

Efficacy of Linezolid Alone or in Combination with Vancomycin for Treatment of Experimental Endocarditis Due to Methicillin-Resistant *Staphylococcus aureus*

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The levels of effectiveness of linezolid, vancomycin, and the combination of linezolid and vancomycin were compared in the rabbit model of endocarditis caused by a clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolate. Vancomycin alone was more effective than either linezolid alone or the combination of linezolid and vancomycin for the treatment of endocarditis due to MRSA.

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in many hospitals has become a major therapeutic challenge. The reliance on vancomycin as the antimicrobial agent of choice for serious infections and its subsequently increased utilization has led to concerns about its reduced effectiveness and the development of resistance. In fact, *S. aureus* isolates with intermediate resistance to vancomycin and two recent clinical isolates of *S. aureus* with high-level vancomycin resistance have already been documented (8, 13). The emergence of these resistant isolates underscores the need for the development of new antimicrobial agents that can provide an alternative to vancomycin for the treatment of multidrug-resistant *S. aureus* infections.

Linezolid, the first oxazolidinone approved for human use, has been demonstrated in *in vitro* studies to have antimicrobial activity against gram-positive pathogens, such as *S. aureus*, coagulase-negative staphylococci, *Streptococcus pneumoniae*, and vancomycin-resistant enterococci (3). Linezolid has been shown to be as effective as vancomycin in the treatment of nosocomial pneumonias caused by gram-positive organisms and of skin and soft tissue infections (12, 14). However, animal studies have shown disappointing results in the treatment of *S. aureus* osteomyelitis (10). The effectiveness of linezolid in the treatment of *S. aureus* endocarditis due to methicillin-sensitive *S. aureus* and MRSA has been previously investigated (5, 9). Both studies demonstrated effectiveness of oral linezolid at doses of 50 and 75 mg/kg three times a day (t.i.d.) in comparison to vancomycin, while linezolid at 25 mg/kg t.i.d. was ineffective. In the treatment of MRSA experimental endocarditis, oral linezolid given in doses of 50 and 75 mg/kg led to mean reductions of aortic valve vegetation bacterial counts of 4.42 and 6.06 log₁₀ CFU/g compared to those of untreated controls (5). However, levels of linezolid in the sera of rabbits receiving these doses were considerably higher than those seen with human dosing.

The objectives of this study were to determine whether lin-

ezolid alone can be an effective treatment agent for MRSA endocarditis and to investigate whether linezolid can provide synergistic activity when given in combination with vancomycin.

The rabbit model of aortic valve endocarditis, which has been described previously (4, 11), was used to evaluate antibiotic treatment regimens. Twenty-four hours after transcatheter placement of polyethylene catheters across the aortic valves, New Zealand White female rabbits (3 to 3.5 kg) were injected intravenously (i.v.) through the marginal ear veins with 1 ml of an overnight culture containing 10⁷ CFU of the test organism, MRSA 27619. MRSA 27619 is a homotypic clinical MRSA isolate with the following susceptibilities: MIC of methicillin, >256 µg/ml; MIC of vancomycin, 1 µg/ml; and MIC of linezolid, 2 µg/ml. Blood samples for culture were obtained 24 h later, and the rabbits were randomly assigned to one of the following treatment groups: (i) no treatment (control group), (ii) vancomycin at 30 mg/kg i.v. twice a day (b.i.d.) for 5 days, (iii) linezolid at 75 mg/kg orally t.i.d. on day 1 and then b.i.d. for 4 more days, (iv) vancomycin plus linezolid at the same doses for 5 days, and (v) linezolid at 75 mg/kg orally t.i.d. for 5 days. Linezolid was prepared as a 75-mg/ml oral suspension in a sterile vehicle (Pharmacia Corporation, Kalamazoo, Mich.). Surviving animals were sacrificed by i.v. administration of pentobarbital after a total of 5 days of antibiotic treatment. Rabbits with negative blood cultures at 24 h were excluded from subsequent analysis. To reduce the possibility of antibiotic carryover, rabbits were not killed until at least 18 h after administration of the last antimicrobial dose.

The heart and kidneys were aseptically removed from each rabbit. Aortic valve vegetations and kidney abscesses or infarcts were removed, weighed, homogenized in saline, and serially diluted. Dilutions were plated on Mueller-Hinton agar, and the plates were incubated at 37°C for 48 h. Titers of bacteria were expressed as log₁₀ CFU per gram of tissue. Sterile aortic vegetation and kidney cultures contained ≤2 and ≤1 log₁₀ CFU/g, respectively (the limit of detection). The mean numbers of bacteria per gram of vegetation and kidney in all treatment groups were compared by analysis of variance. The Student-Newman-Keuls test was used to adjust for multi-

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TABLE 1. Outcome of 5-day treatment of experimental MRSA aortic valve endocarditis

Treatment regimen	No. sterile at the following site/total no. of rabbits		Mean bacterial count (\log_{10} CFU/g) \pm SD	
	Valve vegetation	Kidney	Valve vegetation	Kidney
Control	0/8	0/8	10.24 \pm 0.68	8.66 \pm 0.73
Vancomycin	3/8	3/8	3.31 ^{a,b} \pm 3.00	2.23 ^a \pm 2.44
Linezolid t.i.d. for 1 day, for 4 days	0/8	7/8	7.80 ^a \pm 0.99	0.71 ^a \pm 2.02
Linezolid plus vancomycin	0/8	8/8	6.56 ^a \pm 1.03	1.00 ^a \pm 0.00
Linezolid t.i.d. for 5 days	0/8	3/8	6.27 ^a \pm 1.80	1.75 ^a \pm 1.47

^a $P < 0.05$ for all treatment groups versus controls.

^b $P < 0.05$ for vancomycin-treated animals versus those treated with linezolid, linezolid and vancomycin, and linezolid t.i.d.

ple comparisons. A P value of <0.05 was considered statistically significant for all tests.

Blood samples were taken at day 3 to determine the linezolid steady-state concentration in the plasma. One milliliter of blood was drawn 1 h after the first dose of drug (peak) and 8 h later (trough), just prior to the second dose. Samples were spun for 1 min in a microcentrifuge, and plasma aliquots were stored at -20°C . These plasma samples were sent to the Pharmacokinetics Laboratory at the National Jewish Medical and Research Center (Denver, Colo.) for measurement of the concentrations of linezolid in plasma by high-performance liquid chromatography and mass spectrometry, as previously described (5).

Checkerboard synergy testing was performed by using the microdilution method in microtiter trays with cation-adjusted Mueller-Hinton broth. Combinations of linezolid and vancomycin were tested at concentrations of 0.015 to 16 and 0.25 to 16 $\mu\text{g/ml}$, respectively. Microtiter plates were incubated at 37°C and read at 24 and 48 h. The fractional inhibitory concentration (FIC) index was calculated by adding the FICs (MIC of drug A in combination with drug B/MIC of drug A alone) of linezolid and vancomycin. A total of 20 clinical MRSA isolates underwent synergy testing. All strains behaved similarly, with an average FIC index of 2, indicating additive or indifferent activity between the two antimicrobial agents. None of the strains tested demonstrated synergy or antagonism between the two drugs.

The results obtained with the 5-day antibiotic treatment regimen for experimental endocarditis due to MRSA 27619 are presented in Table 1. A total of 40 rabbits infected with MRSA 27619 were assigned to the various treatment regimens. Control rabbits had a mean \pm standard deviation aortic valve vegetation bacterial count of $10.24 \pm 0.68 \log_{10}$ CFU/g, which is comparable to those reported previously from trials of MRSA endocarditis (4). All treatment regimens reduced aortic valve vegetation bacterial counts significantly ($P < 0.05$) compared with the vegetation bacterial counts from the controls. Treatment with linezolid alone, linezolid in combination with vancomycin, and linezolid t.i.d. led to mean reductions in aortic valve vegetation bacterial counts of $2.44 \log_{10}$ CFU/g ($P < 0.05$), $3.68 \log_{10}$ CFU/g ($P < 0.05$), and $3.97 \log_{10}$ CFU/g ($P < 0.05$), respectively, compared to those in untreated control rabbits. However, the most effective treatment arm was vancomycin, resulting in a mean aortic valve vegetation bacterial count of $3.31 \pm 3.00 \log_{10}$ CFU/g, which is a mean reduction of $6.93 \log_{10}$ CFU/g compared with the counts in the control group ($P < 0.05$). Vancomycin-treated rabbits demonstrated

greater mean reductions in valvular vegetation bacterial counts than those in the other treatment groups ($P < 0.05$), and vancomycin also sterilized aortic valve vegetations in three of eight rabbits. In contrast, none of the rabbits treated with linezolid had sterile aortic valve vegetations.

Table 1 also shows the results of kidney tissue cultures for the different treatment groups. All treatment regimens reduced mean bacterial counts in the kidneys significantly compared with those in the untreated control group ($P < 0.05$). Although there was no statistically significant difference observed in the reduction of mean bacterial counts in the kidneys among the treated groups, rates of sterilization of kidney abscesses were better in rabbits treated with regimens containing linezolid. This may indicate perhaps greater drug concentrations of linezolid in the kidneys.

The results obtained for the peak and trough concentrations of linezolid in plasma are presented in Table 2. The average peak and trough levels of linezolid in serum achieved with approved human dosing (600 mg b.i.d.) are 18 and 4 $\mu\text{g/ml}$, respectively (3). Levels of linezolid in the sera of the rabbits were well above the MIC for the test organism (2 $\mu\text{g/ml}$) and were considerably higher in all linezolid-treated groups than those obtained with human dosing. The average peak levels were approximately three to four times higher than those obtained with human dosing, while the average trough levels were five to 10 times higher. The markedly high trough levels observed suggest accumulation of the drug over time. Of note, the treatment regimen including the combination of linezolid and vancomycin lowered the peak linezolid levels in serum compared with the peak levels obtained with regimens with linezolid alone.

In this study of experimental aortic valve endocarditis

TABLE 2. Mean concentrations of linezolid in plasma for peak and trough samples

Linezolid treatment regimen	Day 3 concn ($\mu\text{g/ml}$) in plasma \pm SD at:	
	1 h (peak)	8 h (trough)
75 mg/kg t.i.d. for 1 day, b.i.d. for 4 days	68.63 \pm 7.79	19.12 \pm 3.00
75 mg/kg t.i.d. for 1 day, b.i.d. for 4 days (with vancomycin)	43.58 ^a \pm 3.65	22.76 \pm 3.69
75 mg/kg t.i.d. for 5 days	70.50 \pm 5.75	42.56 ^b \pm 4.30

^a $P < 0.05$ for linezolid-vancomycin versus linezolid-only regimens.

^b $P < 0.05$ for linezolid t.i.d. versus linezolid and linezolid-vancomycin regimens.

caused by MRSA 27619, treatment with vancomycin alone was more effective than that with either linezolid alone or the combination of linezolid and vancomycin. Although in vitro synergy testing revealed additive or indifferent activity between the two drugs, in vivo antagonism was demonstrated by using the rabbit model. The failure to predict in vivo results from in vitro synergy studies has also been described previously with cephalosporin-rifampin (2) and vancomycin-rifampin (1) combinations against *S. aureus* in experimental endocarditis models. The discrepancy between in vitro and in vivo results underscores the variety of different mechanisms involved in in vivo antibiotic interactions, some of which may not be possible to analyze by the use of in vitro data (6). The observed antagonism between vancomycin and linezolid may be explained by the effects of combining a bacteriostatic agent, such as linezolid, with a bactericidal drug. In addition, we observed a reduction in peak linezolid levels in serum with the combination of the two drugs, which suggests that additional mechanisms may be involved in the interaction between the two antibiotics. Further studies may be needed to investigate the interaction between these two antimicrobial agents.

The decreased efficacy of linezolid compared to that of vancomycin in this study was not a result of inadequate linezolid levels in serum, as both the peak and trough levels were well above the MIC for the test organism. In fact, the levels achieved in the rabbit model were considerably higher than those seen with human dosing. These high serum drug levels raise the concern regarding the use of linezolid, which has focused on the occurrence of myelosuppression, in particular thrombocytopenia. Preclinical studies have shown that linezolid produces a time- and dose-dependent reversible myelosuppression (7).

Recent studies have shown linezolid to be as effective as vancomycin in the treatment of infections caused by gram-positive organisms, such as nosocomial pneumonia and skin and soft tissue infections (12, 14, 15). However, there have not been any studies investigating the efficacy of the combination, even though the two antimicrobial agents are probably being used together in clinical practice. Although linezolid may provide an alternative for patients who are intolerant to vancomycin or perhaps serve as a transition from vancomycin therapy to an oral antibiotic, our data suggest that linezolid should probably not be given together with vancomycin, as the combination was less effective than vancomycin alone.

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