore, each species and strain must be individually evaluated, and it is advisable always to perform in vitro sensitivity tests before using the drug for human therapy.

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