ELSEVIER

International Journal of Antimicrobial Agents 18 (2001) 267–270

INTERNATIONAL JOURNAL OF
Antimicrobial
Agents

www.ischemo.org

Short communication

In vitro activity of linezolid against clinical Gram-positive bacterial isolates from Taiwan: an area with a high prevalence of antibiotic resistance

Chi-Tai Fang [a], Shan-Chwen Chang [a,b,*], Yee-Chun Chen [a], Szu-Min Hsieh [a], Wei-Chuan Hsieh [a]

[a] *Section of Infectious Diseases, Department of Internal Medicine, National Taiwan University Hospital, No. 7 Chung-Shan South Road, Taipei 100, Taiwan, ROC*

[b] *Graduate Institute of Clinical Pharmacy, National Taiwan University, Taipei, Taiwan, ROC*

Received 20 March 2001; accepted 26 April 2001

Abstract

The prevalence of antibiotic-resistant bacteria in Taiwan is due to the heavy use of antimicrobial agents in both animal husbandry and clinical practice over the past decades. Minimum inhibitory concentrations (MICs) of linezolid were established for 371 clinical isolates of staphylococci, pneumococci, enterococci and group A streptococci from Taiwan. All isolates tested including those resistant to β-lactams, erythromycin, vancomycin and quinupristin–dalfopristin were uniformly susceptible to linezolid, with MICs ranging from 0.125 to 2 mg/l. Our data support the observation that there is no cross-resistance between linezolid and other classes of antimicrobial substances.

Keywords: In vitro; Gram-positive bacteria; Taiwan

1. Introduction

Linezolid, an oxazolidinone [1], kills bacteria through blocking formation of the initiation complex at the ribosome to inhibit bacterial protein synthesis [2]. Linezolid was developed for the treatment of infections caused by β-lactam- and glycopeptide-resistant Gram-positive bacteria [3–6]. As quinupristin–dalfopristin is not a consistently effective agent against these bacteria [7], linezolid now serves as a last resort treatment [8,9]. In this study, we investigated the in vitro activity of linezolid against 371 non-duplicate clinical Gram-positive bacterial isolates from Taiwan, a country with one of the highest prevalence of antibiotic-resistant bacteria due to the heavy use of antimicrobial agents in both animal husbandry and clinical practice in past decades [10–14].

2. Materials and methods

2.1. Bacterial isolates

The strains tested were derived from various clinical specimens at the National Taiwan University Hospital (Taipei, Taiwan) in 1998 and 1999. They included 53 strains of methicillin-resistant *Staphylococcus aureus* (MRSA), 67 methicillin-susceptible *S. aureus* (MSSA),

* Corresponding author. Tel.: +886-2-2312-3456x5401; fax: +886-2-2397-1412.

E-mail address: sc4030@ha.mc.ntu.edu.tw (S.-C. Chang).

0924-8579/01/$20
PII: S0924-8579(01)00384-3

60 methicillin-resistant *Staphylococcus epidermidis* (MRSE), 60 methicillin-susceptible *S. epidermidis* (MSSE), 60 *Enterococcus faecalis*, 54 *Streptococcus pneumoniae* and 17 *Streptococcus pyogenes*. The isolates were selected randomly and only one isolate was chosen from a patient. Bacterial species identification was based on standard clinical microbiological methods. We did not include *Enterococcus faecium* in this study because *E. faecalis* is the predominant *Enterococcus* species isolated in Taiwan.

2.2. Antimicrobial agents

The following antimicrobial agents were obtained from their manufacturers for use in this investigation, linezolid (Pharmacia Upjohn, Kalamazoo, MI, USA), penicillin G and oxacillin (Bristol-Myers Squibb, Syracuse, NY, USA), erythromycin (Abbott, North Chicago, IL, USA), vancomycin (Eli Lilly, Indianapolis, IN, USA) and quinupristin/dalfopristin (Rhône-Poulenc Rorer, Vitrysur-Seine, France).

Table 1
In vitro activity of linezolid and other antimicrobial agents against Gram-positive bacteria

Organism (number tested)	Antimicrobial agent	MIC (mg/l)[a]			Susceptibility (%)[b]
		Range	50%	90%	
S. aureus					
Oxacillin-resistant (53)	Linezolid	0.5–2	1	2	–
	Penicillin G	8–64	32	32	0
	Oxacillin	4 to >128	128	>128	0
	Erythromycin	1 to >128	>128	>128	0
	Vancomycin	1–2	1	2	100
	Quinupristin–dalfopristin	0.25–1	0.5	1	100
Oxacillin-susceptible (67)	Linezolid	0.5–2	2	2	–
	Penicillin G	0.25–8	1	4	1.5
	Oxacillin	0.125–1	0.25	0.5	100
	Erythromycin	0.125 to >128	0.25	>128	65.7
	Vancomycin	0.5–1	1	2	100
	Quinupristin–dalfopristin	0.25–1	0.5	1	100
S. epidermidis					
Oxacillin-resistant (60)	Linezolid	0.5–2	2	2	–
	Penicillin G	0.5–32	4	16	0
	Oxacillin	4 to >128	16	>128	0
	Erythromycin	0.5 to >128	>128	>128	8.3
	Vancomycin	0.5–2	2	2	100
	Quinupristin–dalfopristin	0.25–2	0.25	1	100
Oxacillin-susceptible (60)	Linezolid	0.25–2	1	2	–
	Penicillin G	0.125–8	0.5	2	10
	Oxacillin	0.06–2	0.5	1	100
	Erythromycin	<0.03 to >128	0.25	>128	50
	Vancomycin	0.5–2	1	2	100
	Quinupristin–dalfopristin	0.125–1	0.25	0.5	100
S. pneumoniae (54)	Linezolid	0.125–0.5	0.125	0.25	–
	Penicillin G	<0.03–4	0.25	2	35.2
	Erythromycin	<0.03 to >128	32	>128	14.8
	Vancomycin	0.125–1	0.25	0.5	100
	Quinupristin–dalfopristin	0.25–4	1	2	88.9
S. pyogenes (17)	Linezolid	0.125–2	1	2	–
	Penicillin G	<0.03–0.25	<0.03	<0.03	100
	Erythromycin	<0.03 to >128	0.06	>128	76.5
	Vancomycin	0.125–0.25	0.25	0.25	100
	Quinupristin–dalfopristin	<0.03–0.5	0.125	0.25	100
E. faecalis (60)	Linezolid	0.25–2	2	2	–
	Penicillin G	0.5–16	2	2	96.7
	Erythromycin	0.25 to >128	>128	>128	21.7
	Vancomycin	0.5 to >128	1	4	91.7
	Quinupristin–dalfopristin	1–32	2	8	3.3

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

[b] Breakpoints for reading as susceptible were, penicillin G, ≤0.12, ≤0.06 mg/l for pneumococci, ≤8 mg/l for enterococci; oxacillin, ≤2 mg/l; erythromycin, ≤0.5, ≤0.25 mg/l for streptococci and pneumococci; vancomycin, ≤4 mg/l; ≤1 mg/l for streptococci and pneumococci, quinupristin/dalfopristin, ≤1 mg/l.

2.3. Susceptibility test

The minimum inhibitory concentrations (MICs) for all bacteria tested except *S. pneumoniae* were determined by using the agar dilution method, as described by the National Committee for Clinical Laboratory Standards [15]. A Steers' replicator was used to apply 10^4 CFUs on to Mueller–Hinton agar containing serial 2-fold dilutions of each antimicrobial agent (from 128 to 0.03 mg/l). For testing the susceptibility of staphylococci to oxacillin, 2% NaCl was added to the medium. The agar plates were incubated at 35 °C for 18 h before reading. For *S. pyogenes*, 5% sheep blood was added and the plates were incubated at 35 °C for 24 h. The broth microdilution method was used for *S. pneumoniae*, as described by the NCCLS [15]. *S. aureus* American Type Culture Collection (ATCC) 29213, *E. faecalis* ATCC 29212 or *S. pneumoniae* ATCC 49619 were used as internal controls. The breakpoints used for determining susceptibility were those defined by the NCCLS [15].

3. Results

The MICs of tested Gram-positive bacteria to linezolid and other antibiotics are summarized in Table 1. All strains tested in this study were susceptible to linezolid, including strains resistant to β-lactams, erythromycin, vancomycin or quinupristin–dalfopristin.

All 240 strains of staphylococci were susceptible to vancomycin. The MICs of linezolid was 0.5–2 mg/l for MRSA, 0.5–2 mg/l for MSSA, 0.5–2 mg/l for MRSE, and 0.25–2 mg/l for MSSE. Of the 54 tested *S. pneumoniae* strains, 35 (64.8%) were not susceptible to penicillin, including 16 (29.6%) penicillin-resistant strains (MICs 2 mg/l). The MICs of linezolid were 0.125–0.5 mg/l for *S. pneumoniae*. Five (8%) of the 60 *E. faecalis* strains tested were resistant to vancomycin, two (3%) were resistant to penicillin G, but 54 (90%) were resistant to quinupristin–dalfopristin. The MICs of linezolid for *E. faecalis* were 0.25–2 mg/l. All 17 *S. pyogenes* strains tested were susceptible to penicillin G, but four (23.5%) were resistant to erythromycin. For *S. pyogenes*, the MICs of linezolid were 0.125–2 mg/l.

4. Discussion

There are still no standard MICs interpretive criteria for linezolid. Jones and Biedenbach suggested that 4 mg/l should be the breakpoint of susceptibility [16]. Using this provisional breakpoint (susceptible, ≤ 4 mg/l), all clinical Gram-positive pathogenic bacteria tested were uniformly susceptible to linezolid, including strains resistant to β-lactams, erythromycin, vancomycin or quinupristin–dalfopristin.

Taiwan has a very high prevalence of antibiotic-resistant bacteria [10–14]. In a 1998–1999 multicentre surveillance study, 76 and 94% *S. pneumoniae* isolates were found to be resistant to penicillin and macrolides, respectively [14]. Of the staphylococcal isolates, 62% *S. aureus* and 63.2% coagulase-negative staphylococci were resistant to oxacillin [12]. Although less than 10% of the enterococci isolates were vancomycin-resistant, around 77% were resistant to gentamicin [12].

We found no cross-resistance between linezolid and other classes of antimicrobial substances, despite the heavy use of a wide variety of antimicrobial substance in Taiwan.

References

[1] Brickner SJ, Hutchinson DK, Barbachyn MR, et al. Synthesis and antibacterial activity of U-100592 and U-100766, two oxazolidinone antibacterial agents for the potential treatment of multidrug-resistant gram-positive bacterial infections. J Med Chem 1996;39:673–9.

[2] Kloss P, Xiong L, Shinabarger DL, Mankin AS. Resistance mutations in 23S rRNA identify the site of action of the protein synthesis inhibitor linezolid in the ribosomal peptidyl transferase center. J Mol Biol 1999;294:93–101.

[3] Eliopoulos GM, Wennersten CB, Gold HS, Moellering RC Jr. In vitro activities in new oxazolidinone antimicrobial agents against enterococci. Antimicrob Agents Chemother 1996;40:1745–7.

[4] Jones RN, Johnson DM, Erwin ME. In vitro antimicrobial activities and spectra of U-100592 and U-100766, two novel fluorinated oxazolidinones. Antimicrob Agents Chemother 1996;40:720–6.

[5] Noskin GA, Siddiqui F, Stosor V, Hacek D, Peterson LR. In vitro activities of linezolid against important gram-positive bacterial pathogens including vancomycin-resistant enterococci. Antimicrob Agents Chemother 1999;43:2059–62.

[6] Zurenko GE, Yagi BH, Schaadt RD, et al. In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. Antimicrob Agents Chemother 1996;40:839–45.

[7] Werner G, Klare I, Witte W. Association between quinupristin–dalfopristin resistance in glycopeptide-resistant *Enterococcus faecium* and the use of additives in animal feed. Eur J Clin Microbiol Infect Dis 1998;17:401–2.

[8] Bostic GD, Perri MB, Thal LA, Zervos MJ. Comparative in vitro and bactericidal activity of oxazolidinone antibiotics against multidrug-resistant enterococci. Diagn Microbiol Infect Dis 1998;30:109–12.

[9] Patel R, Rouse MS, Piper KE, Steckelberg JM. In vitro activity of linezolid against vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*. Diagn Microbiol Infect Dis 1999;34:119–22.

[10] Chang SC, Hsieh WC, Luh KT. Resistance to antimicrobial agents of common bacteria isolated from Taiwan. Int J Antimicrob Agents 1994;4:143–6.

[11] Chang SC, Chang HJ, Lai MS. Antibiotic usage in primary care units in Taiwan. Int J Antimicrob Agents 1999;11:23–30.

[12] Chang SC, Hsieh WC, Liu CY. The Antibiotic Resistance Study Group of the Infectious Disease Society of the Republic of China. High prevalence of antibiotic resistance of common pathogenic bacteria in Taiwan. Diagn Microbiol Infect Dis 2000;36:107–12.

[13] Hsueh PR, Teng LJ, Lee LN, Yang PC, Ho SW, Luh KT. Extremely high incidence of macrolide and trimethoprim-sulfamethoxazole resistance among clinical isolates of *Streptococcus pneumoniae* in Taiwan. J Clin Microbiol 1999;37:897–901.

[14] Hsueh PR, Liu YC, Shyr JM, et al. Multicenter surveillance of antimicrobial resistance of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in Taiwan during the 1998–1999 respiratory season. Antimicrob Agents Chemother 2000;44:1342–5.

[15] National Committee for Clinical Laboratory Standards (NCCLS). Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 5th ed. Approved Standard, M7-A4. Wayne, PA: NCCLS; 2000.

[16] Biedenbach DJ, Jones RN. Disk diffusion test interpretive criteria and quality control recommendations for testing linezolid (U-100766) and eperezolid (U-100592) with commercially prepared reagents. J Clin Microbiol 1997;35:3198–202.