The post-antibiotic effects of linezolid against Gram-positive pathogens

Mine Hosgor-Limoncu, *MD,* ***Safak Ermertcan,*** *MD,* ***Huseyin Tasli,*** *PhD,* ***Sohret Aydemir,*** *MD.*

ABSTRACT

Objectives: To investigate the post-antibiotic effect (PAE) of linezolid against methicillin-resistant and -susceptible staphylococci, vancomycin-resistant and -susceptible enterococci.

Methods: Clinical strains of *Staphylococcus aureus (S. aureus)*, *Staphylococcus epidermidis (S. epidermidis)* and *Enterococcus faecalis* (*E. faecalis)* were isolated from hospitalized patients at Ege University Medical Faculty Hospital between June and September 2005. This study was made between September and December 2005. The PAE of linezolid was determined at the minimum inhibitory concentration (MIC) and 4 times the MIC concentrations for 60 minutes in Mueller-Hinton Broth (MHB). The duration of the PAE was obtained by following the recovery of bacterial growth in antibiotic free MHB measured colony forming units on Mueller-Hinton agar.

Results: All the straines were susceptible to linezolid. The PAE was greater at 4 times the MIC (0.5 - 2.4 hours) that at the MIC (0 - 1.7 hours) for linezolid against all organisms tested. The PAE for linezolid was slightly higher against *E. faecalis* strains than other organisms.

Conclusion: In this study, it was demonstrated that linezolid had a moderate in vitro PAE against *S. aureus, S. epidermidis and E.faecalis strains.*

Saudi Med J 2007; Vol. 28 (4): 551-554

From the Department of Pharmaceutical Microbiology (Hosgor-Limoncu, Ermertcan, Tasli) and the Department of Microbiology and Clinical Microbiology (Aydemir), Faculty of Medicine, Ege University, Izmir, Turkey.

Received 28th August 2006. Accepted 10th December 2006.

Address correspondence and reprint request to: Dr. Mine Hosgor-Limoncu, Associated Professor, Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Ege University, Bornova, Izmir 35100, Turkey. Tel. +90 (232) 3880110/1358. Fax. +90 (232) 3885258. E-mail: minehosgorlimoncu@yahoo.com.tr

The oxazolidinones prepared by organic synthesis are new class of antibacterial agents. These agents have been introduced since 1980. Linezolid is the first member of the oxazolidinones. It inhibits bacterial ribozomal protein synthesis by preventing the formation of the translation initiation complex. Linezolid binds to rRNA, specifically to domain V of the 23S rRNA of the 50S ribosomal subunit, which is encoded by genes (rDNA) present in multiple copies in clinically relevant species. Selection for linezolid-resistant mutants of various species is difficult, as of resistance would likely require mutations in multiple copies of 23S rDNA.[1-4] Nosocomial infections with resistant Gram-positive pathogens have shown a steady worldwide increase over the last decade. Recently these isolates with reduced susceptibility to glycopeptides have also been reported. Since current options for the treatment of Gram-positive infections are limited, the development of novel antimicrobial classes such as the oxazolidinones are urgently needed.[5] Linezolid has potent activity against multidrug-resistant Gram-positive pathogens such as vancomycin-resistant *Enterococcus faecalis (E. faecalis)* (VRE) and *Enterococcus faecium*, penicillin-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus (S. aureus)* (MRSA), and methicillin-resistant *Staphylococcus epidermidis (S. epidermidis) (*MRSE). Linezolid displays nonbactericidal, time-dependant activity in vitro against staphylococci.[1-4] The post-antibiotic effect (PAE) is the suppression or slowing down of bacterial growth after a short exposure to an antimicrobial agent, which is then eliminated from the culture. Determination of the PAE is very important for all new antimicrobial agents such as linezolid as it is a factor that influences the optimal antimicrobial dosing intervals. To exhibit the PAE of an antibiotic, prolongs the interval between the dosages. Finally, compliance of the patient increases, cost of treatment and drug toxicity decreases.[6-10] Therefore, in this study, we aimed to investigate the PAE of linezolid against methicillin-resistant and -susceptible staphylococci, vancomycin-resistant and -susceptible enterococci.

Methods. Clinical strains of *S. aureus, S. epidermidis* and *E. faecalis* were isolated from hospitalized patients at

Ege University Medical Faculty Hospital, Izmir, Turkey between June and September 2005. This study was made between September and December 2005. The test organisms included one MRSA, one methicillin-susceptible *S. aureus* (MSSA), one vancomycin-intermediate *S. aureus* (VISA), the vancomycin-intermediate *S. aureus* strain Mu50, the heterogeneous-vancomycin-intermediate *S. aureus* strain Mu3, one MRSE, one methicillin-susceptible *S. epidermidis* (MSSE), one VRE, one vancomycin-susceptible *E. faecalis* (VSE) and also the reference strains *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, and *E. faecalis* ATCC 29212. Linezolid was provided by Pfizer. Linezolid MICs were determined by microdilution method using Mueller-Hinton broth (MHB) (Merck) according to criteria of the National Committee for Clinical Laboratory Standards (NCCLS).[11] Prior to testing, antimicrobial dilutions were dispensed into the trays. The organisms were subcultured onto blood agar plates and incubated overnight. Suspensions in saline with a turbidity equivalent to that of a 0.5 McFarland standard were prepared, diluted and inoculated into 96-well microtiter plate. The final inoculum density was $5x10^5$ CFU/mL. The plates were incubated for 16-20 hours and the MICs were read.

The presence of PAE was determined for linezolid for representatives of each group of organisms by the method described by Craig and Gudmundsson.[6] A 10^6 CFU/mL of logarithmic phase organisms were exposed linezolid at the MIC and at 4 times the MIC for 1

Table 1 - Strains distribution according to clinical departments and minimum inhibitory concentrations (MICs) of linezolid.

Strains	Samples	Clinic	Linezolid MIC (µg/mL)
Staphylococcus aureus (S. aureus)			
Methicillin-resistant *S. aureus*	Blood	Anesthesiology and reanimatology	1
Methicillin-susceptible *S. aureus*	Wound swab	Dermatology	1
Vancomycin-intermediate *S. aureus*	Blood	Internal medicine	1
Vancomycin-intermediate *S. aureus strain*			0.5
The heterogeneous Vancomycin-intermediate *S. aureus*			1
Staphylococcus epidermidis (S. epidermidis)			
Methicillin-resistant *S. epidermidis*	Blood	Neurology	1
Methicillin-susceptible *S. epidermidis*	Blood	Neurology	1
Enterococcus faecalis (E. faecalis)			
Vancomycin-resistant *E. faecalis*	Rectal swab	Internal medicine	1
Vancomycin-susceptible *E. faecalis*	Blood	Anesthesiology and reanimatology	1

Table 2 - Post-antibiotic effect parameters after one hour exposure of bacterial strains to linezolid in vitro.

Strains	Post-antibiotic effect (hour)	
	1 x minimum inhibitory concentration	4 x minimum inhibitory concentration
Staphylococcus aureus (S. aureus)		
Methicillin-resistant *S. aureus*	0.7	1.3
Methicillin-susceptible *S. aureus*	0	0.5
Vancomycin-intermediate *S. aureus*	0.1	0.5
Vancomycin-intermediate *S. aureus strain*	0.2	0.7
The heterogeneous Vancomycin-intermediate *S. aureus*	0.8	1
American type culture collections (ATCC 25923)	1.7	2.3
American type culture collections (ATCC 29213)	1	1.2
Staphylococcus epidermidis (S. epidermidis)		
Methicillin-resistant *S. epidermidis*	1	2
Methicillin-susceptible *S. epidermidis*	0.3	1.7
Enterococcus faecalis (E. faecalis)		
Vancomycin-resistant *E. faecalis*	1.1	2.4
Vancomycin-susceptible *E. faecalis*	1.1	1.3
American type culture collections (ATCC 29212)	1.4	1.8

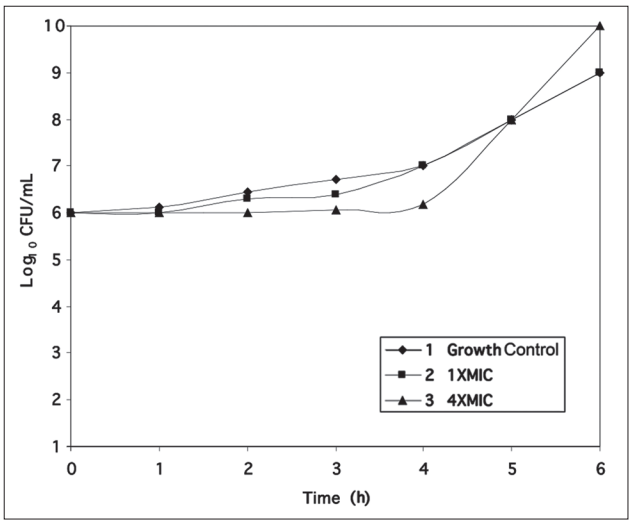

Figure 1 - Growth curves by viable count of methicillin susceptible *Staphylococcus aureus* culture after one hou exposure to linezolid.

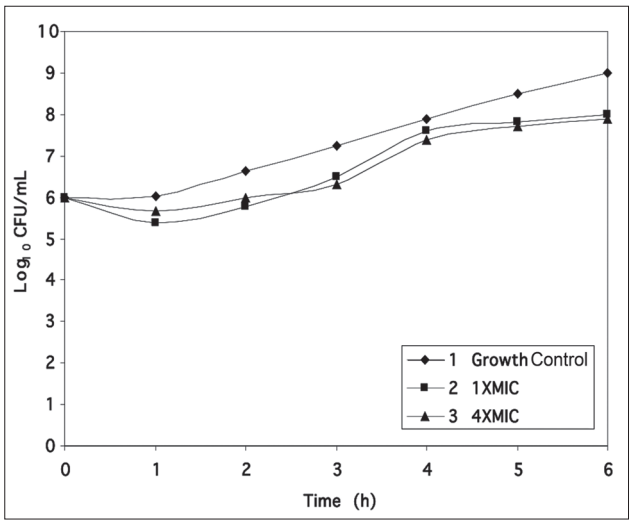

Figure 3 - Growth curves by viable count of Mu50 culture after one hour exposure to linezolid.

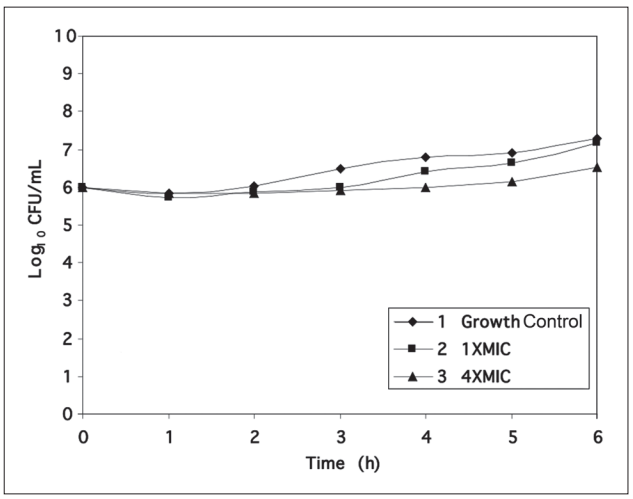

Figure 2 - Growth curves by viable count of vancomycin-resistant *Enterococcus faecalis* culture after one hour exposure to linezolid.

hour at 37°C with shaking in benmari. One test tube of each organism was also used as a growth control. After 1 hour, the drug was removed by centrifuging the solution for 5 minutes at 10000 g. After decanting the supernatant, bacteria were resuspended in fresh pre-warmed sterile MHB using volumes equivalent to the original culture volumes and this washing procedure was performed twice. These new cultures were then incubated at 37°C. Samples were removed for viable counting before washing, immediately after washing and at hourly intervals thereafter. Samples were serially diluted with ice-cold saline and plated onto Mueller-Hinton agar (MHA) to determine the PAE. Colonies were counted after incubation at 37°C for 24-48 hours. All the tests were performed in duplicate. The duration of the PAE was calculated by the equation PAE = T – C, where T is the time required for the count of CFU in the culture exposed to antibiotic to increase 1 $\log_{10}$ above the count observed immediately after drug removal, and C is the time required for the count of CFU in the control culture to increase 1 $\log_{10}$ above the count observed immediately after completion of the same procedure used the test culture for drug removal.

Results. Strains distribution according to clinical departments and linezolid MICs are shown in **Table 1**. Linezolid MICs of all organisms were 0.5-1 µg/mL. All of the strains were susceptible to linezolid. The PAEs after 1 hour exposure to linezolid are presented in the **Table 2** and **Figures 1 to 3.** The PAE range was 0-1.7 hours at the MIC and 0.5-2.4 hours at 4 times the MIC for linezolid against all organisms tested. After 1 hour exposure to linezolid, at 1xMIC, the in vitro PAEs were 0-1.7 hour for *S. aureus*, 0.3-1 hour for *S. epidermidis*, and 1.1-1.4 hour for *E. faecalis.* At 4xMIC, the PAEs of *S. aureus, S. epidermidis,* and *E. faecalis* were 0.5-2.3 h, 1.7-2 h, and 1.3-2.4 h respectively. The PAEs of *E. faecalis* were slightly longer than the staphylococci. The minimum PAEs were detected with MSSA.

Discussion. The linezolid is a member of oxazolidinone which is a unique class of synthetic antimicrobials. It has activity against a wide variety of resistant microorganisms such as MRSA, MRSE, and VRE. Other unique features of linezolid include its novel mechanism of action, for which it does not display cross-resistant activity with other classes of antimicrobials, and the fact that the spontaneous rate of mutation to resistance for it is very low. Also, it was shown that

linezolid had PAE in some studies. If an antibiotic has a PAE, it can be administered in an extended dosing intervals and in this way, cost and toxicity of the drug will be reduced.[10, 12] In this study, we demonstrated that linezolid possess moderate PAE. The PAE was greater at 4 times the MIC (0.5-2.4 hour) than at the MIC (0-1.7 hour) for linezolid against all organisms tested. Among staphylococci, *S. aureus* ATCC 25923 had long PAE while MSSA had very short. The PAE for linezolid was slightly longer against *E. faecalis* strains than other organisms. In a study of Rybak et al,[12] PAE of linezolid was investigated with MRSA, MRSE, vancomycin-resistant *E. faecalis* and vancomycin-resistant *E. faecium* isolates. They found that the PAE of linezolid was longer at 4xMIC (0.2-1.4 hours) than at 1xMIC (0.1-0.8 hour) for all organisms tested. They stated that the PAE for *E. faecalis* was considerably lower than the *E. faecium* and staphylococci isolates. They also indicated that the PAE for linezolid was relatively short. In another study, the PAE range of linezolid was determined as 1.2 to 2.2 hour for staphylococci, and 1.4 to 2.1 hours for enterococci.[10] In the studies discussed above the determined linezolid PAE values were similar to the results of this study. The only difference is longer PAE that was found in *E. faecalis* strains. In an experimental study on mice, PAE of linezolid was determined as 3.6 - 3.8 hours on MSSA strains. They stated that linezolid produced only minimal PAE. Scientists working on this subject found the duration of PAE of linezolid at least 2 hours longer than the in vitro results.[13] There are limited human data on the pharmacokinetics of linezolid. It has 6 hour of serum half-life. Since in vivo PAEs are usually longer than those observed in vitro, linezolid can be given at intervals of 8 to 12 hours or longer.[10, 12]

In conclusion, we demonstrated that linezolid had a moderate in vitro PAE against *S. aureus, S. epidermidis* and *E. faecalis*. Methicillin resistance in *S. aureus* and *S. epidermidis* and vancomycin resistance in *E. faecalis* had no effect upon the in vitro MIC value of linezolid and on the duration of the PAE. The results of this study support that linezolid, newly introduced in our country, an be used twice daily in humans.

Acknowledgments. *The authors wish to thank to Pfizer Inc. for providing linezolid.*

References

1. Donowitz GR. Oxazolidinones. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 5th ed. Philedelphia: Churchill Livingstone; 2000. p. 392-394.
2. Meka VG, Gold HS. Antimicrobial resistance to linezolid. ***Clin Infect Dis*** 2004; 39: 1010-1015.
3. Jacqueline C, Navas D, Batard E, Miegeville AF, Le Mabecque V, Kergueris MF et al. In vitro and in vivo synergistic activities of linezolid combined with subinhibitory concentrations of imipenem against methicillin-resistant *Staphylococcus aureus*. ***Antimicrob Agents Chemother*** 2005; 49: 45-51.
4. Jacqueline C, Caillon J, Le Mabecque V, Miegeville AF, Donnio PY, Bugnon D et al. In vitro activity of linezolid alone and combination with gentamicin, vancomycin or rifampicin against methicillin-resistant *Staphylococcus aureus* by time-kill curve methods. ***J Antimicrob Chemother*** 2003; 51: 857-864.
5. Abb J. In vitro activity of linezolid, quinupristin-dalfopristin, vancomycin, teicoplanin, moxifloxacin and mupirosin-resistant *Staphylococcus aureus*: comparative evaluation by the E-test and a broth microbiol dilution method. ***Diagn Micbiol Infect Dis*** 2002; 43: 319-321.
6. Craig WA, Gudmundsson S. The postantibiotic effect. In: Lorian V, editor. Antibiotics in laboratory medicine. 2nd ed. New York: Williams and Wilkins; 1986. p. 515-536.
7. Dominguez MC, De La Rosa M, Borobio MV. Application of a spectrophotometric method for the determination of post-antibiotic effect and comparison with viable counts in agar. ***J Antimicrob Chemother*** 2001; 47: 391-398.
8. Stubbings WJ, Bostock JM, Ingham E, Chopra I. Assessment of a microplate method for determining the post-antibiotic effect in *Staphylococcus aureus* and *Escherichia coli*. ***J Antimicrob Chemother*** 2004; 54: 139-143.
9. Vogelman B, Gudmundsson S, Leggett J, Turnidge J, Ebert S, Craig WA. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. ***J Infect Dis*** 1988; 158: 831-847.
10. Munckhof W, Giles C, Turnidge JD. Post-antibiotic growth suppression of linezolid against Gram-positive bacteria. ***J Antimicrob Chemother*** 2001; 47: 879-883.
11. National Committee for Clinical Laboratory Standards Methods for Dilution Susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. NCCLS, Villanova, PA, 1997.
12. Rybak MJ, Cappelletty DM, Moldovan T, Aeschlimann JR, Kaatz GW. Comparative in vitro activities and post-antibiotic effects of the oxazolidinone compounds eperezolid (PNU-100592) and linezolid (PNU-100766) versus vancomycin against *Staphylococcus aureus,* coagulase-negative *staphylococci, Enterococcus faecalis, and Enterococcus faecium*. ***Antimicrob Agents Chemother*** 1998; 42: 721-724.
13. Andes D, van Ogtrop ML, Peng J, Craig WA. In vivo pharmacodynamics of a new oxazolidinone (linezolid). ***Antimicrob Agents Chemother*** 2002; 46: 3484-3489.