Efficacy and pharmacodynamics of linezolid, alone and in combination with rifampicin, in an experimental model of methicillin-resistant *Staphylococcus aureus* endocarditis

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**Objectives:** To evaluate the efficacy of oral linezolid, with or without rifampicin, on valve vegetations and secondary foci of infection compared with vancomycin, in the absence or presence of rifampicin, in experimental endocarditis caused by methicillin-resistant *Staphylococcus aureus*. 

**Methods:** Treatment groups were controls (n = 16), linezolid (n = 15), vancomycin (n = 15), linezolid and rifampicin (n = 15), vancomycin and rifampicin (n = 13), linezolid relapse (n = 11) and vancomycin relapse (n = 9). Therapy lasted 5 days in all groups, with survival of animals in the linezolid relapse and vancomycin relapse groups being recorded for an additional 5 days. Blood was drawn to determine the linezolid concentration, and valve vegetations, and kidney, liver, lung and spleen segments were collected for culture.

**Results:** Survival in each individual group was higher than that in the control group; bacterial load in valve vegetations was reduced by all treatment regimens, with linezolid exhibiting bactericidal effects. Bactericidal activity of linezolid was noted in all secondary foci of infection except the lung, where only the combination of rifampicin with linezolid was bactericidal.

**Conclusions:** Orally administered linezolid is effective in limiting bacterial growth in the secondary foci of endocarditis. Co-administration of rifampicin favoured the suppression of bacterial growth in the lung.

Keywords: vancomycin, pharmacokinetics, secondary foci, tissues

**Introduction**

Linezolid is the first member of the oxazolidinones approved for clinical use for infections by resistant Gram-positive cocci. Case studies have described its efficacy in endocarditis caused by methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to vancomycin. In the present experimental study, oral linezolid was administered alone and in combination with rifampicin for the management of MRSA endocarditis. The effect on the bacterial growth in valves and in secondary infective foci was tested.

**Materials and methods**

The applied MRSA isolate was from the blood of a patient with endocarditis. It carried the *mecA* gene as defined by the PCR amplification of a 533 bp of the gene.

Following overnight incubation in Mueller–Hinton broth, the inoculum was adjusted to $1 \times 10^7$ cfu/mL and used for the induction of endocarditis.

MICs were determined by a microdilution technique in Mueller–Hinton broth (Becton-Dickinson, Franklin Lakes, USA) in volumes of 0.1 mL using a $5 \times 10^6$ cfu/mL inoculum (ranges:
0.25–512 mg/L linezolid; 0.125–128 mg/L vancomycin; 0.004–
128 mg/L rifampicin). MIC was defined as the lowest antimicrobial
concentration limiting visible bacterial growth after 24 h of incubation at 35 °C.

The study obtained permission from the Veterinary Directorate of the Prefecture of Athens in accordance with the Greek and European Union legislation. The rabbit model of aortic-valve endocarditis was used; a total of 94 white male New Zealand rabbits were divided into seven study groups and treated for 5 days with the following regimens:

- Group A (n = 16): controls;
- Group B (n = 15): linezolid;
- Group C (n = 15): vancomycin;
- Group D (n = 15): linezolid and rifampicin;
- Group E (n = 13): vancomycin and rifampicin;
- Group F (n = 11): linezolid, as a test of relapse; and
- Group G (n = 9): vancomycin, as a test of relapse.

Administered doses were: linezolid (Pfizer Pharmaceuticals Group, New York, NY, USA) 75 mg/kg/8 h orally which provides in rabbits a kinetic profile similar to that of a 280 mg/kg continuous 24 h infusion; vancomycin (Pharmaserve-Lilly, Athens, Greece) 25 mg/kg/12 h intravenously and rifampicin 5 mg/kg/8 h intravenously.

Surviving animals of groups A–E were sacrificed 12 h after the end of the treatment; those of groups F and G were sacrificed 5 days after the last dose. Aortic valvular and left ventricular friable vegetations and segments from the kidney, spleen, liver and lower lobe of the right lung were removed aseptically either at the time of sacrifice or within 12 h if an animal was found dead.

Excised vegetations and tissue samples were weighed, homogenized in 0.9% NaCl and quantitatively cultured in duplicate onto Chapman agar plates after seven serial dilutions in 0.9% NaCl (10⁻¹ to 10⁻⁷). After incubation for 48 h at 30 °C, the number of colonies was multiplied by the appropriate dilution factor; results were expressed as log₁₀ cfu/g. The lower detection limit was 10 cfu/g. Valve vegetation and tissue cultures without any visible bacterial growth were considered sterile.

Blood samples (1 mL) were drawn at serial time points from the ear veins of rabbits of group B for estimating the concentrations of linezolid after analysis through an HPLC system. The range of concentrations of the standard curve of the purified substance (Pharmacia-Upjohn, Kalamazoo, IL, USA) was 0.39–100 mg/L. All determinations were performed in duplicate. The inter-day variation of the assay was 12.5%. Peak concentration (Cmax) of linezolid and time to reach Cmax (Tmax) were determined directly from the concentration versus time curve, and the elimination half-life (t1/2) by the equation ln 2/k (k is the slope of the concentration–time curve at the terminal log-linear phase).

Tissue viable cells were expressed by their mean ± SE; linezolid concentration and pharmacokinetic parameters were expressed by their mean ± SD. Survival was estimated by the Kaplan–Meier analysis; groups were compared by the log-rank test. Comparisons between sterile and non-sterile valve cultures were performed with the Fisher’s exact test. Comparisons of mean bacterial burden of blood and tissue cultures among the groups were performed by ANOVA and were adjusted according to Bonferroni to avoid random correlations. P values below 0.05 were considered significant.

**Results**

MICs of linezolid, vancomycin and rifampicin for the test isolate were 2, 1 and 2 mg/L, respectively. Median survival was: 2.0 days for group A; >5.0 days for B (P < 0.001 versus A); >5.0 days for C (P < 0.001 versus A); >5.0 days for D (P < 0.001 versus A); and 3.7 days for E (P: 0.014 versus A).

All groups had lower bacterial counts in valve vegetation compared with controls (P < 0.001); the highest reduction was achieved in group B (Figure 1). Sterilization of heart valves was found in six animals of group B (40%, P: 0.007 versus A), in one animal of C (6.7%, P: not significant versus A), in five animals of D (33.3%, P: 0.018 versus A) and in two animals of E (27.3%, P: not significant versus A). All therapeutic regimens were equally effective in the reduction of organ bacterial load in comparison to group A by the end of therapy with the exception of the lung (Figure 1). In the latter, bacterial load was reduced only in group D.

Mean ± SD log₁₀ cfu/g of bacterial load in valve vegetations of group F was 5.03 ± 1.14 mg/L (P: not significant versus group B) and of group G was 4.02 ± 0.79 mg/L (P: not significant versus group C). Three animals of group F (27.3%, P: not significant versus group B) and three animals of group G (33.3%, P: not significant versus group C) had sterile valves.

Mean ± SD serum linezolid at 30, 60, 90, 120, 150, 180 and 240 min after the first dose was 4.88 ± 5.91, 3.83 ± 2.08, 2.35 ± 2.11, 1.64 ± 1.14, 1.61 ± 0.79, 1.22 ± 0.52 and 2.19 ± 1.20 mg/L, respectively. Mean ± SD Cmax was 6.73 ± 4.46 mg/L; mean ± SD Tmax was 0.8 ± 0.4 h and mean ± SD t1/2 was 1.32 ± 0.45 h. Mean ± SD serum linezolid on days 2, 3, 4, 5 and 7 was 1.52 ± 1.57, 2.78 ± 0.82, 7.08 ± 2.57, 10.50 ± 2.55 and 8.78 ± 1.92 mg/L, respectively.

**Figure 1.** Bacterial load of the kidney, liver, lower lobe of the right lung, spleen and of the aortic valve vegetations on death or sacrifice of rabbits with MRSA aortic valve endocarditis. Group A, controls (triangles); group B, linezolid (filled squares); group C, vancomycin (filled circles); group D, linezolid + rifampicin (open squares); group E, vancomycin + rifampicin (open circles). *P < 0.01 among groups.
Discussion

Linezolid is an antimicrobial agent with potent activity against MRSA\(^1\) and could provide an alternative to vancomycin when tolerance is present or when oral switch is desirable. Rifampicin has excellent bactericidal anti-staphylococcal activity, but is nowadays used in combination with other drugs due to the emergence of resistance.\(^7\) The efficacy of linezolid in the eradication of primary and secondary infective foci in experimental endocarditis by MRSA and the significance of the addition of rifampicin were assessed.

Linezolid was bactericidal in vivo with efficacy similar to that of vancomycin; its efficacy was comparable to that reported in previous studies of experimental MRSA endocarditis.\(^5\) Rifampicin did not enhance the overall efficacy of linezolid either in survival or in the reduction of bacteria in valves.

The present study is the first to assess the impact of linezolid on the bacterial load of secondary foci of bacterial endocarditis. Linezolid was bactericidal for the test isolate in the kidney, liver and spleen. When combined with rifampicin, it was also bactericidal in the lung. The latter effect may be attributed to the more efficient penetration of linezolid in lung tissue and epithelial lining fluid. This finding is in accordance with the superior effect of linezolid compared with vancomycin in patients with ventilator-associated MRSA pneumonia.\(^9\)

Trough linezolid concentration in the present study was 2–2.5 times above that in humans.\(^1\) From the second day of the treatment, linezolid concentration was higher than the MIC throughout the entire dosage interval, confirming that effective treatment of infective endocarditis is achieved when trough levels of the antimicrobial agent at the end of therapy are above the MIC.\(^10\)

The present study is the first to indicate that oral linezolid limits bacterial growth in the secondary foci of endocarditis. Co-administration of rifampicin favoured the suppression of bacterial growth in the lung. The efficacy of orally administered linezolid in this animal model indicates that clinical studies in humans are warranted.

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Transparency declarations

None to declare.

References